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Sustainable Agriculture Volume 2

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All contents have previously been published in the journal "Agronomy for Sustainable Development" published by EDP Sciences, France.

ISBN 978-94-007-0393-3 e-ISBN 978-94-007-0394-0
DOI 10.1007/978-94-007-0394-0
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009926164

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

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Part I
Novel Concepts

Emerging Agroscience

Eric Lichtfouse, Marjolaine Hamelin, Mireille Navarrete, Philippe Debaeke, and Agnès Henri

Contents

Abstract Climate change and the recent financial crisis clearly show that humans have entered the anthropocene, an unprecedented era of fast and possibly dangerous changes. Unprecedented changes call for unprecedented thinking. Indeed, agricultural research has been for too long driven solely by the need for higher yields using classical agrosciences, whatever the adverse ecological effects. Agricultural research needs the input of other sciences such as ecological, economic, social and political sciences. These social sciences emerged in agricultural research a few decades ago, but there are currently no precise trends and data on the speed of emergence of specific topics. Therefore, here we report: (1) an analysis of the emergence of topics in the journal *Agronomy for Sustainable Development*, and (2) a review of selected articles published in 2009. First, to analyse topic emergence we studied three data sets: most-cited articles from 1999 to 2009, topic hits in article text from 1999 to 2009, and most-downloaded articles in 2009. We found the following major points. Most-cited articles show that transgenic plants and biofuels are clearly emerging topics from 2007, whereas soil carbon and climate change are the major mainstream topics of the last 10 years. Topic hits analysis allows one to rank topics by mean emergence date, e.g. 2008.3 for 'genetically modified' and 2005.3 for 'irrigation'. Accordingly, the 10 most emerging topics over 1999–2009 are biofuels, genetically modified, conservation agriculture, urban agriculture, sociology, organic farming, carbon sequestration, phytoremediation, mulch and biodiversity. Analysis of most-downloaded articles in 2009 shows the predominance of topics such as carbon, climate, biodiversity, biofuels, pollutants, beneficial microbes, transgenic plants and organic farming. Second,

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_1,

from Lichtfouse et al., Agron. Sustain. Dev. 30 (2010) 1–10. DOI: 10.1051/agro:2009055

we reviewed selected articles published in 2009 with emphasis on emerging topics. We find that sociology is clearly bringing novel and unexpected findings to designing sustainable agriculture. Transgenic crops are highly innovative but show many unknowns that need to be carefully studied using various disciplines. Climate change has many scientifically proven effects on terrestrial ecosystems and agriculture. Here, soil carbon loss should be of particular attention because it rules the long-term fate of many factors such as atmospheric CO_2, erosion, and water and nutrient supply. Biodiversity loss due to industrial monocropping is leading scientists to disclose alternative, more diverse cropping systems that optimise biodiversity, pest control and yield.

Keywords Agriculture • Climate change • Biofuel • Transgenic plants • Biodiversity • Sociology • Organic farming • Conservation agriculture • Urban agriculture • Industrial agriculture • Carbon sequestration • Soil carbon • Crop rotation • No tillage • Beneficial microbes • Citation analysis • Topic emergence analysis • Most-cited articles • Most-downloaded articles

1 Introduction

Climate change and the recent financial crisis clearly show that humans have entered an unprecedented era of fast and possibly dangerous changes. This era is the anthropocene, a term that was coined in 2000 by the Nobel Prize-winning atmospheric chemist Paul Crutzen to point out that human activities now have a global impact on climate and ecosystems. Crutzen has explained, “I was at a conference where someone said something about the Holocene. I suddenly thought this was wrong. The world has changed too much. So I said: ‘No, we are in the anthropocene’. I just made up the word on the spur of the moment. Everyone was shocked. But it seems to have stuck”.

Unprecedented changes call for unprecedented adaptation. Unprecedented adaptation calls for unprecedented thinking. For instance, a major issue is that agricultural research has been for too long driven solely by the need for *higher yields* using monoculture, whatever the adverse ecological effects, such as food and drinking water pollution, biodiversity loss, and pest resistance. Mainstream goals such as higher yields should be challenged and rethought to take into account other factors. Those factors should not be solely defined by classical agrosciences, e.g. plant and soil sciences, but should also include all other sciences that really rule agriculture; for instance, ecological, economic, social and political sciences (De Bon et al., 2009; Wezel et al., 2009; Fleming and Vanclay, 2009; Lamine and Bellon, 2009; Lichtfouse et al., 2009a, b; Veldkamp et al., 2009). In other words, agronomy should not be reduced to a science that improves crop yields but should answer all society’s issues because agriculture is both the foundation and the future of society. Agronomists should rethink the role of agriculture in our society. For instance, studies of farming systems should also include food systems (Gliessman, 2007).

Previous works have attempted to define the core issues of the industrial agricultural society: technology without wisdom; and the tragedy of the global commons: soil, water and air (Lal, 2009a, b); artificialisation and painkiller solutions; climate change and outdated society structures; and society dependence and sustainable agriculture (Lichtfouse, 2009a–c). In the book *Sustainable Agriculture* we have gathered 53 review articles that cover major advances in agrosciences (Lichtfouse et al., 2009a). Four recent books also report major contributions in emerging agrosciences (Lichtfouse et al., 2009d–g). Here, we analyse recent topical trends in the journal *Agronomy for Sustainable Development*. Topical trends are assessed using three indicators: (1) most-cited articles 1999–2009, (2) topic hits on the journal website over 1999–2009, and (3) most-downloaded articles in 2009. We then briefly review selected journal articles published in 2009.

2 Most-Cited Articles 1999–2009

A first means to assess topics in the journal is to look at most-cited articles in the *Journal Citation Reports*. Table 1 shows yearly top-cited articles in the journal *Agronomy for Sustainable Development* from 1999 to 2009. The results indicate that major recent topics are transgenic plants, agroindicators, alternative crop management, beneficial microbes, and topics related to climate change such as biofuels and soil carbon. Whereas

Table 1 Top 3 yearly most cited articles in the journal *Agronomy for Sustainable Development*. Number of citing articles, named cites, are from ISI-thompson on October 22, 2009. The top 5 highest cites are printed in bold. Full references are given in the reference list

Most-Cited Articles			
Year	Cites	Topics	First author - Title
2008	5	Transgenic plants	BONNY. Genetically modified glyphosate-tolerant soybean in the USA...
	5	Transgenic plants	DEVOS. Feasibility of isolation perimeters for genetically modified maize
	4	Agroindicators, N, pesticides	BOCKSTALLER. Agri-environmental indicators to assess cropping and farming...
2007	16	Transgenic plants	DEVOS. Implementing isolation perimeters around genetically modified...
	11	Alternative fertilisation, beneficial microbes	GARG. Symbiotic nitrogen fixation in legume nodules: process and signaling.
	10	Biofuels, climate, carbon	HILL. Environmental costs and benefits of transportation biofuel production...
2006	17	Alternative management, soil, carbon	BERNOUX. Cropping systems, carbon sequestration and erosion in Brazil.
	17	Alternative fertilisation, soil, carbon	HACHICHA. Compost of poultry manure and olive mill wastes as an alternative...
	8	Pollutants, food	GROVA. Effect of oral exposure to polycyclic aromatic hydrocarbons...
2005	19	Alternative management, soil, pesticides	LACAS. Using grassed strips to limit pesticide transfer to surface water.
	18	Agroindicators, biodiversity	CLERGUE. Biodiversity: function and assessment in agricultural areas.
	13	Transgenic plants	COLBACH. Spatial aspects of gene flow between rapeseed varieties and volunteers.
2004	25	Alternative management, biodiversity	MARRIOTT. Long-term impacts of extensification of grassland management...
	21	Alternative management, soil, erosion	LE BISSONNAIS. Grass strip effects on runoff and soil loss.
	17	Soil, carbon, compost	AMIR. Elemental analysis, FTIR and C-13-NMR of humic acids from sewage...
2003	**98**	Soil, carbon, roots	NGUYEN. Rhizodeposition of organic C by plants: mechanisms and controls.
	26	Soil, carbon, fungi	CASARIN. Quantification of oxalate ions and protons released by ectomycorrhizal...
	23	Pollutants, toxicity	ROUT. Effect of metal toxicity on plant growth and metabolism: I. Zinc.
2002	**146**	Alternative management, soil, carbon	SIX. Soil organic matter, biota and aggregation in temperate and tropical soils...
	50	Agroindicators, climate, soil	BRISSON. STICS: a generic model for simulating crops and their water...
	33	Climate, greenhouse	REICHRATH. Using CFD to model the internal climate of greenhouses...
2001	51	Climate, carbon, salt stress	DORAI. Influence of electric conductivity management on greenhouse tomato...
	35	Alternative control	EHRET. Disinfestation of recirculating nutrient solutions in greenhouse horticulture.
	32	Carbon, growth	GUICHARD. Tomato fruit quality in relation to water and carbon fluxes.
2000	**72**	Climate, canopy	WEISS. Investigation of a model inversion technique to estimate canopy...
	52	Alternative control, intercropping	FINCKH. Cereal variety and species mixtures in practice...
	37	Beneficial microbes	BOSSIS. The taxonomy of Pseudomonas fluorescens and Pseudomonas putida...
1999	**73**	Climate, remote sensing	CEROVIC. Ultraviolet-induced fluorescence for plant monitoring...
	44	Climate, canopy	FOURNIER. ADEL-maize: an L-system based model for the integration of growth...
	40	Plant architecture, model	GODIN. Exploration of a plant architecture database with the AMAPmod...

transgenic plants and biofuels are clearly emerging topics from 2007, soil carbon and climate are strikingly apparent as major topics through the whole 1999–2009 period. Soil carbon and climate are also topics of the 4 most-cited articles from 1999 to 2009. Other most-cited topics include agroindicators; alternative crop management, pest control and fertilisation; biodiversity; and pollutants and pesticides. We conclude that transgenic plants and biofuels are clearly emerging topics, whereas soil carbon and climate are the major mainstream topics of the last 10 years.

3 Journal Website Hits

We also studied topics by counting hits over 1999–2009 using the search engine of the journal website (Table 2). As topics refer to queries found in the whole article text, topics do not necessarily represent article topic. We ranked topics by decreasing order of emergence using the mean emergence date calculated by averaging dates weighted by hits. Figures 1 and 2 show examples of the evolution of topic hits with time. Four evolution types were identified: (1) A *plateau*; for

Table 2 Mean emergence date (MED) of topics in articles from the journal *Agronomy for Sustainable Development*. Topics were searched in all article text, and thus do not necessarily reflect the article topic. Topics that showed highest hits in 2009 are printed in bold. Hits in percent refer to the number of articles containing topics measured using the journal website search engine on October 22–23, 2009, versus total yearly published articles. Mean emergence date was calculated as the average of years weighed by hits: $\sum(\text{Hits.year})/\sum\text{Hits}$, thus allowing to sort topics by order of emergence in the journal. Most recent topics have thus most recent date (see Fig. 1). For queries having several words, e.g. genetically modified, we used double quotes operators ("...") to retrieve only answers from words appearing together. AD: Anno Domini

TOPICS	MED AD	2009 %	2008 %	2007 %	2006 %	2005 %	2004 %	2003 %	2002 %	2001 %	2000 %	1999 %
Biofuels	2008.29	5.48	3.39	2.27	0	0	0	0	0	0	0	0
Genetically modified	2008.25	5.48	5.08	2.27	0	0	0	0	0	0	0	0
Conservation agriculture	2007.54	13.70	1.69	0	9.09	0	1.92	0	0	0	0	0
Urban agriculture	2007.22	2.74	1.69	0	0	0	1.92	0	0	0	0	0
Sociology	2007.11	10.96	0	2.27	0	1.59	3.85	1.27	0	0	0	0
Organic farming	2007.05	**38.36**	20.34	22.73	9.09	7.94	0	3.80	5.13	1.43	2.50	0
Carbon sequestration	2006.92	16.44	11.86	6.82	9.09	4.76	3.85	0	3.85	0	0	0
Phytoremediation	2006.87	1.37	6.78	2.27	0	3.17	0	1.27	0	0	0	0
Mulch	2006.62	21.92	28.81	15.91	15.15	3.17	9.62	1.27	5.13	1.43	1.25	1.79
Biodiversity	2006.55	**54.79**	25.42	25	12.12	12.7	7.69	5.06	6.41	5.71	2.50	5.36
Climate change	2006.54	31.51	18.64	9.09	9.09	6.35	9.62	2.53	5.13	2.86	1.25	1.79
Integrated pest management	2006.50	9.59	10.17	11.36	6.06	4.76	0	3.8	0	1.43	0	1.79
Allelopathy	2006.41	5.48	6.78	4.55	3.03	1.59	0	2.53	0	2.86	0	0
Soil erosion	2006.19	21.92	16.95	20.45	15.15	6.35	9.62	1.27	7.69	0	2.50	3.57
Crop rotation	2006.17	**49.32**	30.51	31.82	18.18	12.70	11.54	6.33	12.82	10	2.50	5.36
Transgenic	2006.15	13.70	13.56	13.64	0	4.76	1.92	6.33	0	0	1.25	5.36
Grass strips	2005.92	5.48	3.39	2.27	6.06	1.59	1.92	0	0	1.43	2.50	0
Biocontrol	2005.87	5.48	8.47	4.55	12.12	3.17	3.85	6.33	0	2.86	0	0
Cover crops	2005.76	17.81	16.95	15.91	6.06	9.52	7.69	3.80	14.1	2.86	1.25	1.79
Biological control	2005.70	10.96	10.17	18.18	12.12	7.94	7.69	7.59	0	7.14	2.50	0
No tillage	2005.59	**42.47**	35.59	34.09	18.18	20.63	28.85	18.99	20.51	5.71	3.75	8.93
Weed control	2005.35	27.40	27.12	18.18	24.24	20.63	19.23	13.92	8.97	14.29	2.50	7.14
Intercropping	2005.34	16.44	13.56	18.18	9.09	4.76	5.77	3.80	8.97	4.29	3.75	7.14
Irrigation	2005.21	**39.73**	42.37	43.18	39.39	39.68	51.92	37.97	32.05	28.57	0	0
Biological nitrogen fixation	2005.00	6.85	6.78	11.36	0	0	1.92	3.80	1.28	14.29	0	0
Agroforestry	2004.88	10.96	6.78	6.82	3.03	1.59	1.92	0	3.85	2.86	3.75	7.14
Decision support systems	2004.74	8.22	8.47	6.82	3.03	0	13.46	3.80	6.41	5.71	1.25	3.57
Precision agriculture	2004.06	4.11	3.39	2.27	0	1.59	9.62	1.27	6.41	2.86	0	3.57
Drought stress	2004.01	12.33	5.08	13.64	0	6.35	11.54	10.13	7.69	10	2.5	12.5

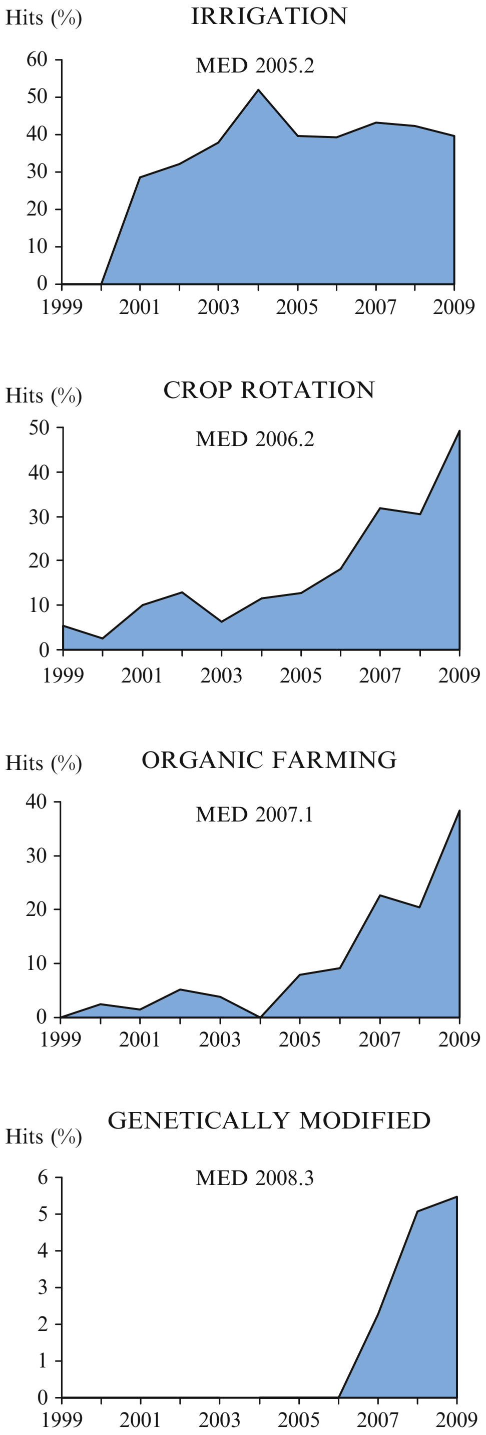

Fig. 1 Emergence of topics in article text from the journal *Agronomy for Sustainable Development*. Note the shift to the right of most emerging topics in the journal. MED refers to mean emergence date (see Table 3 caption). Hits in percent refer to the number of articles containing topics measured using the journal website search engine (see Table 3 caption)

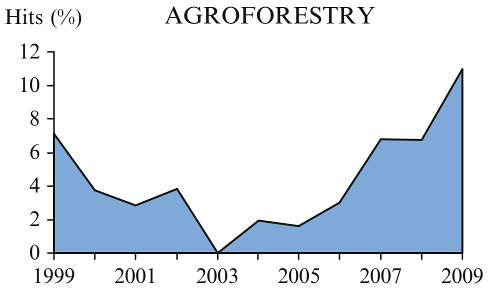

Fig. 2 Evolution of the topic Agroforestry in article text from the journal *Agronomy for Sustainable Development*. Note the decrease until 2003 followed by an increase, suggesting a renewed interest. Hits in percent refer to the number of articles containing topics measured u sing the journal website search engine (see Table 3 caption)

instance, 'irrigation' data shows a plateau with a mean emergence date of 2005.2. (2) A *regular increase* such as that for 'crop rotation' since 2003. 'Crop rotation' has thus a younger mean emergence date of 2006.2. (3) A *sharp increase* such as that for 'organic farming' after 2004. 'Organic farming' has thus an even younger mean emergence date of 2007.1. 'Genetically modified' exhibits the youngest mean emergence date of 2008.3. (4) A *dive-rise* such as that for 'agroforestry', showing a decrease from 1999 to 2003 followed by an increase from 2003 to 2009, suggesting a renewed interest in this topic.

Table 2 shows that according to the mean emergence date the 10 most emerging topics are biofuels, genetically modified, conservation agriculture, urban agriculture, sociology, organic farming, carbon sequestration, phytoremediation, mulch and biodiversity. This finding confirms biofuels and transgenic plants as emerging topics from citation data (Table 1). The predominance of soil carbon and climate is also apparent in most emerging topics.

Urban agriculture, ranking 3 in emergence, and sociology, ranking 4, are of special interest because those topics represent a clear change of thinking. Urban agriculture is challenging the common belief that crops should be cultivated in rural areas. Here, the idea of producing food close to consumers to decrease transportation pollution and costs is clearly elegant and apposite to fight climate change. The emergence of sociology can be explained both by increased interest from agronomists and by the shift in journal topics from 2004 (Lichtfouse et al., 2004). Table 2 also shows printed in bold the 5 top topics according to hits in 2009. Here, biodiversity is found in 55% of articles, crop rotation 49%, no tillage 43%, irrigation 40% and organic farming 38%. These data from one single year cannot be interpreted in terms of evolution, but they probably represent the major concerns of authors in 2009.

To conclude, the 10 most emerging topics according to mean emergence date are biofuels, genetically modified organisms, conservation agriculture, urban agriculture, sociology, organic farming, carbon sequestration, phytoremediation, mulch and biodiversity. Those topics can be roughly classified into two streams of research aiming at a more sustainable agriculture. First, an *analytical stream* that develops technological innovations in plant science such as transgenic plants and biofuels. Second, a *systemic stream* that develops innovative farming practices such as organic farming and urban agriculture. Concepts of the systemic stream are given by Hill and MacRae (1996), Vandermeer et al. (1998), Papy (2001), Dalgaard et al. (2003), Lichtfouse et al. (2009b), and references therein.

4 Most-Downloaded Articles in 2009

Topics of interest for readers of the journal can be evaluated by topics of the most-downloaded articles on the journal website (Table 3). We observe three major categories of topics: (1) topics related to climate change, e.g. biofuels, drought and salt stress, biodiversity, and carbon sequestration in soils; (2) topics related to alternative management, e.g. agroindicators, fertilisation, beneficial microbes, intercropping and organic farming, and (3) topics related to food security, e.g. pollutants, alternative fertilisation and control, and organic farming. The predominance of topics such as carbon, climate, biodiversity, biofuels, pollutants, beneficial microbes, transgenic plants and organic farming agrees with our previous results.

5 Review of Selected 2009 Articles

Here, we review selected articles published in 2009 in the journal *Agronomy for Sustainable Development*. The fast emergence of new disciplines such as social and economic sciences in agronomic research is underlined by Wezel et al. (2009), Lamine and Bellon (2009), and Lichtfouse et al. (2009b). Wezel et al. (2009) reconstruct the historical rise of agroecology following the decline of industrial agriculture. It is noteworthy that these authors observe that the meaning of agroecology changes from one country to another. For instance, in Germany agroecology is mainly a science, whereas in France and in Brazil agroecology is also a green movement that is run by citizens.

5.1 Sociology

Sociology emergence in the journal *Agronomy for Sustainable Development* is highlighted well by Veldkamp et al. (2009), who designed an innovative Dutch project that takes into account the opinion of all stakeholders. The authors develop their ideas on the alternative principle that sustainable development requires a better balance of the triple P values – people, planet and prosperity. Another tantalising example of bridging sociology and agronomy is given by Fleming and Vanclay (2009), who analyse the impact of climate change on farmer discourses. The core issue is nicely underlined by a farmer interview: “What’s sustainable? You’ve got to look at our world as we know it. We’re not in a sustainable position at the moment. That’s why I say ‘what’s sustainable?’ – I don’t know”. The authors found that farmers have four main discourses that guide their decision: money, earth, human responsibility and questioning. Disclosing which discourses are at work in a specific farming system is clearly needed because, to put it simply, farmers will not follow scientists’ advice if they are not convinced that this advice agrees with their discourses. De Bon et al. (2009) show the social benefits of urban agriculture in developing countries. Lamine and Bellon (2009) review the conversion from intensive to organic farming using viewpoints from both agronomists and social scientists. They found that most publications report conversion effects and motivations, whereas few publications study transitions and trajectories. To conclude, the use of sociological tools to study farming systems is bringing unexpected findings to designing sustainable agriculture.

Table 3 Top 30 most downloaded articles from the website of the journal *Agronomy for Sustainable Development*

Most-Downloaded Articles 2009*			
Year	PDF	Topics	First author - Title
2009	1975	Climate, drought stress	FAROOQ. Plant drought stress: effects, mechanisms and management.
2007	1421	Alternative fertilisation, beneficial microbes	KHAN. Role of phosphate-solubilizing microorganisms...
2009	1260	Agroecology	LICHTFOUSE. Agronomy for sustainable agriculture.
2003	970	Pollutants, toxicity	ROUT. Effect of metal toxicity on plant growth...
2006	931	Alternative fertilisation, beneficial microbes	HAFEEZ. Plant growth-promoting bacteria as biofertilizer
2009	786	Alternative management, intercropping	MALEZIEUX. Mixing plant species in cropping systems...
2007	647	Biofuel, climate, carbon	HILL. Environmental costs and benefits of transportation biofuel...
2001	641	Alternative fertilisation, beneficial microbes	IGUAL. Phosphate-solubilizing bacteria as inoculants...
2009	639	Soil, food security	LAL. Soils and food sufficiency.
2009	629	Alternative management, soil	LAL. Laws of sustainable soil management.
2009	628	Climate, biodiversity	FEEHAN. Climate change in Europe. 1. Impact on terrestrial...
2009	609	Climate, carbon, sequestration	BENBI. A 25-year record of carbon sequestration...
2001	522	Climate, carbon, salt stress	DORAI. Influence of electric conductivity management...
2009	497	Transgenic plants	DEVOS. Coexistence of genetically modified...
E-first	495	Organic farming, food security	LAIRON. Nutritional quality and safety of organic food.
2009	493	Organic farming, sociology	LAMINE. Conversion to organic farming...
2009	492	Agroindicators	BOCKSTALLER. Comparison of methods to assess the sustainability...
2009	473	Climate, agriculture	LAVALLE. Climate change in Europe. 3. Impact on agriculture...
2005	446	Climate, drought stress	SAMARAH. Effects of drought stress on growth and yield of barley.
2003	437	Climate, salt stress	BEN KHALED. Effet du stress salin en milieu hydroponique...
2009	436	Climate, soil	JONES. Climate change in Europe. 2. Impact on soil.
2008	427	Alternative control	DORDAS. Role of nutrients in controlling plant diseases...
2009	423	Agroecology	WEZEL. Agroecology as a science, a movement and a practice.
2007	422	Alternative fertilisation, beneficial microbes	GARG. Symbiotic nitrogen fixation in legume nodules...
2009	411	Alternative fertilisation, intercropping	ZUO. Iron and zinc biofortification strategies in dicot plants...
2003	392	Soil, carbon, roots	NGUYEN. Rhizodeposition of organic C by plants...
E-first	391	Alternative fertilisation, food security	SPIERTZ. Nitrogen, sustainable agriculture and food security.
E-first	366	Alternative management, urban agriculture	DE BON. Sustainable urban agriculture in developing countries.
2003	364	Pollutants, beneficial microbes	JONER. Phytoremediation of organic pollutants using mycorrhizal...
2003	341	Beneficial microbes, methods	GAMALERO. Methods for studying root colonization...

* Counts from January 1, 2009 to October 27, 2009. Year refers to year of publication. PDF refers to number of articles downloaded. PDF: portable document format. Full references are given in the reference list. E-first refers to articles online published but not yet issue published; those articles will be published in 2010

5.2 Transgenic Crops

Transgenic crops is typically an emerging topic that has escaped the science sphere to be now a major social, economical and political issue. As a consequence, transgenic crops is therefore a well-suited research topic for agronomists that use sociology and economics. Like all major scientific breakthroughs, e.g. nitro-glycerine and nuclear energy, there are many arguments in favour of genetically modified organisms and many arguments against their use. Devos et al. (2009) analyse policies ruling the coexistence of transgenic and non-transgenic maize in European nations. They found that current isolation distances are excessive, difficult to implement, and not economically viable. Very interestingly, they conclude that 'other scientific issues must be at play'. Such an unknown may indeed be disclosed by the discourse approach of Fleming and Vanclay (2009).

Graef (2009) reviews possible adverse effects of introducing transgenic oilseed rape into Europe. He found that possible adverse effects to be monitored are persistence and spread of herbicide-tolerant oilseed rape, transfer of tolerance to wild relatives, development of herbicide tolerance of weeds, decrease in biodiversity, herbicide pollution, and adverse impact on field organisms and biogeochemical cycles. Hart et al. (2009) demonstrate for the first time the persistence of transgenic crop DNA residues within a soil food web. They found the transgene for glyphosate tolerance in soil arthropods, nematodes and earthworms from a transgenic corn field. The potential of transgene flow from transgenic crops' wild relatives is addressed by Loureiro et al. (2009), who found that hybrids of wheat and its wild relative *Aegilops biuncialis* are formed easily with 9–75% hybridisation rates. To conclude, transgenic crops are highly innovative but show both benefits and drawbacks that need to be carefully studied using various disciplines.

5.3 Climate Change

Climate change effects in Europe are reviewed in three reprints from an European report. First, Feehan et al. (2009) analyse major effects on terrestrial ecosystems and biodiversity. Key trends include northward and uphill shift of plants, birds and mammals; and earlier seasonal events such as flowering, bird nesting and frog spawning. Second, Jones et al. (2009) report major effects of climate change on soils. Key observations include a decrease in soil C over the last 25 years, a higher risk of erosion and projected increases in CO_2 release in the atmosphere. It should be noted that there is much less evidence from soil studies because soil research is usually more difficult and needs more time - and funds - because most soil changes occur very slowly. Here, the major issue is that once adverse changes have occurred, e.g. soil carbon depletion, they cannot be healed fast. Therefore, management options that favour carbon sequestration and soil preservation should be applied (Doumbia et al., 2009; Benbi and Brar, 2009; Hazarika et al., 2009; Pleguezuelo et al., 2009; Tuttobene et al., 2009). Principles for sustainable soil management are given by Lal (2009c, d).

Third, Lavalle et al. (2009) review major effects of climate change on agriculture and forestry. Key trends include the shortening of the growing season in the south with higher risk of frost damage; flowering and maturity of crops occurring now about 2–3 weeks earlier; a higher yield variability due to extreme climate events such as the 2003 summer heat and the 2007 spring drought; a high increase of 50–70% in the water demand in Mediterranean areas; a faster forest growth; and a higher risk of forest fires. Tingem et al. (2009) simulate future crop yield in response to climate change in Cameroon. They found that developing later-maturing cultivars could greatly increase yields of maize, sorghum and bambara groundnut. Farooq et al. (2009) review effects of drought stress on plants. They also propose several solutions to counteract drought stress. To conclude, there are many scientific proofs of the effects of climate change on terrestrial ecosystems. Adverse, long-term effects such as soil carbon loss and erosion should be paid particular attention by agronomists.

5.4 Biodiversity

Biodiversity loss due to adverse effects of industrial agriculture is a major threat to sustainable agriculture. A well-known example is the decrease in bees. Indeed, bees and other insects carry pollen and thus are essential to the reproduction of some crops such as blueberries. Higher biodiversity is also a means to control crop diseases because pathogens and natural antagonists are better balanced. De Cauwer and Reheul (2009) investigate the impact of grassland management on plant biodiversity and invasive species. They found that abundance of non-leguminous dicots decreases with higher intensity use and N supply. They identify several land uses that suppress invasive species and optimise plant biodiversity. Weed diversity and density is also investigated by Izquierdo et al. (2009) and Koocheki et al. (2009). Pelosi et al. (2009) studied earthworm biodiversity in conventional, organic and living-mulch cropping systems. They found that the Shannon-Wiener and equitability indexes were higher in the living-mulch system. To conclude, agronomists are designing alternative cropping systems that optimise biodiversity, pest control and yield.

5.5 Alternative Farming Systems

Alternative management systems involving mixing plants, e.g. intercropping and agroforestry, are reviewed by Malézieux et al. (2009). They found that potential benefits are higher overall productivity, better pest control and better ecological services. Koocheki et al. (2009) analysed the effect of different cropping systems and various crop rotations on weeds. To reduce the weed seed bank they propose rotations that include crops with different life cycles such as winter wheat-maize and winter wheat-sugar beet. To design and evaluate innovative cropping systems on a medium- and long-term basis, new methods are required combining simulation and field experimentation. Here, Debaeke et al. (2009) describe an iterative and rule-based approach to setting up cropping systems in response to a drastic reduction of water, nitrogen and pesticide inputs.

6 Conclusion

Citation analysis shows that transgenic plants and biofuels are clearly emerging topics, whereas soil carbon and climate change are the major mainstream topics of the last 10 years. The 10 most emerging topics according to mean emergence date are biofuels, genetically modified, conservation agriculture, urban agriculture, sociology, organic farming, carbon sequestration, phytoremediation, mulch and biodiversity. In 2009, the highest hits were found for biodiversity (55%), crop rotation (49%), no tillage (43%) and organic farming (38%). Analysis of most-downloaded articles in 2009 shows the predominance of topics such as carbon, climate, biodiversity, biofuels, pollutants, beneficial microbes, transgenic plants and organic farming. We find that sociology is clearly bringing novel and unexpected findings to designing sustainable agriculture. Transgenic crops are highly innovative but show many unknowns that need to be carefully studied using various disciplines. Climate change has many scientifically proven effects on terrestrial ecosystems and agriculture. Here, soil carbon loss should be of particular attention because it rules the long-term fate of many factors such as atmospheric CO_2, erosion, and water and nutrient supply. Biodiversity loss due to industrial agriculture is leading scientists to disclose alternative, more diverse cropping systems that optimise biodiversity, pest control and yield.

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Ants and Sustainable Agriculture

Gero Benckiser

Contents

Abstract 60% of the world's ecosystems are not used in a sustainable way. Modern agriculture is blamed for declining soil carbon and biodiversity. Climate change, habitat fragmentation and other obstacles impede the movement of many animal species, and distribution changes are projected to continue. Therefore, we need alternative management strategies. The colony organisation of social insects, especially of ants, is seen as a model to design an improved agricultural management, because ants are very experienced agriculturists. Ants represent half of the global insect biomass. Their individuals work like a super organism. This article focuses on harvester and leaf cutter ants by considering *Lasius* species. It reviews the organisation structure of social ant communities. Harvester and leaf cutter ants represent a high percentage of the worldwide ant societies. They collect plant saps with carbon nitrogen (C/N) ratios of about 40 for their own nourishment and leaf fragments with C/N ratios of about 100 for fungi gardens and brood nourishment. They sustain huge numbers of individuals with their low N-based organic imports and their colony commensalisms enable them to convert these polymers into lower molecular, partly volatile compounds, adenosinetriphosphate (ATP), and heat. Digging improves water infiltration, drainage and soil aeration. Ants maintain fungi as a food source for the scleroproteinous brood, carry out food preservation, infection control and waste management, and construct with endurance new nests and rebuild them after damage. All these activities move the nest sites far away from the thermodynamic equilibrium. Physical, chemical and biological gradients emerge and the growing populations, together with nest-penetrating mycorrhized plant roots, absorb the released nutrients and form biomass

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_2,

from Benckiser, Agron. Sustain. Dev. 30 (2010) 191–199. DOI: 10.1051/agro:2009026

by lowering energy flows into potentially strong consumer-resource interactions or runaway consumptions. The plant material import of leaf cutter ants, rich in carbon but low in proteins, amounts to 85–470 kg dry weight per year. It keeps the electron donor/acceptor ratio in favour of the electron donor so that denitrifiers can reduce nitrate predominantly to N_2. Ants living in highly N-polluted areas bind the pollutant in the cuticle. In their low N-input environments harvester, leaf cutter and honeydew-sucking ants furnish the N demand of adult ants with the help of N_2-fixing bacteria. The low N-input management of harvester, leaf cutter and honeydew-sucking ants is therefore a resourceful concept for approaching a highly productive agriculture by avoiding soil carbon decline and N_2O emissions increase.

Keywords Ants • Colony organisation • Ecosystem engineers • Biotechnologists • Bio-indicators • Intra- and interspecies communication • Job sharing • Farming and waste management • Plasticity • Distribution in land-use mosaics • C/N management • N_2O emissions

1 Introduction

Nature's functioning depends upon microbial interactions among plants and animals, and their ability to adapt to environmental changes. Their relationship with each other and the environment relies on communication (signal exchanges), and the ability to assume dormancy (cryptobiosis) under unfavourable conditions (Benckiser and Schnell, 2007). The essential role ecosystems have is to provide services to humankind such as nutrient cycling, pest control, pollination, quality of life, and hydrological, atmospheric and climatic regulation. Among the macronutrients, nitrogen, a most unusual element, attracts a lot of interest because it interacts in various ways with ecosystem functioning.

Along its biogeochemical pathway nitrogen is reduced to NH_4^+, and oxidised into NO, NO_2^-, N_2O and NO_3^-. It reaches by leaching, wind, erosion, or transport by animals any part of the Earth's system, no matter where it is introduced. The N cascade is interrupted only when nitrogen is incorporated into biomass, humus and clay minerals. The manifold nitrogen links to the environment make an effective control on farm and landscape level difficult. Instead, N_2 nitrogen increasingly returns as NH_3, NOx and N_2O into the atmosphere, where visibility decreases, the stratospheric ozone is depleted, and global warming and precipitation of acidity increase. Acidity in soils changes the biodiversity (Raubuch and Beese, 2005) and an alternative soil C/N management is required (Crutzen et al., 2007; Fargione et al., 2008; Searchinger et al., 2008).

This review portrays the self-organised living together predominantly of harvester, leaf cutter and honeydew-sucking ants, with their nest functioning based on organic imports high in carbon but low in nitrogen; and it is asked whether a reduction of soil carbon decline and greenhouse gas emissions could be approached by adapting their C/N management.

2 Foraging, Nutritional Behaviour, Intra-/Interspecies Communication and Symbiont Management in Ant Colonies

Ants, order *Hymenoptera*, family *Formicidae*, play among insects a demonstrated role in transporting and pooling nutrients (Mueller et al., 2005). They are highly mobile, skilful tillers of soil, dispensers of seeds and microbial propagules, transmitters of N_2-fixing bacteria, predators, ecosystem engineers, fungi growers, waste managers, biotechnologists, pest controllers, soldiers, and reproducers (Balasubramani and Kannaiyan, 1991; Jouquet et al., 2006; Philpott et al., 2006; Benckiser, 2007; Hölldobler and Wilson, 2009). Ants such as *Atta colombica*, *A. laevigata*, *A. volenweideri* and *A. sexdens* colonies comprise 1–2, 3, 5, 4–7, 5–8 $\times 10^6$ individuals, respectively, and are organised in clear patterns of colonisation and succession (probalistic patchiness). The myrmicine tribe *Attini* has developed during the last 50 million years a nest management based on organic imports high in carbon and low in protein, and gained expertise in fungal farming. Despite the manifold capabilities ants have, ecosystem models mostly neglect them (Moore et al., 2007; Brussard et al., 2007; Osler and Sommerkorn, 2007).

2.1 Nest Organisation and Functioning

For constructing and repairing nests, which are built in a self-organised way without a central control - the queen is merely an egg-layer not an authority figure (Gordon, 2007) - ants must have at minimum the skill to find a suitable site, to evaluate several potential sites for choosing the best one, and to bring the entire population there safely (Hölldobler and Wilson, 1990; Pratt et al., 2002). In an Australian wheat/pasture rotation 5100 to 14860 ant nests per ha are constructed annually and 100 to 370 kg soil turned around, altering the soil structure, oxygen diffusion and metabolism of the nest environment as drastically as in conventionally treated agricultural sites where only a few *Myrmica* and *Lasius* species survive (Wang et al., 1995; Cannon, 1998; Lobry de Bryuyn, 1999; Dauber, 2001; Bucher and Marchesini, 2004; Wagner et al., 2004). Ant nests, reconstructed in late autumn so that they are smaller than at the height of summer, are damaged four times more by conventional agriculture than by no-tillage systems. Ants assemble for digging only when a certain signal threshold concentration is surpassed. The higher the group size, the more body contacts or chemical signals stimulate individuals to dig, and at an assembling volume plateau (V_s) of around 0.2 spontaneous motor activity or low-dimensional disorder of individual ants translates into a directed mobility, physiological rhythm, or coordinated digging (Miramontes et al., 2001; Hölldobler and Wilson, 2009). With nest enlargement the signal concentration decreases and the digging frequency, α, slows down. Ants not engaged in building activities cause feedbacks with snowballing effects on relocation and deposition of building blocks and only the colony level with its specific behavioural rules, activity controls, and high genetic and phenotypic plasticity (fitness), not the individual ant, has the global knowledge of the final nest structure, which tentatively ends in a circular form (Roces, 2002; Detrain and Deneubourg, 2006; Benckiser, 2007).

In conclusion, digging of ants changes soil structures as drastically as human activities (Hölldobler and Wilson, 2009). The mound distribution in a German meadow of *Lasius flavus*, a quince yellow, distributed throughout Europe, small-scale vegetation mosaics creating ant species (Fig. 1), shows that ant activities are not only significantly influencing the soil structure and vegetation in the nest surroundings, but as Fig. 1 suggests, in the whole meadow. The vegetation on the mounds is restricted to a few, mostly therophytic (annual) plant species and only a sub-sample of the surrounding grassland vegetation. More excessive is the digging of leaf cutter ants in South America, as the nest pictures in Hölldobler and Wilson (2009) reveal. The nest sizes of *Atta spp.* reach 26.1 to 67.2 square metres and soil depths down to 7 to 8 metres by excavating approximately 40 tons of soil per nest and constructing 6-m-long tunnels that connect an estimated harvesting area of more than one hectare.

Fig. 1 Distribution of *Lasius flavus* mounds in a meadow near the village Münzenberg, Hesse, Germany (photo Gero Benckiser)

2.2 Foraging and Nutritional Aspects

Foraging, similarly coordinated to digging, starts early in the morning with a small group of patrollers, which leaves the nest, meanders around, and after successfully returning, stimulates other ants to join them for foraging (Alonso and Agosti, 2000; Detrain and Deneubourg, 2006). Polyphagous harvester and leaf cutter ants collect plant saps and/or solid leaf fragments with wide C/N ratios. Stressed plant parts are preferred, because they seemingly meet the food requirement of fungi gardens better (Currie et al., 1999; Meyer et al., 2006; Hölldobler and Wilson, 2009). Fungal staphylae, low in N, are the best balanced blends of nutritional components for the brood. Adult ants receive more than 90% of their energy requirement through imbibed plant saps, which are temporarily stored in the gut of returning ants and freely shared with intranidal individuals by regurgitation (‘communal stomach’). During a day ants always return to the same foraging area but the colony level alone can rapidly and at any time tune the right numbers of foragers to places of current food availability.

Acromyrmex spp. and *Atta spp.* subsist entirely on food low in nitrogen and abstain from supplementation by arthropod prey (D'Ettorre et al., 2001; Bucher and Marchesini, 2004; Hölldobler and Wilson, 2009). From the harvested solid food particles and plant saps, separated from each other by ant mouthparts, infrabuccal pockets and the proventriculus (Cannon, 1998), *Acromyrmex subterraneus* and *A. crassispinus* can hydrolyse cellulose, starch, maltose and sucrose while their fungal associates affect laminarin, xylan and the phenol fraction but not cellulose. *Pogonomyrmex molefaciens* and *Messor pergandei* collect seeds of grasses. At a low supply of desirable seeds they also consume less desirable seeds and non-seed plant material such as leaves. Honeydew-sucking *Lasius niger* and *L. flavus* species receive major proportions of nitrogen through intestinal, N_2-fixing bacteria (Fischer et al., 2001; Zients et al., 2005) and *Camponotus compressus* stimulates seed germination to receive sugars and urea (Shetty, 1982). *C. herculeanus* circumvents N restrictions by spraying formic acid into wounds of preys and digesting proteinous macromolecules extraorally (Zhou et al., 2002). Foregut protease-lacking ants such as the fire ant, *Solenopsis richteri* Forel, avoid protein-rich diets (Ricks and Vinson, 1972) and queens contribute to nutrition by dispersing fungal symbionts from parent to offspring nests (Cannon, 1998; Green et al., 2002). Plant saps in the crop of *Camponotus pennsylvanicus* may contain $1208 \pm 169\ \mu g$ sugars, $5 \pm 0.7\ \mu g$ glycogen and $520 \pm 115\ \mu g$ nitrogenous material (Cannon, 1998). Glycogen, presumably digested as it is consumed, is low-concentrated in the gut of *C. pennsylvanicus* similarly to lipids and lipoidal compounds (hormones, pheromones, antibiotic substances), which are crucial in colony recognition. In respect to lipids and lipoidal compounds it is assumed that they are directly shunted into postpharyngeal glands, having oily contents (Cannon, 1998; Zients et al., 2005), but some ants such as *S. richteri* Forel produce lipases in the mandibular and salivary glands, foregut, midgut, and hindgut that correlate with the "grease-loving" feeding habits of this insect (Ricks and Vinson, 1972).

In conclusion, ants can quickly recover from threats and nitrogen limitations by employing fungi and N_2-fixing symbionts, being carnivorous, and/or linking nutrition and parts of the regulatory network to the environment. Redundancy occurs only at a "critical minimum" of essential resources (commodity of space, habitat, right type of food or nest destruction through agriculture; Liebig's law, the principal tenet of population ecology; Fillman and Sterling, 1985; Showler et al., 1990; Zients et al., 2005; Hölldobler and Wilson, 2009).

2.3 Bacterial Partners

Ants hatch endosymbiotic α-, β-, γ-*Proteobacteria*, *Flavobacteria* and *Actinomyces*, which provide them with nitrogen, essential amino acids, vitamins, antibiotics and other supplements (Zients et al., 2005, 2006; Stoll et al., 2007; Hölldobler and Wilson, 2009). *Blochmannia,* a γ3-subgroup Proteobacterium, resides in densely filled pouch-like ant structures (bacteriocytes) between the epithelial midgut cells and intestines, where it fixes N_2. The *Blochmannia* genome, reduced to 450–800 kb, controls N_2 fixation, the biosynthesis of essential amino acids, acetyl-CoA and tyrosine (required for cuticle sclerotisation), the urease that cooperates with the glutamine synthetase, and sulphate reduction. Despite lacking DNA repair genes the symbiosis between *B. floridanus* and *Camponotus spp.* is relatively stable and decays only when NH_4^+ accumulates in adult ants with finished sclerotisation. Another widespread α-Proteobacterium among insects is *Wolbachia*, that dwells in the ovaries of worker ants and queens and has an influence on the rearing ratio of male and female ants. *Wolbachia*, closely affiliated with *Rickettsae* and *Rhizobiales*, cooperates with strains of the genera *Escherichia*, *Salmonella*, *Yersinia*, *Bartonella*, *Pantoea*, *Sodalis Mesorhizobium*, *Agrobacterium* and *Blochmannia* for the ants' benefit. Work and matter fluxes in the environment of ants and their microbial associates are coordinated by intra- and inter-exchanged mechanical and chemical signals, e.g., the height of honeydew, phloem saps, pheromones, increasing CO_2 nest concentrations, various non-identified substances released from a Dufour or poison gland, cuticular hydrocarbons, the nest temperature, which may vary in temperate regions with colonies of less than fifty workers between 16 and 21°C (*Myrmica punctiventris* Roger; *M. rubra spp.*) and in warmer regions with large colonies of 10^6 and more individuals between 23 and 32°C (*Formica polyactena*, *Camponotus mus*, *Solenopsis invicta*), 'thigmotaxis' and odometry (Way, 1963; Banschbach et al., 1997; Currie et al., 1999; Ruano et al., 2000; Benckiser, 2007; Bollazzi and Roces, 2007; Bollazzi et al., 2008; Hölldobler and Wilson, 2009). By modifying attenuation and degradation of signalling chemicals the nest temperature acts indirectly (Shaw et al., 2006).

2.4 Fungal Farming

High-input systems should focus more on yield with less fertiliser N, but even low-input systems require N to increase yield level and yield stability. Sustainable production systems on low N inputs have to be developed and to reach this goal cross-linked research on different scales, from single cells to crops, to diverse cropping and farming systems are required. In developing more environmentally-friendly agricultural systems ideas could be deduced from fungal farming, which is described here.

Lasius fulginosus cooperating with the fungus *Cladosporium myrmecophilum* and the ant farmers of the *Attini* genera *Leucoagaricus* and *Leucocoprinus*, which hatch basidiomycetes of the family *Lepiotaceae*, exemplify ant-fungi symbioses (Hölldobler and Wilson, 2009).

Concerning the quality of harvested plant material the ant and fungus have conflicting requirements and the decision is generally made in favour of the fungus, more or less irrespective of the attractiveness of the plant sap found during harvesting. From time to time ascomycetes of the family *Escovopis* infect basidiomycetal fungi farms (Mueller et al., 2005). Leaf cutter ants are successful in reacting to infection events by:

(a) sequestration of the fungus garden from the environment,
(b) an intensive monitoring of the fungus garden,
(c) an early abatement of pathogens with filamentous antibiotic-producing bacteria of the genus *Pseudonorcardia* carried on the cuticle,
(d) hatching an array of auxiliary microbes for disease suppression and other pro- and prebiotic services (Lopes and Orduz, 2003),
(e) dispersing clonal fungi across many farmer generations,
(f) maintaining reservoirs of genetically variable cultivars,
(g) switching from time to time to novel cultivars,
(h) sharing domesticated fungal cultivars with distantly related ant species,
(i) a very specific and genetically predetermined feeding behaviour on mycelial fragments,
(j) production of antibiotic substances in metapleural glands with different roles in fungus culturing (phenylacetic acid suppresses bacterial growth; myrmicacin, a hydroxydecanoic acid, inhibits the germination of spores of alien fungi; indol acetic acid, a plant hormone, stimulates mycelial growth, and 20 recently found compounds spanning in addition to keto acids, alcohols and lactones the whole range of carboxylic acids, from acetic acid to long-chain fatty acids), and finally,
(k) a well-organised waste management accounting for approximately 10% of the work that accurately separates waste managers, consisting of a transporter caste, a worker caste and further partitions within the nest, from in- and outside moving ants with other duties, as well as
(l) sensitive reactions of sympatric fungi growers on faecal droplets not derived from their own domesticated fungus. The ingredients of faecal droplets, used to manure newly established fungi gardens, help to identify undesirable basidiomycetal strains. Substrate and microbial imports, not harmful for the ant but for the fungus and less quickly identified, may be rejected too late and thus harm nest functioning (Herz et al., 2008).

3 The Low N-Input Concept of Harvester and Leaf Cutter Ants

3.1 Ants in Land-Use Mosaics

Ants are competitors with each other. They live on honeydew, are predators, even cannibals, or collect within radii of less than one metre to a few hundred metres plant saps and leaf fragments with wide C/N ratios (Hölldobler and Wilson, 1990). Their densities and compositions in agricultural sites depend on human activities and are predictable in a typical German agricultural land-use mosaic such as the "Lahn-Dill Bergland" with arable, fallow, grassland fields and forest sites (Table 1; Dauber, 2001; Braschler, 2005). Twenty-seven ant species were found there between 1997 and 1998. They belonged to migrating forest species (*Myrmica ruginodis*, *M. lobicornis*, *L. acervorum* and *Formica fusca*), cursorial active species (*F. rufibarbis*, *F. cunicularia*, *F. pratensis*, *F. polycatena*, *F. rufa*, *F. fusca*, *F. rufibarbis*, *F. cunicularia*, *F. pratensis*, *F. polyctena*, *F. rufa*, *F. sanguinea*, *M. ruginodis*, *M. lobicornis*, *Lasius platythorax* and *L. acervorum*), peat land and humid meadow species (*M. vandeli*), thermophylic species (*M. sabuleti*, *M. schencki*, *M. rugulosa*, *Tetramorium caespitum*, *Tapinoma erraticum* and *L. alienus*), and relatively resistant species against agricultural vagaries (*M. scabrinoides*, *M. rubra*, *M. ruginodis*, *L. flavus* and *L. niger*), whereas in tropical countries harvester and leaf cutter ants, which can be agricultural pests, dominate the scene (Fillman and Sterling, 1985; Showler et al., 1990).

3.2 Organic Matter Imports and Their Conversion

Harvester ants collect plant saps and transport them in the crop to the nest to feed non-foraging nest mates by regurgitation. In one g of collected plant saps 542 to 718 mg carbon, 13.8 to 20.5 mg nitrogen and 58.0 to 94.0 mg ash contents have been determined (Cannon, 1998). The C/N ratios of plant saps and leaf particles, also transported into the nests, are between 40 and 100. The anabolic-catabolic nest cascade (Fig. 2) narrows them to 37–25 (refuse material). Thereby cellulose, fibre, phenol, true protein and the insoluble N fractions may decrease by about 47.7, 9.1, 26.1, 60.8, 10.9 and 10.9%, respectively, while lignin, crude protein, ash content, total N, soluble N, and soluble inorganic and organic N may increase by about 4.9, 31.9, 324.6, 31.9, 363.2, 150 and 461.5%, respectively. Digging in harvester ant nests enhanced the ash content of the

Table 1 Total and mean species richness of replicate sites and standard deviations of ants and four other taxa in different land-use types sampled with pitfall traps in the landscape mosaic of Hohenahr-Erda, Central Hesse, Germany. Different letters indicate that values are significantly different ($P < 0.05$, unequal N HSD-Test; for details see (Dauber et al., 2005)

		Arable land (n = 12)	Grassland (n = 12)	Fallow land (n = 10)
Carabids	total	75	57	65
	mean	26.6 ± 3.7a	19.3 ± 4.9b	20.4 ± 4.6b
Staphylinids	total	71	70	97
	mean	18.8 ± 6.1a	21.7 ± 7.0a	33.3 ± 6.3b
Ants	total	17	17	21
	mean	6.3 ± 2.5a	7.0 ± 3.4a	9.1 ± 3.2b
Isopods	total	6	10	12
	mean	1.1 ± 1.0a	2.1 ± 0.8b	3.0 ± 1.5c
Diplopods	total	9	10	11
	mean	2.0 ± 1.2a	2.7 ±1.4a	4.0 ± 0.9b

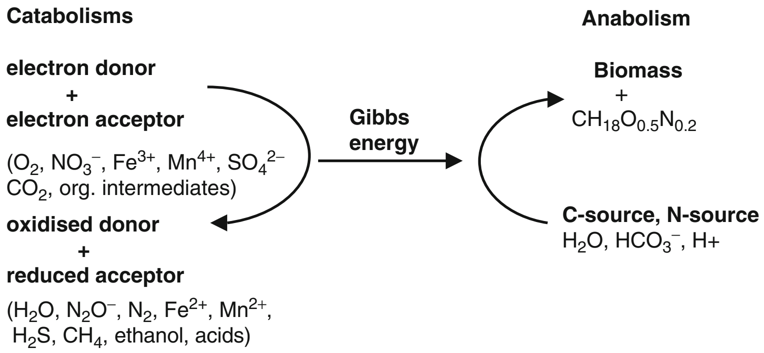

Fig. 2 Relationship between ana- and catabolism

deposited refuse material by 71–74.6%, in which total nitrogen increased by 5.2–17% and total carbon decreased by 10.1–34.3% (Cannon, 1998; Wagner and Jones, 2006). The soil chemistry in leaf cutter nests of the 24 known *Acromyrmex* and 15 known *Atta* spp. may alter similarly to in the harvester nests. Volatile organic intermediates (Vespermann et al., 2007), CO_2 and numerous other signal compounds, released into the nest environment, coordinate there the soil functioning (digging, foraging, waste management and infection avoidance). Nest-penetrating, mycorrhized plant roots and the growing nest commensalisms consisting of multiplying microbes, the cuticle sclerotising brood, and adult ants absorb the available nutrients, lower the energy flows into potentially strong consumer-resource interactions or runaway consumptions and develop super organisms (Ben-Jacob, 2003; Hölldobler and Wilson, 2009). The continuous organic imports into harvester and leaf cutter nests, characterised by C/N ratios between 40 and 100, keep the electron donor/acceptor, carbohydrate/nitrate ratio in favour of the electron donor (Jones and Wagner, 2006). At such carbohydrate/nitrate ratios denitrifiers of the nest environment will reduce accumulating nitrate to N_2, as field studies in plots, which have received varying amounts of mineral and organic fertiliser, and studies in other environments have shown (Tiedje, 1988; Simarmata et al., 1993; Ratering et al., 2007; Falkowski et al., 2008). N_2O emissions are lowest at a carbon/nitrate-N ratio of about 50 and from earthworms, which prefer to ingest organic matter with narrow C/N ratios, it is known that comparably high N_2O emissions can be released (Rizhiya et al., 2007).

3.3 Human and Ant Farming – A Comparison

Farmers, being competitive on the global market, apply high rates of mineral N fertilisers to strongly reduced, fully mechanised and pesticide-treated crop rotations, increasingly grown on large sized fields with various soil types, slopes and valleys. Fertilisation brought a quantum leap in yield improvement, yet concomitantly narrows the C/N ratio and, e.g., increasing amounts of N_2O are emitted from palm- and soybean-biodiesel farms with soil carbon debts of 702 to 3452 Mg CO_2 ha^{-1} (Crutzen et al., 2007; Fargione et al., 2008; Searchinger et al., 2008). A better carbon/nitrogen management is required (Schmidt-Rohr et al., 2004; Honermeier, 2007). Approximately 50 to 60 million years ago and long before the Romans, who perceived the importance of the C/N ratio and started to culture certain crops and trees with critical microbial rhizosphere associates (nitrogen-fixing bacteria, mycorrhizal fungi), ants began to domesticate fungi and to control the carbohydrate/nitrate ratio in their nest environment (Hölldobler and Wilson, 1990). Today's research ties in with this long tradition and investigates more detailed root exudate-consuming, antibiotic-producing, disease-suppressant, plant health-amending bacteria and fungi (Benckiser, 1997; Gómez-Gómez and Boller, 2002; Lopes and Orduz, 2003; Franken and George, 2007), soil carbon stabilisation (Von Lützow et al., 2007), N-demand-net-N-mineralisation-gross-N-release balancing (Icoz and Stotzky, 2008; Kooijman et al., 2008), *terra preta* (Marris, 2006), extended crop rotations (e.g., red clover-red clover-potato-wheat-field beans-wheat-rye or corn field beans-wheat-barley-clover/grass-clover/grass; Honermeier, 2007; Malezieux et al., 2009), the harvester and leaf cutter-CN-nest management (Jones and Wagner, 2006; Wagner and Jones, 2004), soil worm behaviour (Sticht et al., 2006; Rizhiya et al., 2007), recuperation of shifting cultivation systems (Addiscott, 1995), and the domestication of low N-input varieties (Ruiz et al., 2008). Recent findings show that in extended crop rotations with legume fields the nitrogenase, which stimulates soil phosphatases, affords a substantially lower activation energy ($\sim$103 kJ mol^{-1}) than the nitrogenase enzyme itself ($\sim$210 kJ mol^{-1}) and that N_2-fixing plants and ants, which dispense N_2-fixing bacteria among legumes (Balasubramani and Kannaiyan, 1991), have a similar geographical (temperature-dependent) distribution (Banschbach et al., 1997; Houlton et al., 2008). Whether the activation energy of the nitrogenase in N_2-fixing bacteria of honeydew-sucking ants or in organically fertilised fields also needs substantially lower activation energy than the nitrogenase enzyme itself has to be proven.

3.4 Reduction of Carbon Decline and N_2O Emissions

Nowadays farmers try to reduce soil carbon decline and N_2O emissions by converting conventionally treated fields into organic farming systems or by employing fertilisers with nitrification inhibitors such as dicyandiamide (DCD), nitrapyrin or 3.4-dimethylpyrazole phosphate (DMPP) (Duxbury et al., 1982; Nelson and Huber, 2001; Honermeier, 2007). Nitrification inhibitors should only temporarily block the ammonium monooxygenase and denitrification and not impair other essential soil processes. A study in DMPP- and DCD-N-fertilised, unfertilised, and control field plots over 3 years could not fully exclude that nitrification inhibitors impair essential soil processes (Weiske et al., 2001); and accordingly fresh ideas from the low N-input management of harvester and leaf cutter ant nests, which live in 26–67 m^2 sized nests with subterranean tunnels, ducts, fungus chambers and residue dumps, and where millions of individuals are digging, doing indoor farming, pest control and waste management, are of interest, though farmers know from agricultural tradition and experience that habitat-specific microbial communities beyond carbon sequestration and growth promotion can rapidly convert into detrimental ones and destabilise yields in various ways (Young and Crawford, 2005). Besides the difficult management of biological systems a substantial proportion of variation in species richness and functioning is still statistically explained in terms of a few environmental variables (Brussard et al., 2007; Moore et al., 2007; Osler and Sommerkorn, 2007) and a predictive theory for messaging molecules, signal transduction, integrated genome regulation and synergisms in subsystems is at the very beginning (Jones et al., 2006; Detrain and Deneubourg, 2006). However, these areas are a challenge for biologists, agriculturists, physicists, (bio-) chemists and ecologists and may prove to generate exciting results about the manipulative power that synergistic systems can exert over each partner (Hölldobler and Wilson, 2009).

4 Conclusion

Harvester and leaf cutter ants keep invaders low by antibiotics and by importing organic material, low in nitrogen but rich in carbon, by storing nitrogen temporarily in the cuticle and by keeping the nest C/N ratio in favour of the electron donator (Fig. 3).

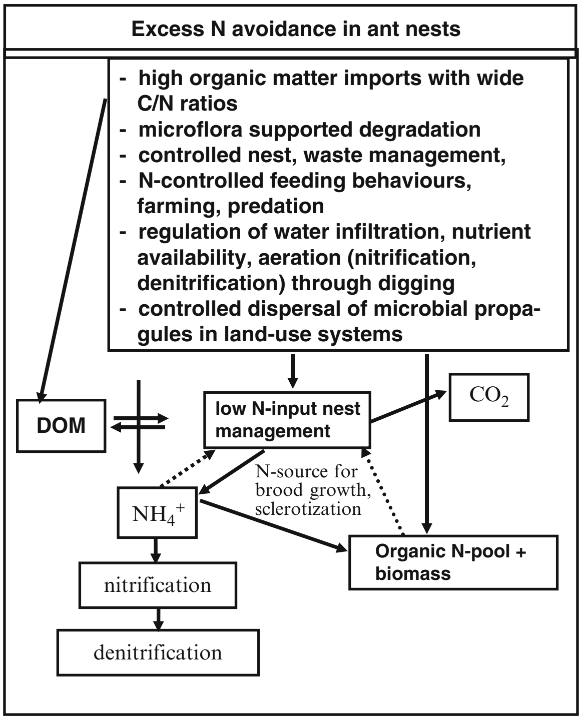

Fig. 3 Ant activities and possible exertions of influence on dissolved organic matter (DOM), inorganic and organic N pools, CO_2 production, and gross N fluxes

There are few existing data, and more detailed studies are imperative, but let us speculate that the N_2O/N_2 ratio under leaf cutter nest conditions is wide and N_2O is predominantly reduced to N_2. Harvester and leaf cutter nest sites produce on a low N-input basis high biomasses, similarly to minerally fertilised agricultural soils, and accordingly such environments are seen as an ideal study ground for receiving hints how highly productive, conventional agricultural systems such as biofuel farms could avoid carbon decline and minimise greenhouse gas emissions (Benckiser, 2007; Hölldobler and Wilson, 2009). Organic farming systems, on the other hand, could learn from ant environments how their soil C and N management could be improved and food webs better used for pest control as Fig. 4 reveals.

Figure 4 exemplifies by the cooperation of stinging tree ants, genus *Crematogaster*, which hatch in their nests made from leaves and fixed between

Fig. 4 Synergisms between Rufous Woodpeckers, *Micropternus brachyurus* (**a**), ants of the genus *Crematogaster* (**b**) and plant juices sucking mealybugs, family *Pseudococcidae* (**c**), which all reside in the tree-nest (**d**), made of leaves. (the ant nest-photo, Top Slip, Western Ghats, Kerala, South India, Gero Benckiser; bird-, ant- and mealybug-photos, Wikepedia)

twigs mealybugs, family *Pseudococcidae*, with the medium sized, brown Rufous Woodpecker, *Micropternus brachyurus*, how agro-ecological management could progress in its development (Vishnudas, 2008). Mealybugs living in moist, warm climates, protected by a secreted powdery wax layer, suck plant juices from a variety of subtropical trees (*Gymnocladus dioicus*, *Dalbergia latifolia*, *Aporusa lindleyana*, *Erythrina indica*, *Grevillea parallela*, *Mallotus alba*, *C. verum*, *Cinnamomam malabatrum*, *Olea dioica*, *Gliricidia indica*, *Hopea parviflora*, *Terminalia bellarica*, *Bischofia javanica*, *Syzygium cumini* and *Lagestroemia microcarpa*). The ants use the honeydew-producing potential of mealybugs nutritionally and foster and protect them in their paper-wasp-like nests. The plant juice sucking mealybug being supported by *Crematogaster ssp.* impairs coffee plantations and both considered as pests and killed with pesticides.

The emerging demand for organic shade-grown coffee all over the world is forcing planters to reduce the use of toxic chemicals in coffee plantations. This helps maintain reasonable numbers of *Crematogaster* ant colonies and, as followers, many forest-dwelling species such as the Rufous Woodpecker. The high conservation value of shaded coffee plantations with their diversity of canopy tree species providing critical habitats would be maintained and this is recognised. Proper agro-ecological management practices are starting to develop and are being popularised amongst planters.

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Agroecology as a Science, a Movement and a Practice

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Contents

Abstract Agroecology involves various approaches to solve actual challenges of agricultural production. Though agroecology initially dealt primarily with crop production and protection aspects, in recent decades new dimensions such as environmental, social, economic, ethical and development issues are becoming relevant. Today, the term 'agroecology' means either a scientific discipline, agricultural practice, or political or social movement. Here we study the different meanings of agroecology. For that we analyse the historical development of agroecology. We present examples from USA, Brazil, Germany, and France. We study and discuss the evolution of different meanings agroecology. The use of the term agroecology can be traced back to the 1930s. Until the 1960s agroecology referred only as a purely scientific discipline. Then, different branches of agroecology developed. Following environmental movements in the 1960s that went against industrial agriculture, agroecology evolved and fostered agroecological movements in the 1990s. Agroecology as an agricultural practice emerged in the 1980s, and was often intertwined with movements. Further, the scales and dimensions of agroecological investigations changed over the past 80 years from the plot and field scales to the farm and agroecosystem scales. Actually three approaches persist: (1) investigations at plot and field scales, (2) investigations at the agroecosystem and farm scales, and (3) investigations covering the whole food system. These different approaches of agroecological science can be explained by the history of nations. In France, agroecology was mainly understood as a farming practice and to certain extent as a movement, whereas the corresponding scientific discipline was agronomy. In Germany, agroecology has a long tradition as a

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_3,

from Wezel et al., Agron. Sustain. Dev. 29 (2009) 503–515. DOI: 10.1051/agro:2009004

scientific discipline. In the USA and in Brazil all three interpretations of agroecology occur, albeit with a predominance of agroecology as a science in the USA and a stronger emphasis on movement and agricultural practice in Brazil. These varied meanings of the term agroecology cause confusion among scientists and the public, and we recommend that those who publish using this term be explicit in their interpretation.

Keywords Agroecosystem • Agronomy • Ecology • Food system • Rural development • Scientific discipline • Sustainable agriculture • Systems approach

1 Introduction

The term 'agroecology' was first used in two scientific publications by Bensin (1928, 1930), and most recently in books by Gliessman (2007) and Warner (2007a). Between these dates, there are 80 years of history and confusion around definitions. The term agroecology has been used increasingly in scientific literature in recent years. For instance, the occurrence of the root agroecolog* or agro-ecolog* in the Web of Science increased from six in 1991 to 141 in 2007. The figures in a CAB abstracts search include two records in 1971; 102 in 1991; and 208 in 2007. This clearly indicates a great potential source of new information and perspective in agriculture and food systems. The term agroecology is currently used with quite different meanings in science, and also as describing a movement or agricultural practices. We recognise that confusion around the world concerning the term agroecology is partly due to translations, but also to meanings among and within different cultures. Our objectives are to explore and discuss three major uses of the term agroecology: science, movement and practice. Based on the historical development of agroecology in various time periods and geographical contexts, we discuss the evolution of its different meanings with examples from Europe (France and Germany), North America (USA), and South America (Brazil). To complete this analysis of the history of agroecology as a scientific discipline, we also consider some important reports that cover similar issues but where the term agroecology is not explicitly mentioned. The intent is to clarify use of the word "agroecology" and to urge colleagues to be explicit in definition when the term is used in the future.

2 Two Major Historical Periods of Agroecology

2.1 The 'Old Age' of Agroecology: 1930s–1960s

The word "agroecology" emerged at the beginning of the 20th century. Thereafter, both its definition and scope evolved significantly. This path primarily can be related to the evolution of two disciplines from which agroecology is derived, agronomy and ecology, but also to other disciplines such as zoology and botany/plant physiology, and their applications in agricultural and environmental issues. The term agroecology or agroecological was firstly used by Bensin (1928, 1930, 1935) (Table 1), a Russian agronomist, who suggested the term 'agroecology' to describe the use of ecological methods in research on commercial crop plants (Bensin, 1930 cited in Klages, 1942). Agroecology would hence be preliminarily defined as the application of ecology in agriculture – a meaning which is still used.

In the 1950s, the German ecologist/zoologist Tischler (1950, 1953, 1959, 1961) published several articles in which he used the term agroecology. He presented results of agroecological research, in particular on pest management, and discussed unsolved problems concerning soil biology, insect biocoenosis interactions and plant protection in agricultural landscapes, including also non-cultivated habitats. His book was probably the first to be actually titled 'agroecology' (Tischler, 1965). He analysed the different components such as plants, animals, soils, and climate, and their interactions within an agroecosystem as well as the impact of human agricultural management on these components. This approach combines ecology, especially the interactions among biological components at the field or agroecosystem level, and agronomy with a focus on the integration of agricultural management.

Between the 1930s and 1960s related studies were published, without using the word agroecology in the

Table 1 Important works in the history of Agroecology (adapted from Gliessman, 2007)

Year	Author	Title
1928	Bensin	Agroecological characteristics description and classification of the local corn varieties chorotypes[1]
1928	Klages	Crop ecology and ecological crop geography in the agronomic curriculum[2]
1930	Bensin	Possibilities for international cooperation in agroecological investigations[2]
1930	Friederichs	Die Grundfragen und Gesetzmäßigkeiten der land- und forstwirtschaftlichen Zoologie[1]
1938	Papadakis	Compendium on crop ecology
1939	Hanson	Ecology in agriculture[2]
1942	Klages	Ecological crop geography[1]
1950	Tischler	Ergebnisse und Probleme der Agrarökologie[2]
1956	Azzi	Agricultural ecology[1]
1965	Tischler	Agrarökologie[1]
1967	Hénin	Les acquisitions techniques en production végétale et leurs applications[2]
1973	Janzen	Tropical agroecosystems[2]
1976	INTECOL	Report on an International Programme for analysis of agro-ecosystems[3]
1978	Gliessman	Memorias del Seminario regional sobre la agricultura agricola tradicional[3]
1979	Cox and Atkins	Agricultural ecology: an analysis of world food production systems[1]
1981	Gliessman et al.	The ecological basis for the application of traditional agricultural technology in the management of tropical agroecosystems[2]
1983	Altieri	Agroecology[1]
1984	Douglass (ed.)	Agricultural sustainability in a changing world order[1]
1987	Arrignon	Agro-écologie des zones arides et sub-humides[1]
1987	Conway	The properties of agroecosystems[2]
1989a	Altieri	Agroecology: A new research and development paradigm for world agriculture[2]
1990	Gliessman (ed.)	Agroecology: researching the ecological basis for sustainable agriculture[1]
1991	Caporali	Ecologia per l'agricoltura[1]
1995	Altieri	Agroecology: the science of sustainable agriculture (3rd edition)[1]
1997	Gliessman	Agroecology: ecological processes in sustainable agriculture[1]
2003	Dalgaard et al.	Agroecology, scaling and interdisciplinarity[2]
2003	Francis et al.	Agroecology: the ecology of food systems[2]
2004	Clements and Shrestha (eds.)	New dimensions in agroecology[1]
2007	Gliessman	Agroecology: the ecology of sustainable food systems[1]
2007a	Warner	Agroecology in action: extending alternative agriculture through social networks[1]

[1] Book.

[2] Journal article.

[3] Conference proceedings or report.

title, that in effect applied the meanings of agroecology that predominated in this period. The German zoologist Friederichs (1930), who also worked in the tropics, published a book on agricultural zoology and related ecological/environmental factors for plant protection. This book presented different pest management strategies, including biological control and the role of natural habitats for pest management, and evaluated the economic impact of pest damage. His approach was very similar to that of Tischler. A second important book on agroecology was published by the U.S. agronomist Klages (1942), whose article in 1928 (Klages, 1928) may be one of the first papers dealing with agroecology without explicitly using the term, and this included research on the distribution of crop plants using a physiological basis. He also analysed the ecological, technological, socioeconomic and historical factors influencing their production; his vision is quite different from that of the zoologists. Although Klages (1942) used the term agroecology only once, his contribution and that of Friederichs (1930) can be seen as the basis for later publications about agroecology. Thus the first scientists to introduce agroecology were rooted in the biological sciences, particularly zoology (Friederichs, 1930) and agronomy and crop physiology (Klages, 1928, 1942; Bensin, 1928, 1935).

At the end of the 1960s, the French agronomist Hénin (1967) defined agronomy as being 'an applied ecology to plant production and agricultural land management'. This is not far from Bensin's definition, without actually using the word 'agroecology'. The Italian scientist Azzi (1956) defined 'agricultural ecology' as the study of the physical characteristics of environment, climate and soil, in relation to the development of agricultural plants, e.g. the quantity and quality of yield and seeds. However, he did not include entomological aspects in his analysis. The foundation of his work was already laid 30 years before (Azzi, 1928, 1942).

2.2 Expansion of Agroecology: 1970s–2000s

From the 1970s agroecology continued to be defined as a scientific discipline, but also gradually emerged both as a movement and as a set of practices beginning in the 1980s (Fig. 1). Here the general trends toward movements and practical applications are described, with specifics discussed in the 'country' examples since they are generally case-specific. We also observe the close association today between focus on agroecology and work in sustainable agriculture, often by the same people in science and development.

Concerning agroecology as a scientific discipline, greater historical detail from the 1970s until present is given in Hecht (1995), Francis et al. (2003) and Gliessman (2007). They noted that through the 1960s and 1970s there was a gradual increase in applying ecology to agriculture, partially in response to the Green Revolution that created greater intensification and specialisation. During this period an important influence also derived from research on traditional farming systems in tropical and subtropical developing countries (e.g. Janzen, 1973). Organic farming as an alternative model was discussed in relation to agroecology, for example by Rosset and Altieri (1997) and Guthman (2000). This period was marked by an increasing interest of an ecological point of view on agriculture. The key concept of agroecosystems emerged in the 1970s. It was suggested by the ecologist Odum (1969, quoted in Altieri 1995), who considered them as 'domesticated ecosystems', intermediate between natural and fabricated ecosystems. Since the beginning of the 1980s, agroecology has emerged as a distinct conceptual framework with holistic methods for the study of agroecosystems. Agroecology became defined as a way to protect natural resources, with guidelines to design and manage sustainable agroecosystems (Altieri, 1989a; Gliessman, 1997). Conway (1987) further developed the concept and identified four main properties of agroecosystems: productivity, stability, sustainability and equity. As its influence grew, agroecology contributed to the concept of sustainability in agriculture, mainly applied at the level of the farming system and supported in the proceedings of a conference edited by Douglass (1984), and later expanded by Gliessman (1990) and Altieri (1995). During the 1990s, agroecological research approaches emerged, several textbooks were published, and academic research and education programmes were put into motion, in particular in the USA. Recently, higher education programmes in agroecology have been developed in the USA and Europe.

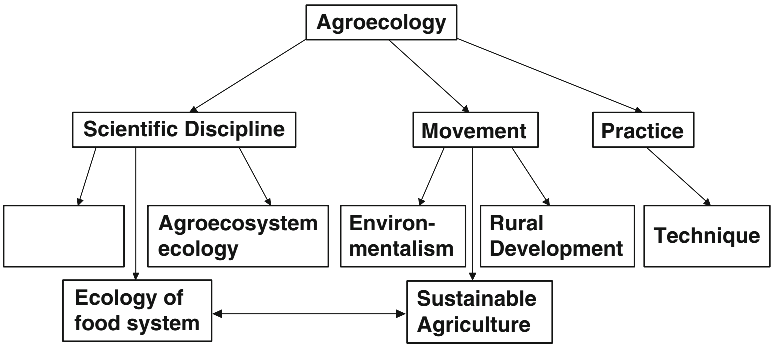

Fig. 1 Diversity of current types of meanings of agroecology

Finally, agroecology as a scientific discipline went through a strong change, moving beyond the field or agroecosystem scales towards a larger focus on the whole food system, defined as a global network of food production, distribution and consumption (Gliessman, 2007). In this perspective, producers and consumers are seen as actively connected parts of the system (see also Hill, 1985). This entails a new and larger definition of agroecology as 'the integrative study of the ecology of the entire food systems, encompassing ecological, economic and social dimensions, or more simply the ecology of food systems' (Francis et al., 2003). However, we observe that more restricted definitions of agroecology as a discipline, focussing on the field or agroecosystem scales, are still favoured in different countries as described later.

In general, the environmental movements in the 1960s often emerged as a consequence of the unexpected impacts of industrialised agriculture after the Green Revolution. Researchers with narrow focus on short-term yields and economic returns considered environmental and social factors to be externalities. Public policies rarely considered the environmental impact of agriculture, nor the social consequences of a uni-dimensional rural development focussed on production and economics. This environmentalism was primarily concerned with the impacts of toxic substances, in particular pesticides, on the environment. Other non-agricultural topics of these environmental movements included industrial pollution, nature conservation, and distribution of benefits. Nevertheless, in the 1960s, 1970s and 1980s the term agroecology generally was not used to explicitly describe a movement. This started in the 1990s, especially in the USA and Latin America, when the word started to be used to express a new way to consider agriculture and its relationships with society.

Almost in the same period a third word usage emerged, that of recognising a set of agricultural practices which aims at developing a more "environmental-friendly" or "sustainable" agriculture. An international example is described in LEISA (2008). One of the origins of agroecology as a practice was laid during the 1980s in Latin America. It was seen as the basis for an agricultural development framework, supported by ecologists, agronomists and ethnobotanists working especially in Mexico and Central America. Agroecology helped local farmers to improve their indigenous farming practices as an alternative to a high input, chemical-intensive agriculture promoted by international corporations (see Altieri, 1989a, 1995; Gliessman, 2007). Practices such as conservation of natural resources, adapted soil fertility management and conservation of agrobiodiversity are the practical basis for the different agroecological movements in Latin America (see country example Brazil). Another example for agroecology as a practice is described by Arrignon (1987), who illustrates technical, more adapted methods in agriculture such as water and livestock management or anti-erosion measures as a basis for rural and sustainable development in arid and subhumid areas. Today there are many different types of movements sharing this view which do not explicitly use the term agroecology, including those of multinational chemical and seed companies that define their new-generation products and transgenic crops as essential to long-term sustainability.

3 Examples from Different Countries

3.1 Case Study of the USA

In the USA, a long history of agroecology as a science began with the work of the agronomist Bensin (1930, 1935), concurrent with research of crop physiologist Hanson (1939) and agronomist Klages (1942). Thus the foundation in the USA was mainly laid in agronomy. According to Hecht (1995), a second advance in agroecology in the 1960s and 1970s was shown by a gradual increase in applying ecology to agriculture. At the same time as the Green Revolution – with its non-ecological, chemical-intensive practices, maximum yield breeding strategies, and monoculture specialisation – there was a reaction that promoted a renewal of agroecology. Since the early 1970s there has been an enormous expansion of literature with an agroecological perspective. For instance, Cox and Atkins (1979) provided a broad overview and in-depth analysis concerning the dynamics of agroecosystems, including political, economic and energy-related questions. An important influence was injected from research on traditional farming systems in developing countries, especially in Latin America (e.g. Gliessman et al., 1981; Altieri 1989a, for more examples see Hecht, 1995). Researchers recognised that traditional management of agroecosystems in these countries represented ecologically based strategies for agricultural production, often linking crop and animal production as well as natural resources. Later, the scientific focus of agroecology gained influence in U.S. west coast universities, incorporating entomology, agronomy and ethnobotany (Altieri, 1993). Agroecology had matured to look more closely at the potentials of building biological connections in agriculture.

In the first half of the 20th century, biological interactions were examined as part of the emergence of ecology as a science, but they were strongly neglected from the 1950s to the 1970s due to the large use of pesticides in crop protection. After two to three decades of decline, the evidence of negative pesticide drawbacks revived the study of ecological agriculture that was tied to advanced knowledge in biology. In this period, agroecology contributed to the emergence, definition and consolidation of the concept of sustainable agriculture (Douglass, 1984; Altieri, 1989a, 1995; Gliessman, 1990, 1997). Finally, the initial definitions for agroecology were expanded to "the ecology of food systems" (Francis et al., 2003, Gliessman, 2007).

The environmental movement in the USA appeared in the 1960s (Altieri, 1989a, 1995; Hecht, 1995). Environmentalism was primarily concerned with the impacts of toxic substances, in particular pesticides, on the environment. The publication of Rachel Carson's book 'Silent Spring' was one of the sparks in the early 1960s, providing a critical appraisal of the relationships among agricultural technology, science and nature (Carson, 1964). Today, agroecology plays an important role as a movement towards extending alternative agriculture, through agroecological partnerships between farmers and extension specialists in social networks (Warner, 2007a).

Agroecological partnerships as a movement (Warner, 2007a) suggest that the term 'agroecological practices' was commonly used to describe techniques for improving product quality while avoiding environmental impacts (Warner, 2005, 2007a, b). Other authors identify traditional knowledge systems (e.g. Norgaard, 1984; Raza, 2007) and resource-poor small farmers (e.g. Altieri, 2002) as target groups for agroecological transitions. Warner focuses on the heart of industrial agriculture, where conventional growers employ seasonal foreign workers. He works toward realizing Rachel Carson's 'dream', setting agroecology in action through socio-technical networks associating producers, extension agents and consumers-citizens who support such alternatives (Warner, 2007a).

In summary agroecology in the USA was first explored by scientists concerned with environmental pollution from agriculture who built up a scientific corpus based on analyses of traditional and conventional practices. As this expanded to larger scale agriculture, the research evolved into a scientific discipline and laid a foundation for agroecological movements in supporting sustainability, rural development, and environmental improvement, all of which helped to promote agroecological practices.

3.2 Case Study of Brazil

In contrast to the USA, the foundation of agroecology was laid in Brazil with different types of movements, based on traditional agricultural practices, and not from science. These movements emerged in the 1970s as different forms of alternative agriculture, from a critical appraisal of the effects of agricultural modernisation on farmers and subsequently towards promoting family farms, as well as food sovereignty and autonomy. In the 1970s, the agronomist Lutzenberger (1976) strived to influence environmentalists and farmers' groups, often supported by NGOs and the Catholic Church, towards an alternative agriculture. With his "Brazilian ecological manifesto: 'The end of future?'", Lutzenberger launched a crusade against contamination by pesticides. He subsequently became involved in practising and teaching organic agriculture (Lutzenberger, 1981). This movement grew from concern about environmental deterioration, and also traditional and small farmers' social exclusion from agricultural modernisation (Norgaard, 1984). Lutzenberger later served as national secretary of the environment for Brazil.

Early in the 1980s, both the first "National meeting of alternative agriculture" (convened by the Federation of Brazilian Agronomists) and the creation of the "Advisory body and services to projects in alternative agriculture" (AS-PTA) formalized a network of organisations in 10 Brazilian states (Canuto, 1998). AS-PTA's work led to the first 'National Meeting of Agroecology' in 2001. Among its objectives, this meeting aimed at making agroecology more visible and at lobbying in the national elections. As a result, a Brazilian law in December 2003 gave formal recognition to agroecology under the umbrella of organic farming (Bellon and Abreu, 2006). In this law, participatory guarantee systems (Oliviera and Santos, 2004) and political dimension of agroecology (Byé et al., 2002) were described as important to support small farmers and foster rural communities.

Byé et al. (2002) highlight the appearance of the Ecovida network of agroecology in three states of south Brazil in 1998, as a militant process contributing to a social alternative. For Ecovida, the objectives were to break with organic agriculture third-party certification systems and formal markets, and to approach local markets through a partnership with consumers. Likewise, the Association of Organic Producers from Paraná (AOPA), created in 1995, became the Association for the Development of Agroecology in 2004. The National Articulation of Agroecology (ANA) appeared in 2002 as a space for convergence of movements, networks and organisations from civil society, bringing together groups involved in concrete experiences to promote agroecology and sustainable development in various regions in Brazil.

The Agroecological Movement of Latin-America (MAELA) declared in 1998 its "opposition to degrade nature and society". It advocated "the management and control of natural resources without depending on external inputs (chemicals and genetically modified organisms)" and indicated "its assistance to promote, exchange and extend local experiences of civil resistance and to foster the generation of alternatives to use and maintain local varieties" (MAELA, 2000, quoted by Sevilla Guzmán, 2001).

The Brazilian technical assistance and rural extension public policy also promotes the implementation of agroecological principles through participatory approaches (MDA, 2004). This contributes to the new paradigm 'sustainable rural development', wherein agroecology is considered as one eligible approach (Sevilla Guzmán, 2001, 2002). In public extension services (EMATER) from the southern state of Rio Grande do Sul, agroecology generates a wide disciplinary matrix integrating various forms of knowledge, skills and experiences from distinct social actors, thus giving support to the new paradigm of rural development (Caporal et al., 2006). The authors are extension workers trained in Spain by the sociologist Sevilla Guzmán (2001, 2002). However, they also differentiate "agroecology" from a specific farming type, such as a production system or an agricultural technology (Caporal and Costabeber, 2000), and focus the term on alternative or sustainable agriculture for smallholders. To support their arguments and experiences, they refer to scholars such as Altieri (1989b) to optimise agroecosystems as a whole and not one single production system or activity. They also refer to Gliessman (2007) in arguing that when converting to ecologically based management, the transition levels cannot be attached to any one specific farming situation.

In general, social movements based on sovereignty and autonomy of the local populations are considered as very important and found as a common outlook in Latin America (e.g. see Caporal and Costabeber, 2000). Today in Brazil, this is stronger than ever in the southern federal states of Rio Grande do Sul, Paraná (Caporal and Morales Hernandez, 2004), and Santa Catarina where agroecology was recently institutionalised. This institutionalisation of agroecology (Brandenburg, 2002) is also criticized. For Abramovay (2007), agroecology cannot be the official doctrine at state level, since such a position would counteract scientific progress or impede the development of other production regimes, namely those that tend to serve several objectives in a given institutional context.

On the scientific side, the Brazilian Association of Agroecology (ABA) was created in 2004 (Zonin, 2007). Agroecology was officially recognised recently as a science by the Brazilian Agricultural Research Corporation (EMBRAPA, 2006). For them, agroecology is the ecology of food systems, following Francis et al. (2003), and historically emerging from alternative agriculture and small family farms. The research dimensions integrate renewed conceptual bases and methods, grounded in the work of Altieri (1995) and Gliessman (1997). Emphasis is on agroecosystems and agrobiodiversity in family farms, using systemic, interdisciplinary and participatory approaches, and also to better integrate indigenous knowledge. Agroecology is seen both as an emerging science and as a field of transdisciplinary knowledge, influenced by social, agrarian and natural sciences, especially applied ecology. However EMBRAPA also clearly stated that agroecology should be a foundation to promote sustainable agriculture and rural development (EMBRAPA, 2006). This was strongly initiated through education programs promoted by Altieri and Sevilla Guzman.

As EMBRAPA now recognises agroecology both as a scientific perspective and as a social movement, research workers are re-connected with family farmers' situations, but also with extension workers and high level agricultural training curricula. A final example for a combined scientific and movement approach for agroecology is the Latin American Scientific Society of Agroecology (SOCLA, 2007). Its goal is to promote the development of agroecology as the scientific basis of a sustainable development strategy in Latin America which emphasises food sovereignty, conservation of natural resources and agrobiodiversity and empowers rural social movements.

In summary, agroecology in Brazil was first a movement for rural development and environmental aspects in agriculture. This stimulated a search for alternative practices, now more often called agroecological practices, which were also related to the dynamics of organic farming. In recent years, agroecology has also been considered a scientific discipline, based on an adaptation of the U.S. scientific interpretation of agroecology with an integration of social dimensions.

3.3 Case Study of Germany

Germany has a long history of the scientific discipline of agroecology. From 1930 to present, most research on agroecology has been located within the different faculties of agricultural science and has provided much of the major foundation for agroecology as a science. The starting point was applied zoology with questions concerning crop protection (Friederichs, 1930), and later the ecology of agricultural landscapes (Tischler, 1950, 1953, 1959, 1961, 1965; Heydemann, 1953). Brauns (1985) further developed and broadened agroecology by analysing industrialisation of agriculture and environmental impacts such as herbicides, fertilisers, and water pollution on agroecosystems and their agro-biocoenosis. Agroecological research was extended to the Tropics and Subtropics by Koch et al. (1990). They described the need to thoroughly analyse site parameters, and biocoenosis in agroecosystem, to be able to elaborate strategies and management options for more sustainable local cropping systems.

In addition to the classical ecological factors within an agroecosystem, Schilke (1992) presented, the economic and political influences on agriculture, as well as social consequences for the rural population. Although Schilke does not provide any definition of agroecology, and although his book is not a classical scientific book but rather was written for high school students, he considers this field as the ecology of the food systems (as in Francis et al., 2003). The most recent book by Martin and Sauerborn (2006) finally combines basic ecology and applied agronomy such as farming, crop production and crop protection.

In Germany, a still more restrictive use of the term agroecology in relation to scale is commonly used, and this can be described as agroecosystems ecology or ecology of the agricultural landscapes, or even restricted to the field scale (Fig. 1). For example, Martin and Sauerborn (2006) described agroecology as the science of the conditions for the existence of organisms in the environment, which is managed by man toward the production of certain crops. This is in agreement with the tradition of Tischler (1965), who defined agroecology as the science of life events in the agricultural parts of the landscape. The definition of agroecology provided by the Department of Crop Science (section of agroecology) at the University of Göttingen (2008), probably summarises best both the evolution and the most common current definition of agroecology in Germany: 'Agroecological analyses focus on plant and animal communities, food web interactions, and conservation biology in temperate as well as tropical agricultural landscapes and agroecosystems'. This meaning is widely used in Germany, as compared to the USA where a broader definition including food systems tends to overshadow the narrow field or landscape meaning.

Thus far, the term agroecology as related to movements is more or less nonexistent in Germany. Normally the terms 'environmental movement' or 'ecological movement' are used. These started in the 1970s in opposition against different types of environmental pollution from industry or from nuclear power stations (Brüggemeier and Engels, 2005). In the 1980s the objectives of these movements expanded to include topics such as nature conservation, death of forests in Germany due to acid rain, destruction of tropical forests, or destruction of the ozone layer. Thus far, in only very rare cases some NGOs (e.g. AGRECOL, 2008) speak of an agroecological movement; yet these NGOs are working almost exclusively in Latin America.

To summarise for Germany, agroecology is almost exclusively considered within the scientific sphere with a relatively similar interpretation today as used in the past. The focus ranges from field to landscape analyses, mainly based on ecological and biological scientific approaches.

3.4 Case Study of France

In France, agroecology is not established as a specific scientific discipline. The French Institute for Agricultural Research (INRA), the largest research organization in agriculture in Europe, has not yet positioned itself nor provided a definition of agroecology, even if the word is becoming more broadly used. At present, the term is used with meaning not far from that of Altieri (1995), focussing on the analysis of agroecosystems for the design of agricultural systems. Agroecology is mentioned only once in the general conclusions of a recent French book on agronomy (Doré et al., 2006). This rare use of the word agroecology in science seems to be strongly related to two historical trends. The first is that agronomy until recently was clearly separated in education and research institutions from the scientific disciplines dealing with crop protection and breeding, and to some extent from ecology. This did not favour integration with agronomy, in particular knowledge about the biotic components of the agrarian system, which have only been integrated in recent years. The second factor is the long history of the discipline of agronomy in France (Robin et al., 2007). Hénin (1967) has given the already mentioned definition of agronomy, and Sebillotte (1974) gave scientific ground to the discipline, agronomy in France has emphasized a systemic approach to agriculture. This led to the development of two scientific directions in agronomy: (i) an analytical direction towards the ecophysiology of plant species, and (ii) a more holistic direction considering the entire agroecosystem, including attention to farming techniques and economic and environmental performances as well. Although the cultivated field, or the plot, was considered as the primary level of attention, other larger scales such as the farm level and the association among farm units and the rural territory also became relevant to agronomy. This includes issues such as maintenance of soil fertility, quality of product at harvest, and the environmental consequences of agricultural practices such as pollution from nitrate or phosphate. Through these changes, agronomy in France has become enriched with knowledge and concepts from other disciplines, especially from the social sciences, but yet has not changed its name thus far. As a consequence, there were several similarities in regard to conceptual work between U.S. agroecology (sense of Altieri, 1995) and agronomy in France: holistic approach, integration of non-production dimensions and including the social dimension such as analysis of farmers' attitudes and practices. These are among the trends within agronomy in France, which have lasted for more than three decades. But it is clear that what is considered by others as characteristics of agroecology (sense of Francis et al. (2003) and Gliessman (2007), and particularly the ecology of the food system remains unrecognized in France.

Nevertheless the term 'agroecology' is more and more used in France. Interestingly, the English, German and Spanish web pages of Wikipedia (2008) present agroecology as a science, whereas the French web pages define agroecology as a practice, and a certain type of agriculture, which does not only respect the various ecosystems, but integrates the economic and social dimensions of human life. In fact, in many cases the term agroecology is used for describing, in a sometimes very vague manner, a way to produce more ecologically sound agricultural products in the field, or at the farm level. Sometimes agroecology is even used as a synonym for organic or ecological farming. A practice-oriented approach to agroecology has emerged over the past two decades. Although Arrignon (1987) did not give a precise definition for agroecology, he described technical, more adapted methods in agriculture as a basis for rural development in arid and sub-humid areas. This has been expanded by various institutions, with a definition of agroecology as an approach to integrate more ecological aspects into agriculture, and with special emphasis on the field scale, e.g. soil fertility conservation, water management, closed and improved nutrient cycling. Different French research institutions such as CIRAD (2007), which focuses on tropical and sub-tropical agriculture or the Non-Governmental Organisation Agronomes & Vétérinaires Sans Frontières (2007) have implemented this concept within their research and technical programmes. The latter does not give a more precise definition of agroecology than "an integrated approach of crop production, animal production and the environment". For CIRAD on the contrary, agroecology has been defined far more precisely as a technology which brings agriculture closer to the wild ecosystems, and uses the term ecological engineering (Capillon, 2006). This context is mainly based on conservation agriculture with no-tillage and mulching. Recently, the more plot-oriented approach has been expanded to include the level of field to farm linkages. It may be worth noting that this set of technologies was first applied by CIRAD outside France, for instance in Brazil, before being applied in Southeast Asia and Africa, and then re-imported to France. This technology context is now incorporated in CIRAD publications, with agroecology as a scientific discipline (sense of Altieri (1995)). In the same sense, but not within a research framework, authors like Rabhi (2007) defined agroecological techniques including recycling organic matter and using natural organisms to control pests and diseases. The "mother earth" and its organisms have to be respected in applying 'agroecological techniques', inspired by natural processes, for agricultural production (Rabhi, 2007). Humanism and solidarism are also important. Also for the editors of Terre and Humanisme (2007), agroecology is more a philosophy of ethics, for it also includes societal aspects.

As in Germany, the environmental movement in France started in the 1970s. Topics varied from environmental pollution from industry and from nuclear power stations. Some concerns did exist about industrialized agriculture, and different agricultural trade-unions or NGOs promoted alternative agriculture systems. But their actions were hardly realised in broader environmental movements until recent years. If one can presently speak of an agroecological movement in France, it is more in the sense to promote organic farming or more ecological friendly agriculture.

To sum up for France, agroecology was first seen as an alternative way to practice agriculture. In parallel, the evolution of the French scientific discipline agronomy gradually incorporated parts of what is called agroecology in other countries. In recent times, agroecology as a scientific discipline is on the way to becoming established with a similar interpretation as in Germany.

4 Discussion

4.1 Today's Scientific Discipline of Agroecology: A Variation in Definitions and Scales

An interesting aspect in the different concepts and the resulting research in agroecology is the range of different spatial scales as well as the mix of disciplines that have been employed over the past 80 years. The many different definitions and descriptions shown in the publications in Table 1 demonstrate how agroecology has changed from focus on the plot or field scale (1930s to 1960s), to the farm, to landscape agroecosystems, and to farming and food systems scales from the 1970s into the 2000s (Fig. 2). Yet the plot and field scale approach persists up to present, with a narrow definition of applying ecological principles to farming practices by some researchers. Currently, the definitions of agroecology given by Francis et al. (2003) and Gliessman (2007) go beyond the concrete spatial scale of field and farm and expand into the full dimensions of the food system. This dimension requires multi-scale and transdisciplinary approaches and methods, to include the study of food production systems, processing and marketing, economic and political decisions, and consumer habits in society. None of these can be confined nor attributed directly to a certain level of scale, but all are connected intimately with each other across scales and through time in different and complex ways.

Although agroecology as a science evolved significantly and definitions have been articulated, a large diversity still is found in approaches and definitions in different countries and regions of the world. One of the broadest definitions was provided by Francis et al. (2003) with agroecology as "the integrative study of the ecology of the entire food systems, encompassing ecological, economic and social dimensions", or more simply "the ecology of food systems". This approach possesses a degree of originality and inclusiveness, however clear concepts or new analysis models are not yet defined which combine the different dimensions covered by this broad umbrella for various types of analyses. A second definition, integrating the food system, is provided by Gliessman (2007) with "the science of applying ecological concepts and

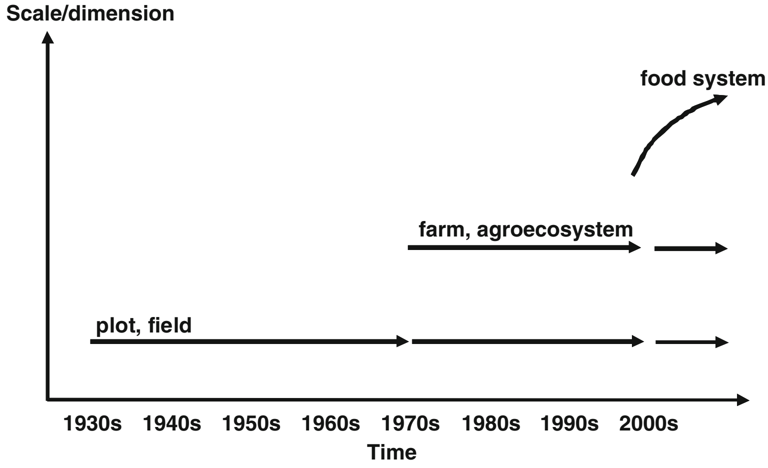

Fig. 2 Temporal changes in scale and dimension in the definition of agroecology and in applied research

principles to the design and management of sustainable food systems". This definition clearly emphasises the practical application. The definitions of Francis et al. and Gliessman are in part based on prior definitions and descriptions from Altieri (1989a, 1995). Ruiz-Rosado (2006) calls agroecology a transdiscipline because of its systems thinking and systems approach, using methods and advances from various disciplines and taking into account local knowledge where ecological, social and economic concepts and principles are applied in a reasonable manner. Similarly, Buttel (2007) describes agroecology as an interdiscipline that includes the social and human sciences as well as the ecological and agricultural sciences. Dalgaard et al. (2003) defined agroecology as "the study of the interactions between plants, animals, humans and the environment within agricultural systems" in covering "integrative studies within agronomy, ecology, sociology and economics", From their analysis of the situation and state of the art, they concluded that agroecology could clearly be considered as a scientific discipline. A common point in all these approaches is that if someone wants to practice this new discipline – or interdiscipline, or transdiscipline – its operational tools and concepts are still under development and difficult to identify.

Another new possible theoretical approach could follow the holon concept of Bland and Bell (2007), where every system at any level of spatial scale is composed of smaller subsystems, and in turn is a component of larger supersystems, even if this is still difficult to translate into reality. Due to the need to tackle the problems of boundary and change, which are evident for all agroecological research questions, Bland and Bell argue that agroecologists need to take into account how intentionalities seek to create holons (an intentional entity) that persist amid the ever-changing ecology of contexts, and how boundaries can be recognized based on how intentionalities draw and act upon them. This concept needs to be further developed and operationalised.

A more restricted approach in agroecology defines the system boundaries as field, farm, and landscape agroecosystems, without taking into consideration interactions with society, politics and economy. This is almost congruent with Conway (1987), although he also considers sustainability and equity as key properties of a system. A definition was presented in the case study of Germany. Among agroecosystems approaches the concepts and methods will vary depending on the definition of an agroecosystem. At the smallest level of scale, agroecological approaches are restricted to the plot or field scale. Here research almost exclusively analyses crop-insect and crop-weed interaction with a particular emphasis on natural processes, as well as impact of pesticides. For others, the farm is seen as equivalent to an agroecosystem, and still others view an agroecosystem at the scale of a local or regional landscape where agriculture is practised. According to each different choice of scale, applied research methods will vary accordingly. Based on our four country examples, it seems that these two more restricted approaches dominate in France and to a certain degree in Germany, where the mix between science and social movement in agroecology is less pronounced than in Brazil and the USA. One could conclude that in the areas where science and social movements are mixed, science is more value-laden and needs to incorporate social sciences if its goal is to be achieved.

One major constraint needs to be mentioned in evaluating the use of the term agroecology as a scientific discipline. Germany and USA used the term relatively early, thus it was found in many publications thereafter. However, many more publications exist which did not use the term agroecology in either the title or text, but which can clearly be seen as related to what we now accept as agroecology, based on current definitions. In this sense the use of the terms "agricultural ecology", "agrarian ecology", "ecological agriculture", "crop ecology" or "ecological crop geography" must indeed be mentioned. In countries such as France, it is even more difficult to consider all relevant publications, as synonymous key words for agroecology took a long time to enter the literature and it is very difficult to determine when and where they were used. In general, it can be assumed that there are other valuable publications that clearly recognize agroecology as a science, but which could not be considered here because of the difficulty in identifying them.

One unique example from nearly a century ago [first published in 1915] is the novel *Herland* by Charlotte Perkins Gilman (1992) in which a 2000-year-old society has practiced many of the key concepts in agroecology in producing food, maintaining a clean environment, and solving the challenges of population and food equity. Another is the futuristic agrarian society described by Marge Piercy (1976) in *Woman on the Edge of Time*. Both of these novels would never appear in a literature search, since they do not use the term agroecology, nonetheless they represent another way to visualise and study whole systems and their complex interactions.

Is there any concern about the confusion and lack of acceptance of agroecology as a science? All sciences evolve in their contents and definitions, and this evolution should not be considered as a problem. Nevertheless, at present there are multiple definitions, and different objects, concepts, levels of scale, and research methods. Although this can be seen as richness, our experience reveals that this rich diversity is also a source of misunderstanding. So the question persists, "Is agroecology a science?". Dalgaard et al. (2003) provide convincing evidence, but only as applied to their specific and narrow definition of agroecology as confined to practices and farming systems. It would be erroneous to apply their conclusion to agroecology in general, without considering the great differences among the different meanings and definitions when applying the conventional scientific criteria of communalism, universality, disinterestedness, originality and doubt. As a result, one should not automatically assume that "agroecology" is a science without giving a precise meaning to the word.

4.2 Agroecology as a Movement or a Practice

What is an agroecological movement? So far, it is not possible to clearly answer this due to a broad variety of these movements as illustrated in the different country cases. An agroecological movement can be a farmers' group working for food security, sovereignty, and autonomy. Or it could be a more political movement of the local population for rural development (Brazil). Or it can be a farmers' group movement for extending alternative agriculture through social partnerships to better respond to ecological and environmental challenges within relatively specialised agricultural production systems as in the USA. These movements are clearly action-oriented, and in general happen in response to higher common goals such as sustainable development and sustainable agriculture.

Much of the early project work that today we could call "an agroecological movement" did not actually use the term at all, and often they were within the framework of larger environmental activities. This causes certain unbalance in the country studies. In the USA, for example, Hecht (1995) mentions that the environmental movements of the 1970s enhanced agroecology as a science. In contrast, such relations between movement and science were never established in Germany, thus knowledge about similar movements is confined to publications using the term agroecology.

In general, agroecological practices are seen as new, modified, or adapted practices or techniques that contribute to a more environmentally friendly, ecological, organic or alternative agriculture. They are used to improve traditional or indigenous agriculture in developing countries. The different practices are appropriate to their related objectives, definitions, and to certification in the case of organic farming. For traditional agriculture, practices are mainly for soil fertility and organic matter management or resource conservation, or techniques for low external input systems. Biological pest management or soil fertility enhancing techniques are major objectives for environmentally friendly, organic or alternative agriculture. Conversely, agroecology challenges the interpretation of organic farming as mere input substitution rather than redesign of the system (Rosset and Altieri, 1997), and also may soften the division made by ecoagriculture between productive and natural areas (Altieri, 2004).

As with science, the lack of precise definition of agroecological movements or practices may be seen as a weakness. Indeed, everyone supports the goal of a more sustainable agriculture, and thus everyone could claim that his or her own movement could be called agroecology. The same type of difficulty is recognised in describing agroecological practices. At present, an agroecological practice is one that is not ecologically harmful, although this may not be scientifically grounded. In some cases, the environmental problems which practices are supposed to solve are not clarified, adding more to the confusion.

4.3 Combined Use of the Three Definitions

In many countries there is a combined use of the term "agroecology" as a movement, as a science and as a practice, and in most situations they are strongly intertwined. In Germany, agroecology has a long tradition as a scientific discipline, and the term is not associated with a movement or with practices. In the USA and in Brazil, agroecology is used to describe all three activities, with a predominance toward science in the USA and a stronger movement and/or practice emphasis in Brazil. In France, agroecology was mainly known until recently as a practice. In the countries where the agroecological movements are well established, the idea of practises is strongly connected, or even incorporated, into these movements. Here, they merge for the objective to develop and assist a transition into sustainable agroecosystems (e.g. Wojtkowski, 2002; Gliessman, 2007) and also with other models such as traditional, alternative or organic farming. There is large overlap in use of these several terms.

In this sense agroecology encourages farmers and extensionists to participate in the design of new systems, and also contribute to social movements. This is particularly the case for Brazil, and to certain extent for the USA and France. In these situations, there is often a link between a political vision (the movement), a technological application (the practices) to achieve the goals, and a way to produce the knowledge (the science). A key-point here for the scientists is to assess how these tight connections may influence the science of agroecology, where there will be application to meet a political vision using a set of technological practices. This association raises serious questions for some who have seen science more as an objective activity that is somewhat disconnected from practice. For example, when the science agroecology is defined as the scientific basis of a sustainable development strategy which emphasises food sovereignty, conservation of natural resources and agrobiodiversity and empowers rural social movements, the science itself may appear as an advocacy activity that will be impacted by diverse goals and applications of results. Instead of considering agroecology as a general matrix including the wider range of disciplines (Caporal et al., 2006), collaborations between agricultural, natural and social scientists should help to clarify such embedded interpretations of agroecology. One must ask, of course, whether this connection between the science and the practice is any different from our accepted linkages between research and recommendation, for example studies of fertilizer rates, types of pesticide that are effective, or scheduling of irrigation. Especially in the USA, where land grant university faculty are often involved in both research and extension, there is often a close connection between these activities.

4.4 Agroecology, a History of Oppositions?

In analysing the historical evolution of agroecology, either as a science, movement or practice, it becomes evident that different topics, discussions or debates in certain periods seem to have provoked major changes or reactions within agroecology. The common ground in the U.S. evolutions in agroecology was to find a scientific basis for new alternative agricultural systems. But why did this concern evolve to a new scientific discipline in the USA, but not in France, at least not so soon, where the concern was also present? Maybe because the holistic definition of agronomy, and the included concepts in France could welcome part of the changes needed to target this goal, which was not the case in the USA. Therefore a rupture was sooner necessary in the USA, leading to the creation of a new scientific discipline. For the case of Brazil, a clear rupture can also be mentioned. The expansion of different farmers' agricultural movements in the 1980s and 1990s was finally translated into agroecological movements whose common interests have been canalized under the term agroecology. In addition, to better distinguish their practical approach from industrialised agricultural practices, it seems that the use of the term 'agroecological practices' fulfilled this best. In speaking of agroecological practices since the 1990s, a possibility was found to distinguish them clearly from other conventional practices. Thus, a sort of new identity was created for these practices, which considered for instance more ecological and environmental aspects. In contrast to the USA and to Brazil, the different types of movements have not been the starting point for agroecological movements in France and Germany, or did not provoke a clear reaction into agroecological research. This might be explained by the fact that the agricultural problems in Europe interested or concerned only a limited part of the population. In Brazil on the contrary, a larger part of people are involved in agriculture, and the questions of rural poverty and disparities in agricultural land tenure still are important topics. In the case of Germany the lack of social movement around agriculture can also be explained considering that ecology in general, and in particular ecology related questions to agriculture, have been already well established in research, and thus evolved gradually instead of creating new research fields as for example in the USA. As usual in a scientific community, it can also be noticed that ideas attached to agroecology have crossed national borders. Therefore, many authors attached to a specific institution also gained and communicated knowledge from other countries, for instance through exchanges between Europe and the Americas.

4.5 Future Questions and Challenges of the Scientific Discipline of Agroecology

Many open questions remain for the scientific discipline of agroecology, especially for those who embrace the definition as the ecology of food systems. What new concepts, new models, and new methods need to be developed or adapted in order to grapple with this expanded definition of agroecology? Do we need new competencies for researchers and educators dealing with this more holistic and systemic approach (Lieblein et al., 2007b)? These authors raise the question of how higher education and learning activities will need to be modified to deal with systems, uncertainty, and complexity? Lieblein et al. (2000, 2007a, b) have proposed experiential learning strategies, but still the essential contents of agroecology courses have to be clarified and their applications defined for different cultures and perhaps for different ecoregions. Most authors also demand that the scientific discipline of agroecology should provide results for practical application in the design and management of sustainable agroecosystems. It is important to work out the crucial interfaces between the scientific discipline of agroecology and the needs of different stakeholders. It is important that particular participatory or on-farm approaches be employed, which also take into account the social, cultural and ethical dimensions. Although these questions cannot be answered quickly nor easily, and although a certain misunderstanding in using the term agroecology will persist, it will be intriguing to observe the evolution of the terms and their applications in this rapidly changing learning and development landscape. Moreover, it will be interesting to see how the word will escape the fatal challenge of being marginalized as too vague, confusing, and ineffective by scientists, farmers, environmentalists and consumers who want to express their ecological concerns in relation to agriculture and to move these concerns into effective action.

5 Conclusions

Today there is certain confusion in use of the term "agroecology". We have described the three main definitions of the term: as a scientific discipline, as a movement, and as a practice. Application of the term depends strongly on the historical evolution and epistemology, that provide the foundation, scope and validity of use of the term, in different countries. The most important influencing factors are (i) the existence of strong social or environmental movements, (ii) the existence of different scientific traditions and their evolutions, and (iii) the search for frameworks and concepts to describe new types of practices or movements.

From the historical analysis it became clear that the scientific discipline of agroecology and its scales, dimensions and definitions distinctly evolved from beginnings in the 1930s. We could illustrate that scales, and with them the definitions, expanded spatially over the next 80 years from the plot or field scale to the farm or agroecosystem scale, and finally leaving a concrete spatial scale or place and entering the entire realm of the food system. Today, all these three different scale approaches still exist within agroecology. The preference to any one of these approaches seems to depend in many cases on the historical evolution in different countries, at least in those we could analyse. In spite of the existence of different approaches and definitions, the new views and dimensions brought into agroecology as a scientific discipline will help facilitate the efforts to respond to the actual challenges of agricultural production, because of increasingly applied systems thinking and interdisciplinary research approaches.

Acknowledgements Many thanks to Guillaume Olivier for his contribution to a scientometric analysis, to Lucimar Santiago de Abreu for her inputs in the Brazilian case study, and to Rob Heslop for some English language corrections.

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Adaptiveness to Enhance the Sustainability of Farming Systems

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Contents

Abstract During the last decade the context in which farmers must manage their farm has changed rapidly, and often with little warning. Dramatic price swings for agricultural commodities, more stringent quality requirements, new environmental regulations, the debates surrounding genetically modified crops, extreme climatic events, the demand for energy crops, the revision of the Common Agricultural Policy and the consequences of the financial crisis all create uncertainty regarding future threats and potentials. During such turbulent times, a one-sided focus on efficient production is no longer enough. Farmers also need to be able to cope with unexpected events and to adapt to new developments. Based on a literature review, we identify three strategies that strengthen the adaptive capacity of a farm: learning through experimenting and monitoring its outcomes, ensuring a flexible farm organisation to increase the options for new activities by the farm family, and diversifying to spread risks and create buffers. Implementing these strategies enlarges the farmer's room to manoeuvre and allows identifying transition options. These options do not depend only on the farm itself, but also on the farmer's ability to mobilise external resources and to engage in collective action. Change is then no longer seen as a disturbance, but as a trigger for the reorganisation of resources, and for the renewal of the farm organisation and activities. Implementing these strategies comes at a cost, so that farmers need to tackle the inevitable trade-offs between efficiency and adaptability. However, unless farmers master this challenge they may not be able to ensure the sustainability of their farms.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_4,

from Darnhofer et al., Agron. Sustain. Dev. 30 (2010) 545–555. DOI: 10.1051/agro:2009053

Keywords Adaptive farm management • Adaptive capacity • Evolutionary approach • Complex adaptive systems

1 Introduction

Farmers are faced with increasingly stringent environmental regulations, higher quality standards, detailed animal welfare demands and volatile markets, as well as uncertainty regarding the next reforms of the Common Agricultural Policy. Not only is the number of the demands made on farmers increasing, they are also becoming less predictable. The sources of uncertainty include the increasing frequency of extreme climatic events, the demand for energy crops and the debates surrounding genetically modified crops, as well as public health threats stemming from intensive animal production (e.g., BSE, avian influenza, swine influenza). Thus, although farmers have always had to cope with uncertainty, especially regarding weather patterns and prices, it seems that with globalisation the sources of uncertainty are becoming more diverse and the pace of change is increasing (Urry, 2005). This obviously has an impact on farmers' ability to plan ahead and to manage their farm so as to ensure farm continuity. The rapid pace and the often unforeseeable direction of change may increasingly require farmers to keep their farms flexible to be able to respond to new challenges as they arise.

However, the flexibility and adaptability of a farming system has seldom been the target of research on improving farming practices or designing technical innovations. Most of the developments have focused on increasing productivity, improving product quality, optimising production processes, reducing the environmental impact, minimising costs, or maximising profits. The recommendations derived from research were based on the implicit assumption that the general conditions on a farm and its socio-economic context would remain roughly the same. Offering farmers a stable context was also the goal of government policies which stabilised commodity markets and controlled imports. Although there were changes, these were introduced gradually and in a predictable way.

With liberalisation and globalisation leading to an increasing interconnectedness of markets and sectors, the assumptions of gradual and predictable change are increasingly challenged. The dynamics of the farming system and its context will thus need to receive more attention than they have in the past. To understand these dynamics better, insights derived from complex adaptive systems can be applied to farming systems. The complex adaptive systems view draws attention to the fact that change can be sudden and dramatic and that the links between components are important, rather than the components themselves (Manson, 2001). Systems are also adaptive, i.e. are in constant co-evolution with their environment (Rammel, 2003).

The aim of this paper is to succinctly review three approaches to farming and to indicate how the concepts from co-evolution and complex adaptive systems can be applied to increase our understanding of the sustainability of farming systems. To clarify how the adaptive approach differs from previous approaches, we first briefly review the theoretical concepts and assumptions underlying the engineering approach and the farming systems approach. We then elaborate on the implication of evolutionary and complexity thinking on the understanding of adaptive farming systems. We believe that including the dynamic dimension of evolving farming systems can make an important contribution to understanding how farming systems can be more sustainable in a rapidly changing world.

2 Three Approaches in Agronomy and Farm Management

We distinguish between three broad approaches that co-exist in the farm management discourse: the engineering approach, the farming systems approach and the adaptive management approach. These three are loosely defined and we are aware that we cannot do justice to the wide variety of disciplinary refinements. However, using a broad-brush approach allows highlighting the differences in the underlying assumptions between schools of thought (see Table 1). Although the three approaches were developed consecutively, they can all be found in current research, policy formulation and practice.

Table 1 Key characteristics of the three broad approaches to researching farms and farming systems

Characteristic	Engineering	Farming systems	Complex adaptive systems
Underlying theory	Positivism, reductionism	General systems theory, 'simple systems', system dynamics	Co-evolution, complex systems, adaptive systems
Systems view	Mechanistic systems, Newtonian science	Systemic view: system is made of parts that interact, focus on the parts	Hierarchically nested systems, various temporal and spatial scales, properties of complex adaptive systems: emergence, hysteresis, etc., focus on interaction between parts
Time	Atemporal: time not taken into consideration	Atemporal but some linear projection into the future; no change in the dynamics of a system	Time is a key variable: 'history matters', path dependency, irreversibility
Dynamics considered	Static approach, steady-state, equilibrium view	Static approach, equilibrium view, relationship between elements does not change	Perpetual disequilibrium, non-linear dynamics, adaptability: the dynamics change over time, co-evolution
Context	Irrelevant ('one size fits all'), allows for technological blueprints	Context matters: differences between locations is important, farmer perception needs to be taken into account, focus on agricultural sector	Context is constantly changing, change can be unexpected in strength, timing and direction, due to interactions need to include all sectors, not just agriculture
Inclusion of social sciences	Mostly single discipline-driven, some inclusion of neoclassical economics	Interdisciplinary: inclusion of sociology to address farmer perception, farmer participation, economics include some behavioural notions (e.g., agents are boundedly rational), learning	Interdisciplinary, inclusion of insights from psychology such as mental 'traps' and bias typical to information processing by humans, learning as an on-going and interactive process

2.1 Improving Crop and Animal Production Based on an Engineering Approach

The attributes at the core of this approach are: efficiency, constancy and predictability. A problem such as low crop productivity or a threat from pests is identified, and a technological solution developed. Implementing the solution aims at achieving a predictable outcome such as a specific yield level. This approach to farm management is based on an engineering mindset. The goal is to 'design' a crop or animal production system, so as to turn a variable natural process into one that produces standardised commodities in a reliable, predictable and economically efficient way (e.g., Tsai et al., 1987). To achieve this stability agricultural pests are controlled through pesticides, nutrient competition is reduced through herbicides, natural, multi-species grassland is converted into monoculture, water supply for crops is regulated through irrigation or drainage, and field patterns are reorganised to reduce border effects and increase labour productivity (e.g., Fogel et al., 1974; Gotsch and Rieder, 1990). Recent developments, such as robotics and precision farming, aim at adjusting crop management steps to account for field variability by using technological means such as satellite navigation, sensors, computer models and information technology (e.g., Auernhammer, 2001; Bennis et al., 2008; Slaughter et al., 2008). The goal is to control processes, to reduce the range of natural variation of the farming system, and to stabilise the output of the farm so as to ensure an efficient and stable supply of goods and services (Grumbine, 1994; Holling and Meffe, 1996; Okey, 1996).

The solution to a problem is seen as being direct, i.e. there is a clear relation between cause and effect. It is seen as appropriate, i.e. the system has clearly defined boundaries and changes to the system have no effects outside these boundaries. And it is seen as feasible, i.e. relatively simple, without complex interrelationships. Generally, the focus is on an isolated issue, such as the nutrient supply for a crop (Table 1). This is based on the implicit assumption that there are no side effects on other parts of the farm or other spatial scales, and that the relations shown to be relevant over the short term are also the ones that will be relevant over the long term. The world is thus understood as stable, causal relationships are known, and there are no uncertainties. Farmers are seen as a homogeneous group and as business managers or entrepreneurs, so that farmer decision-making is assumed to comply with the model of economically rational agents (van den Bergh et al., 2000; Gowdy, 2007). Being rational, they will all reach a similar conclusion, which is expected to be comparable to the recommendation developed by scientists.

The normative and prescriptive technological solutions derived from this approach led to the strong increase in productivity in the 1970s and 1980s, both in crop and in animal production. This increase in productivity mainly took place in favourable production environments, i.e. good soils, reliable water supply, and a supportive economic and political framework such as ready access to cheap inputs, government-guaranteed output markets and stabilised prices. These favourable environments led themselves to the implementation of 'technological package solutions' (Norman, 2002).

The example of high yielding crop varieties can be used to illustrate the logic of the engineering approach. These are selected to comply with the DUS criteria (distinctiveness, uniformity, stability). These varieties are bred in centralised breeding facilities, with a few large companies claiming exclusive commercial rights to new plant varieties. The quality criteria are mostly breeder-driven and focus on efficiently producing a standardised quality, as required in industrial food processing. They achieve a high yield by being able to make best use of synthetic fertilisers, and tolerate herbicide application. Their performance is thus dependent on large-scale use of fossil fuel-based inputs and processes that help in limiting environmental variability (Tilman et al., 2002; Boody and DeVore, 2006; Wolfe et al., 2008).

2.2 Applying Systems Thinking to Farming

The farming systems approach is generally characterised by an increased sensitivity by technical scientists to the complexity and variability of farmers' production environment, the recognition by economists of the limitations of the rational decision-making approach, the heterogeneity of farmers, and the recognition that it is not sufficient to optimise individual crops or animal production systems, as the farm needs to be understood as one system (Bawden, 1995; Hubert et al., 2000; Norman and Malton, 2000; Norman, 2002).

The importance of taking into consideration the farming context became increasingly evident as farmers, especially in less favoured areas, did not adopt the technological packages developed within an engineering approach. Technical scientists thus recognised that both the bio-physical and the socioeconomic components of a farm need to be considered, highlighting the usefulness of an interdisciplinary, systemic and gender-sensitive analysis (Hart and Pinchinat, 1982; Biggs, 1985; Jiggins and Röling, 1994; Dent et al., 1995) (see Table 1).

There was increased awareness of the social nature of heterogeneity between farms and thus the importance of the farmer's perceptions and goals (e.g. Biggs, 1985; Lev and Campbell, 1987; Norman, 2002; Commandeur, 2006; Brodt et al., 2006; Ondersteijn et al., 2006). Linked to these developments was the acceptance by economists that farmers' behaviour could not be understood only through maximisation of profit (Norton, 1976; Colin and Crawford, 2000), so that concepts such as satisficing behaviour (Simon, 1986) and bounded rationality (Kahneman, 2003) were included in the analysis. When making decisions, farmers and farm households also take into account issues such as long-term preferences, security, lifestyle and quality of life (Brossier et al., 1991; Gafsi and Brossier, 1997). Furthermore, farmers were often involved in the research process, e.g. to better understand their norms, values and decision rules (Bellon et al., 1985; Collinson, 2000).

The farm is no longer seen as a mechanistic sum of (more or less) independent parts (Table 1). Instead, a farm is viewed in its entirety, as a system (Osty, 1978; Béranger and Vissac, 1994; Bawden, 1995), as a change in one part of the farm often affects other aspects of the farm organisation. The farming systems approach thus focuses on the interaction between the parts of the system, and on the identification of improvements that were compatible with the whole farming system (Norman, 2002). For example, the livestock farming system approach proposed by animal scientists considers the farmer, the herd and the resources as one socio-technical system (Gibon et al., 1999). This implies that the interactions between its constitutive elements, as well as the self-regulating properties of biological systems, need to be taken into account (see Puillez et al., 2008).

The implications of this different understanding of agronomy and farming systems can be illustrated in how it shapes the selection of crop varieties. Instead of stabilising the production environment to achieve high yields, varieties are selected for being robust in a wide range of conditions, i.e. aim for yield stability across a wide range of temperature, nutrient and water conditions. Standardisation of quality is not seen as desirable, as farmers and consumers have different preferences, and traditional cuisine requires specific qualities found in landrace varieties (Sánchez et al., 2008). Desirable traits are not reduced to yield and qualities, but include system-wide considerations, such as balancing the nutrient needs in the whole crop rotation or the need for straw for animal housing (Wolfe et al., 2008). To take into account these preferences, breeding often takes place in a decentralised setting, with farmer participation (Sumberg et al., 2003; Bocci and Chable, 2008; Østergård et al., 2009).

2.3 An Adaptive Perspective in Understanding Farming Systems

As the long-term environmental and social impact of intensive farming systems became increasingly apparent, the various issues related to ecological, economic and social sustainability came to the fore. This led to more attention given to longer-term effects, and raised the challenge to balance short-term productivity increases with the long-term sustainability of farming systems. It also pointed out that the externalities of on-farm practices need more attention, and that the interaction between the farm and its context needs to be better understood. At a fundamental level, it highlighted the need to account for uncertainty as complex dynamics and interdependencies between sub-systems cannot be fully anticipated (EEA, 2001; Mayumi and Giampietro, 2001; Millennium Ecosystem Assessment, 2005; Campbell, 2008; IAASTD, 2009). With societal and farm dynamics being uncertain, there are bound to be unexpected developments that will require flexible adjustment (Table 1).

There had been early calls for technologies to increase farmers' flexibility, to enable them to cope with changing economic environments (Long, 1984; Chambers, 1991, quoted in Norman, 2002; Lev and Campbell, 1987; Park and Seaton, 1996). Petit (1978, 1981) suggested a theory of adaptive behaviour in farm management, based on the observation that farmers interactively adjust both their objectives and their situations. This was illustrated by studying the patterns of change of farms over long time frames, offering empirical evidence that farm structure, activities and organisation can change substantially in response to on- and off-farm dynamics (Bourgeois and Krychowski, 1981; Levrouw et al., 2007; Cialdella et al., 2009).

Against the multi-dimensional background of socio-economic, political and environmental dynamics, changes and adaptations increasingly seem to be essential elements in any approach towards a sustainable farming system. It is thus necessary to understand the (co-)evolution of a farming system with its environment and how this on-going change is reflected in the internal organisation of the farm and the farmer's goals. Given the uncertainties of future developments, previous concepts that guided research – such as stability, income maximisation, technical fine-tuning or biological optimisation – need to be balanced with concepts such as adaptability, resilience and flexibility.

The theories that might be used to inform an adaptive perspective of farm management are evolutionary theory and complexity theory. The term 'evolutionary' is used for theories that explain the driving forces requiring the adaptations of the system over time, and the mechanisms through which they operate. Evolutionary theories have mostly been developed in ecology. In the context of farms, these theories can help explain how farms generate and adapt to change, and how these processes are intertwined with what happens both at the level of individual farms and the higher level of markets and the farm's environment in general (see Rathe and Witt, 2001). In an evolutionary framework, continual development and change at the farm level is needed to maintain its 'fitness' relative to the systems it is co-evolving with (Cournut and Dedieu, 2004). This on-going change implies that there is no stable state, no single optimal solution, no 'right' development path that can be defined a priori (Rammel, 2003). The evolutionary perspective requires a system to be adaptable, i.e. to be able to perform well according to unknown future conditions and goals that might change over time (Holling et al., 2002; Smit and Wandel, 2006; Rammel et al., 2007; van den Bergh, 2007; Fauvergue and Tentelier, 2008). The objective of management must include initiating and maintaining a diversity of alternative options so as to increase the chance of finding an adaptive response to unpredictable change (Beinhocker, 2006).

The theory of complex adaptive systems is another theoretical approach that focuses on understanding the implication of on-going change, and which emphasises the unpredictability of change (Ison et al., 1997; Levin, 1998; Manson, 2001; Holling, 2001). Complex adaptive systems are systems that involve many components and agents that interact simultaneously and adapt or learn as they interact (Holland, 2006). The theory of complex adaptive systems has been taken up by some researchers in economics and management sciences (e.g., Anderson, 1999; Meyer et al., 2005; Walsh et al., 2006; Teece, 2007), as well as in other social sciences (Stewart, 2001; Urry, 2005). This integration has proven fruitful as it allows one to understand how firms interact with their environment, how the past influences present behaviour, how the components interact (rather than focusing on the properties of the components), and how function is maintained, even though the components may be replaced (Cilliers, 2005; Trigeorgis, 2005). Within natural resource management, it has led to an approach called adaptive management (Lee, 1999; Westley, 2002; Jacobson et al., 2009). Given the similarities in the challenges faced by farmers and other society actors in the face of a rapidly changing context, it would seem that integrating insights from studies of complex adaptive systems and adaptive management can be useful to increase our understanding of the adaptability, resilience and persistence of farming systems.

3 Strengthening the Adaptive Capacity of Farming Systems

Emphasising the adaptive capacity of farming systems is based on the premise that the key to coping with rapid and unforeseeable change is to strengthen the ability to adequately respond to change to sustain long-term survival. However, this needs to be balanced with the ability to take advantage of existing favourable conditions, i.e. to perform under current conditions. The challenge for farm management is thus to balance between long-term adaptability and short-term efficiency (Lev and Campbell, 1987; Giampietro, 1997). Studies of natural resource management indicate that the characteristics allowing a social-ecological system to strengthen its adaptive capacity include the ability of the manager to learn, the flexibility of a system and its diversity.

3.1 Learning Through Experimenting and Monitoring

The existence of uncertainty and surprise as well as their unpredictable nature (Funtowicz and Ravetz, 1993; Folke et al., 2003) requires a continuous learning process that attunes to new information by reformulating hypotheses and models, and understanding activity implementation as experiments (Westley, 2002; Hagmann and Chuma, 2002). For example, there could be different interpretations as to the cause of an animal disease such as calf scour, leading to different assumptions on how to best tackle the disease. Whereas one farmer might rely exclusively on the veterinarian to treat the disease when it appears, another will experiment with different preventive measures and adapt processes on her farm (Magne and Cerf, 2009). Indeed, farmers' choices are constrained by their personality, preferences and competences, but also by external structures such as the social norms, technologies and the natural environment. Acknowledging that there are different valid solutions for each problem allows one to see that a farmer might find some solutions more useful than others depending, e.g., on her priorities, farming style and context. Learning is thus not seen as an objective attempt to understand the 'world out there', but as based on a relational understanding of reality: learning allows for a new perspective of challenges and for perceiving new possibilities.

To increase the number of learning opportunities and to structure them, it is useful to experiment and monitor the outcomes. Experimentation allows a better understanding of current system dynamics; for example, the influence of buffer strips on the insect population and reduction of pest incidence. It also allows widening the repertoire of options in case of changes in the context. For example, a farmer might experiment with mechanical weed suppression to reduce herbicide use or experiment with on-farm processing to see whether it would be compatible with work flow and meet consumer demand (Sumberg et al., 2003). In this framework, quantitative information is often less important than understanding the 'rules of the game' and how these rules change. Unexpected outcomes, active experiments to test hypotheses and monitoring through feedback systems allow farmers to learn about local agroecosystems, about the dynamics of social institutions, and about the potential and limits of various technologies and processes, and thus inform how they actively adapt their farm management.

This approach stresses the role of creativity and imagination, and recognises the crucial role of the farmer for the development of a farm over time. Indeed, what a farm can produce with given resources hinges critically on the conceptions, capabilities and projects of the farmer (Rathe and Witt, 2001; Teece, 2007; Gueringer et al., 2009). The farm is thus no longer seen as a device to exploit economies of scale as a response to technological progress. Rather, farms are interpreted as learning systems whose survival and growth strongly depends on the successful generation and integration of new knowledge.

Learning is not limited to experiments a farmer undertakes to understand a specific aspect of his farming system better. Much learning takes place through discussions with others, and when expanding on local and traditional knowledge (Berkes and Folke, 2002; Sumberg et al., 2003). For example, by building on the experiential knowledge of older farmers, who know which pastures remain productive even during drought, a farmer can broaden his options for action when faced with a similar crisis. But monitoring need not be done on an individual basis. It can involve a range of local stakeholders (Couix and Hubert, 2000). A farm monitoring and study group can provide a collective learning environment, in which ideas are shared and the results of experimentation with delayed lambing, flexible bull finishing and cross-breeding are analysed in detail, thereby confirming or disproving ideas for new practices (Seath and Webby, 2000).

Learning also benefits from combining different types of knowledge, e.g. experiential and experimental knowledge (Scoones and Thompson, 1994), from expanding from knowledge of structure to knowledge of function, from understanding about the dynamics of complex systems, and from understanding the complementarities of different knowledge systems such as scientific and traditional knowledge (Folke et al., 2003). Indeed, local knowledge systems can be based on a different conceptualisation of the world compared to science-based farm management (EEA, 2001; Olsson and Folke, 2001; Macé et al., 2007). It is thus important not to dilute, homogenise or diminish the diversity of knowledge systems, but to nurture diversity (Folke et al., 2003). Farmers' learning can benefit from comparing diverse information sources and perceptions, for example, by discussing new ideas with people belonging to different social groups. Off-farm employment or engagement in community organisations (e.g., church, sports club, hunters, fire brigade) allows access to various information sources, different world views, and different understandings of societal trends, consumer preferences or upcoming changes in agricultural policy. By discussing new ideas with a variety of people and reflecting on their views, a farmer may learn new ways to interprete and explain phenomena and thus discover new options to act (Ison et al., 2000; Ondersteijn et al., 2006).

3.2 Flexibility to Increase Response Options

In management sciences, the concept of flexibility is seen as a means to face uncertainty and thus also defined in relation to adaptive capacity (Reix, 1979). Generally, there is a distinction between operational and strategic flexibility. Operational flexibility refers to the ability of a system to implement changes in the short term when facing surprises. Strategic flexibility refers to long-term choices and to the capacity to change the structure, the resources, and the competences of the farm in anticipation of, or to react to, changes in the environment. For example, farm households not only need to ensure that they can flexibly change their daily or weekly work schedule to respond to changing weather patterns (operational flexibility), they also need to be able to develop new on- or off-farm enterprises (strategic flexibility).

Tarondeau (1999) further identified three sources of flexibility in production systems: the products, i.e., their diversity and exchangeability; the processes, i.e. the organisation of work and of the technical systems that allows for several processes; and the input specificity, i.e. whether different sources of inputs can be combined or substituted rather than depending on one specific input. This concept has been used to analyse the adaptive capacity of farming systems (Bellon et al., 2004; Lopez-Ridaura et al., 2005). As the available work force is often limited on farms, work organisation is of particular importance: what tasks need to be done, who can perform the task, is it possible to hire skilled workers, can work flows be adapted to react flexibly, e.g. to changing weather (for a review see Madelrieux and Dedieu, 2008).

For example, livestock farms in pastoral systems may be specialised and have only dairy cows, or they may keep a flexible mix of dairy cows, suckling cows, heifers and oxen. Work organisation and processes may be based on regular routines and a clear differentiation of tasks to be performed by each worker, or they may be flexible and make room for contingencies. Regarding inputs, a study by Gueringer et al. (2009) shows the possibility of coping with changing fodder availability through spatial management of grassland fields and flexible fodder purchase. The farmers differed in their combination of harvesting technique, i.e. grazing, harvest as hay or harvest as silage to be stocked as wrapped bales, depending on various factors such as grass regrowth, labour availability, quality schemes (hay-only milk), spatial location of the fields (size of field and distance between individual fields), and available storage options. Farmers may also increase their flexibility by adapting stocking density and herd composition (dairy cows, suckling cows, calves) to available fodder and labour, as well as market demand (Lemery et al., 2006).

This illustrates the complexity with which farmers are confronted on a daily basis, in an attempt to remain flexible while maintaining the overall coherence of their farm. Farmers have to maintain various sources of flexibility over the short term, e.g. fodder sources over one season, and over the medium term, e.g. type and quality of milk and meat produced, as well as in the long term. As a study of change patterns on 14 farms during a 50-year period has shown, farms implement a wide set of adaptability options by flexibly organising the workload of family members, changing the structure of animal production and using off-farm employment, as well as various forms of cooperation with neighbouring farms (Cialdella et al., 2009).

Indeed, flexibility does not depend only on processes internal to the farm but also on its capacity to enrol external resources. Chia (2008) has called this ability 'relational flexibility', i.e. the ability of a farm to mobilise external resources through collective action. This might take the form of processing or marketing cooperatives to promote a specific quality label, the purchase of machinery by several farmers, or establishing a company to run a common biogas plant. On a smaller scale it also includes mutual help and exchanges. For example, the flexibility of the management of grassland fields can be enhanced, if the farmer not only considers her own fields, but is able to arrange with a neighbouring farm for, e.g., a seasonal exchange of plots or labour (Gueringer et al., 2009).

Emphasising flexibility thus highlights that the processes on a farm, its work organisation and the products and services marketed by a farm at any given time merely represent one of several ways in which it could be using its resources. Indeed, the 'productive opportunities' of the farm, even with an unchanged set of resources, are not objectively given. A farm is essentially a pool of resources that can be used and combined in different ways (Penrose, 1997). This means that for a farm, it matters how the components are linked and the way in which the resources are used, not just the resources themselves. What matters is how flexible the arrangements are, and whether or not the selected technological paths enable reversibility. To ensure flexible rearrangement, modularity, i.e. the ability to combine subroutines, actions or resources in different ways plays an important role. Modularity allows farmers to respond to change by combining a set of available resources. The novel combination of available building blocks is often more flexible than trying to anticipate each possible situation with a distinct strategy or resource (Holland, 2006).

3.3 Diversity to Cope with Variability

Clearly, flexibility is in part linked to diversity, i.e. the ongoing development and management of a portfolio of alternative capabilities, opportunities and relationships (Smit and Trigeorgis, 2006). Managing complex systems and uncertain future developments implies spreading risks and creating buffers, i.e. not putting 'all eggs in one basket'. The evolutionary potential of a farm, its ability to initiate new development trajectories, builds on the diversity of co-existing activities, its repertoire of alternative options and innovative activities (Rammel and van den Bergh, 2003). This diversity has been shown to play an important role in the reorganisation and renewal process following disturbance (Folke et al., 2003) as it enlarges a farmer's room to manoeuvre.

One way to approach diversity is at the whole-farm level. Here, activities of the members of the farm household can be diversified, which includes both on- and off-farm activities (Bryden et al., 1992; Lemery et al., 2005; Cialdella et al., 2009). A diversity of resources available on the farm, such as family labour, knowledge, networks, arable land, grassland and buildings can be invested in a range of projects. These projects will in part depend on the diversity of opportunities offered by the context, such as proximity of the farm to a city offering employment opportunities or a demand for fresh local products, or a village dairy manufacturing quality labelled cheese or the city council outsourcing services such as composting, snow ploughing or roadside maintenance to farmers. Thus, even if the focus is on the farm, the context needs to be taken into account, as it shapes the diversity of options available. Of course, this diversity can be enhanced by the farmers, especially if they engage in collective action, e.g. such as establishing a cooperative to process and package their produce (Chia, 2008; Cialdella et al., 2009). Also, diversity is not objectively given, but depends on the creativity of farmers to be innovative and creative. Indeed, different perceptions (Magne and Cerf, 2009) and interpretations of societal trends can make the farm and community more robust.

Unfortunately, strategies to build diversity are not yet well understood (Penrose, 1997), as most research efforts have focused on efficiency and specialisation. More research is needed to understand diversification and pluriactivity, the coordination and interaction between the activities leading, e.g., to challenges in work organisation (Fiorelli et al., 2007; Madelrieux and Dedieu, 2008). Indeed, farmers increasingly complain of too high work pressure and lack of work-free time (weekends, vacations). The challenge in building diversity is thus not only to coordinate labour peaks and to ensure the flexibility necessary to accommodate unpredictable events, but also to ensure quality of life through a satisfactory work-life balance for all members of the farm family.

Another way to approach diversity is at the technical system level. Here, the focus is on the role that diversified resources, production processes and type of products play to secure the system and to allow its evolution. For instance, a study has shown that creating and maintaining a diversity of land resources can play a key role in the management of a dairy farm, especially to reduce the sensitivity of milk production to climatic variations (Andrieu et al., 2008). Similarly, a system that allows for a diversity in crops (Østergård et al., 2009) or herd management (e.g., composition, age, uses, etc.) seems to be more resistant to periodic forage shortages, especially when considered at the herd level and over the long term (Tichit et al., 2004).

Diversity can also be approached at the functional level (Elmqvist et al., 2003; Walker et al., 2006). The focus is then not on diversity to allow various responses to change, but on ensuring that a function on the farm, such as ensuring adequate nutrient supply for crops, can be supplied by different processes, such as synthetic fertilisers, compost or adequate crop rotations. At the farm level, functional diversity means that the farm can perform several functions, i.e. is multifunctional, thus addressing a range of societal demands (Wilson, 2008; OECD, 2009). This approach allows one to avoid the sectoral approach to farming, which tends to focus on food, fibre and fuel production, and widen the perspective to the provision of public goods and services such as ecosystem services, cultural landscape or climate change mitigation.

Maintaining diversity in the current activities as well as maintaining diversity of future options implies that not all resources are used efficiently at any one point in time. In other words, there will be apparently redundant resources that are maintained 'in case' something happens, when they might be useful. Keeping unused buildings, machinery or land involves costs and thus reduces efficiency in the short term. However, diversity and redundancy are an insurance against uncertainty and surprise, and need to be actively nurtured to allow for reorganisation and renewal. A farm will thus have to pursue parallel strategies by exploiting today's capabilities and at the same time exploring new projects. In stable periods, the focus is likely to be on exploiting current strengths while during turbulent periods it will be more important to assess which of the projects are likely to meet the demands of the new context. For example, whereas until the mid-1990s livestock farmers were able to focus on the production of standardised quality, since the BSE crisis in the late 1990s the quality specifications, documentation and traceability requirements have increased dramatically. A farmer that had a range of marketing channels and had experience with quality production thus adapted more easily to the changes than a farmer that had focused exclusively on mass production of standard quality (Chia, 2008). Diversity thus implies a dynamic process involving multiple parallel strategies, which allow one to adjust or switch between various alternative paths as the strategy unfolds.

Putting diversity to use implies that decision rules need to change over time, to adapt to changes in requirements, preferences or context. Farmers are thus faced with the challenge of deciding which rules to change when, and how to change them. Holland (2006) has called them the 'credit assignment problem' and the 'rule discovery problem'. The first problem arises because overt information about performance and efficiency is often irregular and partial. Indeed, why a strategy was successful is hard to establish, since the strategy is the result of a long line of choices extending over space and time. Holland (2006) uses the metaphor of a game of chess, where a winning player has little information about which moves along the way were critical to success. The rule discovery problem arises when it becomes obvious that some of the farmer's decision rules are ineffective. Rules serve as tentative hypotheses about the farmer's environment. As that environment evolves, some rules will be progressively disconfirmed. The question then is: how to select a new, more adequate decision rule. As these challenges show, maintaining an appropriate level of diversity, and making use of that diversity are not trivial management issues.

4 Conclusion

Farmers have always had to cope with a certain level of change and unpredictability and thus needed to be flexible and adapt to new circumstances. However, with globalisation, sectors and countries are increasingly interconnected, leading to spill-over effects, so that change is different both in speed and variety. Adaptability is no longer just one factor enhancing competitiveness on the market, it has become a key aspect of farm survival.

Enhancing adaptability goes against the recommendations derived from an engineering approach to farm management, which tend to create simplified, specialised farms. Their impoverished diversity limits their capacity to adapt to societal change. Coping with ubiquitous change also demands broadening the perspective found in farming systems, to integrate the options built through collaborative actions by several stakeholders, as well as emphasising dynamics and adaptability.

Understanding the ability of farms to be adaptive raises the challenge to identify and develop methods to capture the dynamics of a system, and analyse which characteristics strengthen or threaten the ability of farms to adapt. Participatory methods are a promising avenue, as most disciplinary scientific models cannot capture the complexity of relationships or their dynamics, whereas farmers juggle them on a daily basis. Thus, researchers face the dual challenge of developing adequate theories and methods to understand the dynamics of co-evolution, as well as ensuring that their recommendations are relevant to real-world decision-making.

Learning to live with change and uncertainty requires a fundamental conceptual shift, from assuming that the world is in a steady state to recognising that unexpected change is the rule. Farms and farming systems undergo constant reorganisation, with phases of more or less fundamental modifications. Through our literature review we have shown that learning, flexibility and diversity, in their various forms, play a key role in the strategies of farm households to cope with change. The goal of these strategies is both to recognise the opportunities offered by change and to implement them by initiating transition processes.

As learning, flexibility and diversity require resources, they are costly. Farmers thus face the inevitable trade-offs between efficiency and adaptability. Ensuring the economic, social and ecological sustainability of farms is a dynamic and complex adaptation process, in which strategies and contexts co-evolve.

Acknowledgements We would like to extend our gratitude to the two anonymous referees and to the editor for their constructive criticism and helpful suggestions, allowing us to improve on the paper's earlier version. We also thank the participants at the IFSA 2008 conference in Clermont Ferrand for providing so many case studies illustrating the adaptability of farms.

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Economics of Biosecurity Across Levels of Decision-Making

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Contents

Abstract Biosecurity is a concept that has important economic, social, ecological and health-related dimensions. By biosecurity we mean protection of production, ecosystems, health and the social infrastructure from external threats caused by pests, pathogens and diseases of various forms and origins. The fact that more goods, transport platforms and people are moving around the globe at increasing speeds provides unforeseen possibilities for rapid spread of different types of organisms. This is exacerbated by changes in the production structures and climate. As a result, both the benefits and the risks of changes in the food system cross borders more often, leading to an increased demand for biosecurity policies. Economics can be related to biosecurity in at least three fundamental ways. First, many of the ultimate or proximate causes of bioinvasions create economic welfare. Second, bioinvasions result in various types of impacts, many of which are economic by nature – or at least may be measured in economic terms. Third, the negative impacts of invasions or their probability of occurrence can often be either avoided or reduced. These biosecurity policies themselves have economic implications, which often may be quite different from those caused by the biological hazard itself. A few reviews of separate components of economics of biosecurity exist, but there have been no attempts to review the big picture. Instead, the previous reviews have concentrated on different components of biosecurity such as invasive species or animal diseases. Our aim is to look at the issue in broad terms, draw some commonalities from the research conducted, and identify areas in which economic analyses have primarily been conducted and in which areas there remains work to do. The review includes about 230 studies from all areas of biosecurity

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_5,

from Heikkilä, Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010003

up to the year 2008. The review finds that study of economics of biosecurity is growing steadily, but is still relatively concentrated on narrow questions, few countries, few species/diseases and few journals.

Keywords Biosecurity • Economics • Invasive Species • Pests • Diseases • Review

1 Introduction

Biosecurity is a concept that has important economic, social, ecological and health-related dimensions. By biosecurity we mean protection of production, ecosystems, health and the social infrastructure from external threats caused by pests, pathogens and diseases of various forms and origins. In this paper, we call these external biological threats collectively 'biological hazards'. An entry of a biological hazard is referred to as an 'invasion'.

The ultimate global forces that accelerate invasions by biological hazards include changes in production structure and land use, climatic change and globalisation (see, e.g., Daily and Ehrlich, 1996). In this context globalisation refers to the increasing integration of markets for goods, services and capital throughout the world. This leads to an increased volume of transactions and reduced trade barriers and transaction costs, all driven by the gains of trade. The fact that more goods, transport platforms and people are moving around the globe at increasing speeds also provides unforeseen possibilities for rapid spread of different types of organisms. This is exacerbated by changes in the production structures and climate. As a result, both the benefits and the risks of changes in the food system cross borders more often, leading to an increased demand for biosecurity policies.

Some of the organisms that are moved provide positive economic impacts on the receiving economic system, and some provide negative economic impacts. Some organisms produce both (often intended) positive impacts as well as (often unintended) negative impacts, with the net impact being sometimes difficult to determine. Nonetheless, the fact that species transfer is an issue that cannot be defined as dominantly beneficial or dominantly harmful makes biosecurity policy challenging to design and implement.

Economics is related to biosecurity in at least three fundamental ways. First, many of the ultimate or proximate causes of bioinvasions create economic welfare. Be it related to transport, tourism and trade, or to urbanisation, migration and agricultural intensification, many outcomes of economic decision-making provide incentives or means for species and diseases to be moved around the world. Second, bioinvasions result in various types of impacts, many of which are economic by nature – or at least may be measured in economic terms. These include damage to production processes, health and the environment (see, e.g., FAO, 2001). Third, the negative impacts of invasions or their probability of occurrence can often be either avoided or reduced. These biosecurity policies themselves have economic implications, which often may be quite different from those caused by the biological hazard itself.

A few reviews of separate components of economics of biosecurity exist, but there have been no attempts to review the big picture. Instead, the previous reviews have concentrated on different components of biosecurity such as invasive species (Born et al., 2005; Gren, 2008; Heikkilä, 2006; Olson, 2006) or animal diseases (Elbakidze, 2003). Our aim is to look at the issue in broad terms, draw some commonalities from the research conducted, and identify areas in which economic analyses have primarily been conducted and in which areas there remains work to do.

The review includes 231 studies from all areas of biosecurity up to the year 2008. The review finds that study of economics of biosecurity is growing steadily, but is still relatively concentrated on narrow questions, few countries, few species/diseases and few journals. The following priority themes (in no particular order) are suggested for further study: (1) sophisticated economic policy instruments to mitigate biological hazards; (2) rigorous economic prioritisation frameworks; (3) clearer understanding of the distribution of biosecurity impacts and the associated incentives; (4) closer links with the costs and methods of prevention to the economic risk; and (5) the role of human behaviour in biosecurity and how that behaviour can best be directed.

The rest of the paper is organised as follows. In Section 2 we briefly define biosecurity and economics of biosecurity in order to clarify which questions and topics are included in the review. In Section 3 we first discuss existing reviews, and then review the existing studies based on the framing of the research question. In Section 4 we draw some conclusions as well as give recommendations for further study.

2 Biosecurity

Biosecurity provides an appropriate framework for managing the risks presented by biological hazards. It can be defined as the exclusion, eradication and effective management of risks posed by pests and diseases to the economy, environment and human health (Biosecurity Council, 2003). The main targets for biosecurity policies include environmental, commercial, cultural, human health-related, and social objectives. The FAO (2003) sees biosecurity as the "process and objective of managing biological risks associated with food and agriculture in a holistic manner". Policy is here defined as any instrument or institution that is working to reduce the risks (i.e. either probability or impact) of biological hazards. Although parts of biosecurity, this review excludes studies on genetically modified organisms (GMOs) and diseases that are confined solely to humans.

The four main elements that biosecurity policies protect include human health, animal health, plant health and environmental health, including the health of the built environment. Each category will be shortly discussed below. However, it is worth noting that many biological hazards are not limited to a certain category. Instead, a particular species or disease may cause detrimental impacts in several ways and in several sectors. For instance, avian influenza is of concern for human, animal and environmental health.

The human health aspect of biosecurity includes zoonotic diseases (diseases communicable between animals and humans) and biological food safety. Hazards may be divided into bacteria (e.g. salmonella), viruses (e.g. avian influenza and HIV/AIDS), protozoa (e.g. the malaria parasite *Plasmodium* spp.), fungi and worms (e.g. trichinosis), and prions (e.g. bovine spongiform encephalopathy, BSE) (Delfino and Simmons, 2000). The importance of biological hazards that threaten human health is vast (see e.g. Wolfe et al., 2007). For instance, smallpox was perhaps the largest single cause of death in the 20th century, and the Spanish flu of 1918 (H1N1-type avian influenza) killed approximately 40–50 million people and attacked the young disproportionately (IMF, 2006). Today, on a global scale communicable diseases are estimated to cause about a quarter of all mortality and morbidity, and even in industrial countries one-third of the population acquire annually a disease caused by a foodborne microbe (WHO, 2007).

The human health component includes not only physical/biological health, but also social health. This refers to human wellbeing separate from mere infection by a disease. For instance, many animal diseases and government policies related to animal diseases are likely to affect social health. This may be due to, for example, movement restrictions that result in social isolation, or disposal of infected animal carcasses (e.g. Rossides, 2002; Mort et al., 2005; Dixon, 2007). Welfare reduction may also result from loss of cultural heritage and spiritual or religious values when, for instance, a traditional ecosystem is transformed into an ecosystem dominated by exotic species.

Animal health is another component of biosecurity, including animal diseases as well as animal predators, parasites and parasitoids. The importance of animal health is likewise vast. For instance, in North America and Europe calories of the human diet are mainly obtained from animal products (Carvalho, 2006). Examples of diseases threatening animal health include classical swine fever, rinderpest, Newcastle disease and Aujeszky's disease. In addition, zoonotic diseases are an animal health issue as well as a human health issue. For instance, Ebola haemorrhagic fever has caused the death of thousands of gorillas in Gabon and the Republic of Congo (Bermejo et al., 2006).

The plant health aspect of biosecurity includes plant diseases and pests. The injurious effect may be direct, as in the case of pests that directly affect the plant, such as pathogens, parasites and herbivores, but it may also be indirect, as in the case of weeds that compete for biotic or abiotic resources, or any other organism impacting on the plant indirectly (Schrader and Unger, 2003). Plant production provides the basis of the human food chain, and it is therefore a crucial target of biosecurity. In addition, forest products are a source of significant revenue for many countries, both in the developed world (timber, recreation, biodiversity, berries) as well as in the developing world (forage, construction, fuelwood, biodiversity, medicine). About 75% of calories in a balanced human diet are from plant products (Carvalho, 2006). For instance, the famine in Ireland in the 1840s was caused by *Phytophtora infestans* – a fungus that arrived from North America and destroyed the Irish potato harvest (Schrader and Unger, 2003).

Health of the environment and built environment is the fourth component of biosecurity. Invasive alien species may be harmful to environmental health through various kinds of biotic or abiotic interactions (including, for instance, predation, competition, interbreeding and spread of diseases). For example, the *Arundo donax* giant reed, which is currently under consideration for bioenergy production in Europe, has been found to have detrimental ecosystem impacts in California riversides (Hundley, 2007). We also include the built environment within environmental health. Examples are provided by, for instance, damage to roads, runways and parks caused by rabbits (Gebhardt, 1996) or damage to power-plant intake pipes by the zebra mussel (Pimentel et al., 1999, 2005; Connelly et al., 2007).

The aspects of biosecurity discussed above, as well as the concepts of biosecurity, food safety and dietary safety are clarified in Fig. 1. The figure illustrates that there are components of food safety not included within the definition of biosecurity, as well as several other factors affecting the health of humans. For instance, human health is partly affected by biosecurity (e.g. foodborne microbes and zoonotic diseases), partly by non-biological aspects of food safety (e.g. acute chemical substances), partly by dietary safety (e.g. accumulating chemical substances), partly by the way of life not related to the environment or biological hazards (e.g. sports, stress), and partly by the environmental factors surrounding us (e.g. noise, temperature). While it is true that, for instance, exotic plant pests can drive changes in risks of natural forces (e.g. fire regime), such feedbacks are not presented in the figure.

Biosecurity is primarily about managing risk and uncertainty. Risk in economic terms is a continuum of possible events and future states of the world. Put simply, the economic risk is the probability of an event (or future path) multiplied by the economic costs and benefits that ensue if that path materialises. The risk is thus affected by the likelihood of an event as well as the net cost (costs less benefits) of that event. The probability and the impacts are usually not known with certainty.

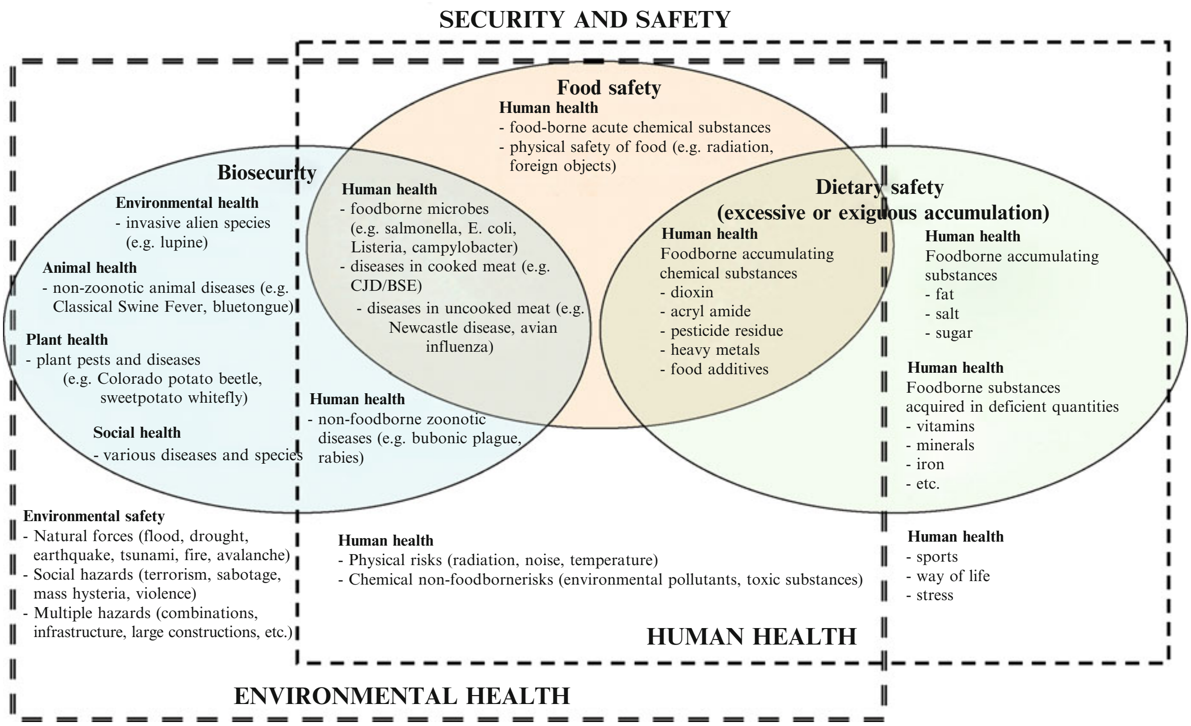

Fig. 1 Biosecurity, food safety and dietary safety

Uncertainty may be divided into uncertainty regarding biological and natural processes and uncertainty regarding human factors (Heal and Kriström, 2002). Regarding natural processes, there may be uncertainty and natural stochastic variation in the invasion process and subsequent spread processes. Uncertainty regarding the human factors arises from human preferences and decision-making as well as from the functioning of the society and its institutions. These include issues such as how biosecurity hazards and policies affect human systems, and how some unknown economic values (such as the value of life or the discount rate) affect the evaluation. Thus, even if we knew the science behind certain natural science phenomena with certainty, the impacts of such phenomena on the various components of human societies may remain uncertain. For instance, it is unknown how the continued presence of the biosecurity hazards would affect the production patterns or land use in the longer run. In addition, there may be uncertainty related to which policies are needed to address the problems, how those policies impact on the issue in question and what the costs of undertaking these policies are (Heal and Kriström, 2002).

An important implication here is that the risk is an endogenous process that is affected not only by the biological processes, but also by the human activities that create the risk as well as human reactions to the risk. For instance, it has been shown that socio-economic variables are significant in explaining the invasion process in the context of plants (Dehnen-Schmutz et al., 2007; Dalmazzone, 2000), marine species (Weigle et al., 2005), insects (Work et al., 2005) and infected meat imports (Wooldridge et al., 2006). So risk is as much an economic question as a biological one (Mumford et al., 2000). It is a matter of allocating resources so that the negative effects of risk are reduced enough compared with efforts required to reduce the risk.

Despite risk and uncertainty, we would like to maximise social net benefits. There are several actions that we may take to deal with risk. First, risk may be avoided. This can be achieved by, for instance, not using (or trading) a potentially risky good and thus foregoing the utility from the good. Second, the realisation of the risk may be avoided. This requires increased surveillance at all levels, extra care in the use of the good, and quality management in the entire production and consumption chain. Third, we may accept the presence of the risk. This means getting prepared to face the consequences and manage them in the case that the risk materialises. Fourth, risk may be transferred by, for instance, hedging, insuring or diversifying.

Biosecurity thus involves protection of various environmental and anthropogenic elements in an uncertain world against the threat presented by biological hazards. The primary question in most economic studies (albeit not explicitly stated in most of them) is: what should we do about this risk?

3 Economics of Biosecurity

3.1 Reviews

There have been a few reviews of separate sections within the economics of biosecurity. Olson (2006) reviews economics of terrestrial invasive species. He suggests that there has been rapid development in the field, but uncertainty, spatial modelling, prevention, trade, and conflict between private and public incentives are areas where more sophisticated analyses are needed. Gren (2008) reviews invasive species studies in relation to two questions: (1) how to set targets for species damage mitigation; and (2) which policy instruments are best in achieving the targets. Her results indicate that strategies for prevention, control and damage reduction are complementary, and ignoring any one of them may impose social costs. She also argues that there is a lack of empirical applications and of studies evaluating current regulations.

There are also two reviews on cost-benefit types of studies. Born et al. (2005) review the economic cost-benefit evaluations of invasive species. Their main conclusions are that: (1) studies mostly have methodological shortcomings; (2) assessments are mostly ex-post rather than ex-ante; (3) prevention is hardly reflected in the analyses; and (4) uncertainty is insufficiently addressed. They conclude that most studies "focus on ex-post evaluation, on control measures, on few countries, on agriculture, and on use values" (Born et al., 2005).

Similar conclusions are reached in a review by Heikkilä (2006), who makes three basic observations on studies on invasive species in an agricultural context. First, the policy alternatives evaluated are often simply undertaking some policy versus not undertaking it. The most common analysis seems to be between a current protective policy versus abandoning it. Wider policy options are generally either not available or not analysed. Second, it is often only the direct, easily monetised costs that are included in the quantitative analysis. Costs that are more difficult to analyse – such as trade or employment effects – are often ignored altogether, even though in the cases where they are included they turn out to be very important. Third, sensitivity analyses and treatment of uncertainty are in many cases inadequate, if conducted at all. Heikkilä (2006) also notes that in many cases invasive species cost-benefit studies are not written by economists. The same can be argued to apply to research on animal diseases. In fact, Adamson and Cook (2007) go so far as to argue that standardised approaches to economic evaluations have not been developed and economic reviews are generally undertaken by consultants. As a result, the economic foundation of the studies is not as strong as it could be.

Elbakidze (2003) reviews the economics of agricultural biosecurity in relation to bioterrorism and argues that especially in relation to foot and mouth disease surveillance and detection (early actions) are overlooked in studies compared to vaccination and slaughter (reactive actions) – an idea shared by Born et al. (2005) in relation to invasive species. McInerney et al. (1992) concluded that economic studies on livestock disease concentrate on cost-benefit analyses of control programmes or gross estimates of costs of particular diseases, neither of which offers an adequate basis for economic decisions. There are also reviews on economics of specific biological hazards; for instance, on bovine viral diarrhoea virus (Houe, 2003) and parasitic diseases of production animals (Perry and Randolph, 1999).

Economics is about the scarcity of resources, and how to allocate the available resources in the best possible way to maximise the objectives set by the decision-maker. It has been noted that the role of economics can be to evaluate a chosen policy, to identify the most cost-effective measures to attain a specified goal, or to prioritise between different goals and measures (Goldbach and Alban, 2006). Embedded in this notion is the fact that there are various hierarchical levels at which economic decisions need to be made. Ideally, research that attempts to answer such questions would involve simultaneous considerations of the management strategy, objectives within the strategy and the tools to achieve those objectives in the best possible manner. The objective, after all, should be to maximise (social) net benefits subject to given constraints.

However, individual papers rarely discuss the whole management issue. Instead, the research question is often limited to, for instance, evaluation of specific management alternatives. Therefore, in this review the existing studies are organised categorically, based on the aims of the research setting in which they study biosecurity economics. The first category is priority-setting between different kinds of hazards confronting human society including, for instance, biological, chemical, social and military hazards. The second category is priority-setting between different kinds of biological hazards; for instance, between invasive species and animal diseases, which are often administered in separate ministries. The third category is divided into three subcategories. The first subcategory is evaluation of management strategies to deal with a biological hazard; for instance, prevention versus adaptation. The second subcategory is the cost-efficiency of different management methods; for instance, questions such as the optimal tax level or the optimal pre-emptive slaughter radius around infected premises. The third subcategory deals with the evaluation of the available policy instruments aiming to achieve the desired outcome of the chosen management strategy – for instance, whether we should use economic instruments or command and control instruments. Finally, the fourth category deals with the assessment of impacts (usually costs) of specific invasions or outbreaks, and is often done given the prevailing strategies, policy instruments and management methods. Such assessments usually do not include any comparisons but rather an outright monetary evaluation of the incurred or simulated costs.

These categories are presented in Fig. 2 and reviewed in more detail below. Additionally, two examples are discussed. The first example deals with a very common setting in management strategy assessment of category 3, namely the choice between prevention versus adaptation strategy. The second example deals with import risk, and discusses the types of studies dealing with this risk. Whilst ideally the question would be tackled as an entity, the examples demonstrate how individual studies often address more specific questions.

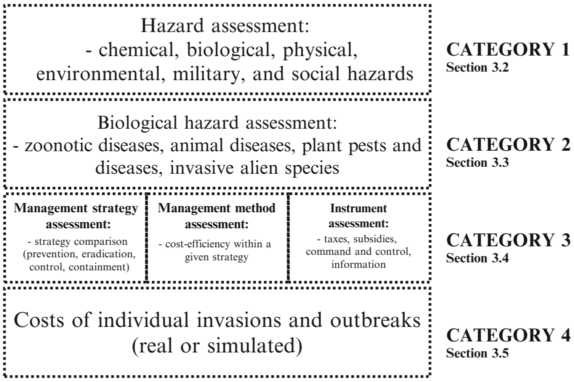

Fig. 2 Categorisation of economic biosecurity studies

3.2 Category 1: Prioritisation Between Different Types of Hazards

Renn (2006) divides hazardous agents into biological agents, physical agents (radiation, temperature, noise), chemical agents (pollutants, carcinogens), natural forces (drought, flood, tsunami, hurricane, fire, avalanche, earthquake), socially communicative hazards (terrorism, sabotage, mass hysteria, violence), and complex hazards (combinations of the above). How the society's resources are divided among these hazards is to a large extent a political question, and has not to our knowledge been studied academically. Having said that, we do not see any intrinsic reasons why such an exercise could not be undertaken. Naturally, comparison and estimation of risks and costs associated with such a wide variety of hazards is complex, and no easy solutions for comparison are available. The risks and impacts of such hazards may show such variety that they are, in addition to being difficult to value, very difficult to compare with one another. For instance, comparing the impacts of a materialising military hazard with the loss of welfare from burning animal carcasses may prove to be a daunting exercise.

Yet, resource allocations are constantly made at the level of the government as decisions regarding allocation of resources between, say, ministries of the environment (invasive species) and agriculture (health of production animals and plants) are made. Studies on individual hazards can achieve deeper understanding of the specific circumstances, but it would be worthwhile having analyses of the larger issues and trade-offs present at higher levels to assist such resource allocation issues. For instance, how resources should be allocated regarding chemical versus biological hazards in food products would already be a prioritisation question in this category. This has, indeed, been studied to some extent (Valeeva et al., 2007, 2006).

3.3 Category 2: Prioritisation Between Different Types of Biological Hazards

In this category the focus is purely on biological hazards. These can be divided into hazard categories as discussed in Section 2 (human, animal, plant and environmental health). Economic prioritisation can take place at two levels: (1) between different categories and (2) within the categories. As far as we are aware, prioritisation between the categories – for instance, comparing the economic risk presented by animal diseases to risks posed by plant diseases – has not taken place. However, for comparison of benefits from protection against different kinds of biological hazards, see Waage et al. (2005).

Instead, some comparisons within the hazard categories have taken place. In fact, such actions are also already politically sought for within animal diseases: the European Union is seeking to harmonise animal health policy and is looking for a single legal framework to bring together trade, imports, animal disease control, nutrition and welfare. It aims to introduce a scheme for classifying and prioritising animal diseases and disease prevention according to their economic or human health impact (European Commission, 2006). Somewhat similar plans are underway regarding plant health and invasive alien species and, for instance, risk-based surveillance overall is becoming increasingly popular (McKenzie et al., 2007), but these trends have not yet materialised as clearly as in the case of animal health.

The problem in lack of such assessments is that the resource allocation may be inefficient. As some kind of allocation is in any case made, if it is not based on (economic) assessment of the risks, it is based on something else. For instance, Virtue (2007) points out that in Australia too many species have been declared weeds in order to effectively contain or eradicate them all. Australian regions are argued to focus on widespread species for various reasons, including a long history of control, high visibility, political pressure, perceived impacts, shared burden of control, familiarity, and agricultural bias. Economic prioritisation has rarely played a part in such choices (Virtue, 2007). Similarly, risk is not the primary determining factor in control of many animal diseases (Rosengren and Heikkilä, 2009).

A few rigorous economic studies exist that prioritise hazards within certain categories. In addition, there are several studies that include some sort of risk ranking of biological hazards, including their economic impacts, but where the methodologies are less rigorous. In the first of the two more rigorous studies that we are aware of, Moffitt and Osteen (2006) study prioritisation of invasive species threats under uncertainty. They examine different decision rules and develop a priority list of potential agricultural crop pests in the United States to demonstrate the criteria. The other example is Cook (2005), who studies the critical level of investment in pest exclusion in Western Australia. He provides a framework that allows prioritisation of pests based on damage and production cost increases, and also identifies a critical level of expected damage associated with the pest that can be used as a ceiling for incursion expenditure. If this level is exceeded, other control options such as containment or adaptation should be considered. Using a static partial equilibrium model, the study considers a range of host plants.

Various hazard ranking studies are usually somewhat less rigorous from an economic perspective. This type of ranking usually follows the basic structure of Covello-Merkhofer risk assessment, including release, exposure and consequence assessments, which are combined to form a risk estimate (Peeler et al., 2007). The approach has been applied to, for instance, weeds in the US (Parker et al., 2007) and in Europe (Weber and Gut, 2004), to ants (Harris, 2005) and wildlife pathogens (McKenzie et al., 2007) in New Zealand, and to plant pests in Western Australia (Cook and Proctor, 2007). In these assessments the basic themes relate to what components are included in the risk ranking, how they are scored and aggregated, and how the opinions are formed.

Hazard ranking is based on separate components that together form the ranking order. The components typically include the probability of entry (or invasion or introduction or outbreak), the probability of spread (or invasiveness), and the likely consequences, which may or may not be measured in monetary terms. In most cases the ranking is on an arbitrary scale (e.g. 1 to 5), and the overall rank is obtained by summing up the different component values (e.g. Harris, 2005). Also, multiplicatory scoring is used, in which case the rank approaches zero if any of the individual components does so. This approach may be augmented by a measure for the feasibility of control. For instance, Virtue (2007) augments the risk measure by a containment feasibility measure, which is the product of control costs, current distribution and persistence. Rank is then obtained by dividing the risk measure by the control feasibility measure. The priority assessment is usually done using either expert opinion on its own (Weber and Gut, 2004; Cook and Proctor, 2007) or combined with a literature review (McKenzie et al., 2007). As pointed out by Cook and Proctor (2007), different juries are likely to produce different results.

Such prioritisation assessments are not without both theoretical and methodological problems, and the resulting rank order does not act as a decision-rule in itself. The rank order often illustrates the risk presented by the organism, not the benefit that would be achieved when investing a unit of control in it. Even so, a formal framework that approaches the problem in a standardised manner and helps in thinking through the issues involved is better than basing the decisions on, for instance, history, personal opinion or political pressure. A more detailed discussion on risk ranking is presented in Heikkilä (forthcoming).

3.4 Management Strategies, Methods and Policies

Study of biosecurity economics in category 3 is ideally a continuum and a holistic exercise in which the management strategy, the management methods to undertake that strategy and the policy instruments to obtain the desired outcome are evaluated simultaneously. However, this ideal is seldom achieved, at least in individual research papers. Instead, the choice between strategies, management methods or policy instruments is often made given the other factors. Hence in this review the studies are divided according to the objectives of the studies.

3.4.1 Category 3a: Assessment of Management Strategies Within Hazards

Biological hazards may be managed through various strategies, including prevention, eradication, containment and control (CBD, 1992; Council of Europe, 2003; Shine et al., 2000). If prevention fails, the most extensive measure is to eradicate the entire invasive population or the disease. Within the realm of certain animal diseases and in the case of some plant pests or diseases, eradication may be an automatic action if the hazard is encountered. In the case of invasive species threatening the environment, such decisions are likely to be made much more on an ad hoc basis. For examples of eradication successes and failures, see Genovesi (2005) and Simberloff (2003). If eradication is found not to be feasible or economical, it is possible to try and contain the hazard to a given area and thus prevent any further spread. In containment, the objective is to protect the nearby areas, either inside the country in question or in neighbouring countries (Council of Europe, 2003). If this is also not possible, the final alternative is to control the hazard such that its population size, density or prevalence remains below some threshold level.

Although, for instance, the guiding principles of the United Nations Convention on Biological Diversity (CBD) advocate eradication as the next best thing if prevention fails, in the context of invasive species very few eradication programmes have been carried out in Europe. The reasons for this include inadequate national laws, unclear responsibilities among authorities and opposition by animal rights groups (Genovesi, 2005). In contrast, for animal diseases the case is somewhat different. Heath (2006) points out that all high-income countries have eradicated all new animal diseases provided that there has been political will to do so. For the poorer countries this is not the case, as private producers are not necessarily interested in diseases that cause large trade disruptions but where production losses may be modest. In other words, there is a divergence between private and social incentives.

In addition, invasions that are not targeted immediately when a hazard is first encountered and thus become widespread can be extremely costly to eradicate (see e.g. Cowan, 1992; Genovesi, 2005). Hence eradication is often feasible only when the size of the invading population is relatively small and it is geographically restricted (Council of Europe, 2003). Invasion dynamics are not the only issue to account for, however. Perrings (2000) establishes the conditions under which allowing establishment and spread of invasive species is optimal and emphasises that the relative costs and benefits of native and non-native species are important.

The society should explicitly account for the tradeoff between present expenditures to protect ourselves from the invasion, and future expenditures to control or reduce the damage from the few harmful invasions (Jensen, 2002). For instance, most studies on foot and mouth disease are argued to concentrate on post-outbreak disease management, and less attention has been paid to pre-event surveillance and detection. No studies have been conducted that assess the benefits of surveillance and detection relative to associated damage (Elbakidze and McCarl, 2005, see also Born et al., 2005).

Several economic studies exist where one management strategy (often the current strategy) is compared to an alternative strategy. Wider comparisons of various alternative strategies are much less common. Examples of comparisons for more than two strategies include, for instance, McInerney and Kooij (1997), and Rich and Winter-Nelson (2007). The most common strategy options that are evaluated include some sort of preventative action versus reactive control. These strategies have been studied by, for instance, Burnett et al. (2007), Fernandez (2008), Persson and Jendteg (1992), Maijala et al. (2005), Cembali et al. (2003), Ranjan et al. (2008), Cook et al. (2007), and Heikkilä and Peltola (2007).

Other economic strategy comparisons that have been undertaken include, for instance, eradication versus control (Eiswerth and van Kooten, 2002; Olson and Roy, 2002; Andersson et al., 1997), eradication versus containment (Cacho et al., 2008; Sharov and Liebhold, 1998; Sharov, 2004), detection versus control (Mehta et al., 2007), control versus no control (Fasina et al., 2007), vaccination versus alternatives (Mukhebi et al., 1999; Kobayashi et al., 2007; Berentsen et al., 1992), regionalisation versus no regionalisation (Paarlberg et al., 2007), and eradication with restoration versus adaptation (Zavaleta, 2000).

In most cases the strategy alternatives are seen as substitutes that are compared against each other. Much more rarely has a study incorporated strategy choices as complements in order to determine the optimal mix of strategies. Such a study was undertaken for prevention and control by Burnett et al. (2008) to determine the optimal intertemporal allocation of resources. Also, Pifafi and Rousmasset (2007) provide an integrated framework where optimal prevention is combined with optimal pest removal. They show how optimal prevention depends on minimised costs resulting from the failure of prevention, including damage costs. Other studies on complementary strategies include Burnett et al. (2006), Leung et al. (2002), and Kim et al. (2005).

The methods employed in these assessments show a wide (but thin) variety including, for instance, optimal control theory (Burnett et al., 2007), Monte Carlo simulation (Heikkilä and Peltola, 2007; Russell et al., 2006; Niemi et al., 2008), linear programming (Hastings et al., 2006) and game theory (Fernandez, 2008). Objects of the studies include a wide variety of different biological hazards.

The optimal management strategies have been found to vary in space and time. Location has been found to matter for the largest cost components (Persson and Jendteg, 1992) as well as for optimal strategies (Burnett et al., 2007). For instance, James and Rushton (2002) argue that in many studies (Netherlands, Italy, Germany, etc.) it has been shown that eradication of foot and mouth disease is more economical than vaccination in Europe, whereas in developing countries stamping out seems not to be feasible or desirable. Similarly, it is argued that in Nigeria, even at the expense of loss of export markets, the best method for H5N1 control is vaccination (Fasina et al., 2007). The optimal strategy has also been found to be time-varying (Hastings et al., 2006) as well as to depend on species characteristics (damage, growth rate) and varying from do nothing to a high level of effort (Mehta et al., 2007).

Costs that should be included in a thorough economic assessment of biosecurity management strategies, and that have variedly been included in the strategy assessments, comprise (after Burnett et al., 2007; Persson and Jendteg, 1992; Niemi et al., 2008; Cembali et al., 2003; Mehta et al., 2007; Kobayashi et al., 2007; Fasina et al., 2007): (1) infrastructure and administration cost; (2) search and detection cost; (3) cost of pest or disease control; (4) illness treatment costs (human, animal); (5) illness costs (human); (6) loss of productive capacity (human, animal, plant, environment); (7) cost of business interruption and demand switches; (8) price movements; and (9) compensation payments and other income transfers.

When the distribution of costs and benefits of management strategies has been studied, it has generally been found to be very uneven. For instance, Mukhebi et al. (1999) find that 91% of the costs of heartwater disease are borne by the commercial farmers and Cembali et al. (2003) find that the nurseries who pay for the analysed virus prevention programme receive benefits of \$0.5 million (0.2% of total benefits), whereas growers and consumers who do not pay for the programme benefit \$80 million and \$147 million, respectively. Similar findings are made by Gutrich et al. (2007) in the case of fire ant in Hawaii as well as Mangen and Burrell (2003) in the case of classical swine fever in the Netherlands. Also, Niemi and Pietola (2004) note that in the case of pig diseases, the privately optimal solution is not necessarily the one that is socially desirable.

Uncertainty is more and more included in the assessments in this category, but treatments of it as well as the implications of uncertainty differ widely. Uncertainty is important to account for, because it is often related to the outcome of the events, although not in a straightforward manner. It has, for instance, been found that uncertainty in initial population size (Mehta et al., 2007) and in timing and nature of invasion (Ranjan et al., 2008) has a substantial impact on the optimal strategy. On the other hand, Russell et al. (2006) find that in 60% of the cases considered in their study, the best policy did not change when uncertainty was introduced. Similarly, Jensen (2002) finds that his conclusions hold regardless of whether the costs are known for certain or only in distribution.

Example of Category 3a Problem Framing: Prevention Versus Adaptation

Prevention (or mitigation, avoidance, pre-emptive control) and adaptation (or reactive control, treatment, amelioration) are perhaps the most widely studied strategy alternatives. There is a general agreement that on most occasions preventative actions are the best strategy, given the uncertainties involved and the difficulties in eradicating most species and diseases reactively. For instance, Fernandez (2008) finds that cooperative and preventative abatement is optimal to all other strategies. This approach is put forward by, for instance, the intergovernmental scientific advisory body established by the CBD (Perrault and Carroll Muffett, 2001) as well as the European Union in relation to many animal diseases.

For instance, Leung et al. (2002) use stochastic dynamic programming to study how to devote resources between prevention and control efforts given uncertain invasion events. They apply their general model to the case of zebra mussel (*Dreissena polymorpha*) – an aquatic species that spread from Europe in ship ballast and has subsequently spread rapidly within the United States. The species damages power plants by blocking their water intake pipes. The authors show that the society could be made better off by spending up to 0.3 million dollars annually to prevent the invasion of a single hypothetical lake with a power plant. This figure can be compared with the 0.8 million dollars that the US Fish and Wildlife Service spent on all aquatic invaders in all US lakes in 2001 (Leung et al., 2002).

The costs of prevention comprise, for instance, costs of surveillance, labelling, import restrictions, compensation payments and post-monitoring. Often the benefits of not having the pest around outweigh these costs, but this is by no means inevitable (Mumford, 2002). In such a case, continued efforts to prevent the hazard from invading consume the limited resources and may possibly lead to other, more dangerous, hazards not being targeted with sufficient resources. Several European countries have, for instance, voluntarily renounced their protection systems regarding specific species (Heikkilä, 2006). On at least one occasion cost-benefit analysis has been in favour of denouncing a preventive policy (MacLeod et al., 2005).

Elbakidze and McCarl (2005) show that optimal pre-event detection depends on likelihood of disease introduction, spread rate, relative costs, ancillary benefits and effectiveness of mitigation. For slowly spreading diseases, investment in pre-outbreak activities is optimal only for very high outbreak probabilities. A somewhat similar conclusion is reached by Burnett et al. (2008), who suggest that it would be worthwhile spending money on finding small populations rather than to attempt to prevent all future introductions. Of course, investments in prevention and adaptation are not necessarily mutually exhaustive. For instance, Kim et al. (2005) show that prevention and control are complementary when the population size is small, but become substitutes as the population size increases.

In addition to biological factors, relative costs and benefits, and effectiveness of mitigation there are three further factors affecting the strategy choice between prevention and adaptation. The first of these is uncertainty. Mahul and Gohin (1999) note that if there is little uncertainty it may be worthwhile to wait and see until uncertainty is resolved. Thus, in practice adaptation may be preferred over prevention because of uncertainty. The relationship between resources invested in prevention and benefits thus acquired is very uncertain. Risk in this context has two dimensions: (1) the probability and level of damage associated with the biological hazard; and (2) the opportunity cost of resources spent on prevention of the biological hazard, which may or may not make an entry attempt. Finnoff et al. (2007) show that a risk-averse (i.e. precautionary) manager is likely to invest less resources in prevention and more in adaptation because the return for the investment in prevention is less certain. Hence adaptation may appear as the less risky management strategy, even though it is often also the more expensive one (Finnoff et al., 2007, see also Shogren, 2000 and Perrings, 2005).

The second additional factor is human adaptation, which is important to take into account when considering the merits of adaptation. In a relatively early theoretical analysis, Butler and Maher (1986) argue that by not taking the actions by the victims of an externality into account, the society may end up devoting too many resources to prevention. Similarly, Margolis et al. (2005) show that unaccounted for private actions result in the regulator choosing a level of prevention (tariff level) that is larger than is socially optimal. Shogren (2000) argues that once we acknowledge that people do adapt, assuming otherwise may lead to biased results.

Finally, the chosen objective of strategy optimisation may affect the desirability between prevention and adaptation. Barrett and Segerson (1997) note that besides Pareto efficiency there are other objectives that policies may seek, including minimising damage subject to a budget constraint or minimising expenditure subject to a given damage level. They show that under these constrained objectives some factors affecting the relative desirability of prevention versus adaptation may affect the decision differently from how they function under Pareto efficiency. For instance, in contrast to Pareto efficiency, under a budget constraint a reduction in uncertainty about the effectiveness of adaptation may lead to a decrease in the level of prevention and an increase in the level of adaptation. Adaptation may also be preferred if the government pays for prevention and has a strict budget constraint. Therefore, if public money can be more productively invested in preventing entry by Hazard B, it may be that Hazard A is best controlled through adaptation paid for by producers, although prevention might also in this case be a socially optimal strategy.

Lichtenberg and Penn (2003) – albeit in relation to chemical hazards – argue that prevention is not always the most cost-efficient strategy in the case of agricultural pollution. This is so when there are multiple sources of emissions, multiple sites affected and a widely adopted precautionary approach to uncertainty. More generally, based on the above discussion it might be argued that adaptation is relatively more attractive when: (1) the invasion probability is relatively low and the probable damage modest; (2) the effectiveness of adaptation is high; (3) adaptation strategies and incentives of individuals are taken into account; and (4) the decision-maker dislikes uncertainty regarding the outcome of the policies. Furthermore, as Waage and Mumford (2008) suggest, perhaps a greater focus should be put on building resilience to invasion (through, for instance, developing pest and disease resistance and vaccines as well as through diversification of production) rather than building walls around the protected systems.

3.4.2 Category 3b: Cost-Efficiency of Management Methods

Cost-efficiency of different management methods within strategies is the next category reviewed here. Here, the management strategy (and perhaps the method) has already been agreed upon, but the question is how to utilise it most efficiently. This includes questions such as what is the economically optimal eradication radius in a disease outbreak, when and to what extent should vaccination be used, how much pesticide should be used to control a plant pest, and so forth. Separate questions also include how the cost-effectiveness of the management method is affected by, for instance, resistance to chemical control substances and antibiotics or changes in the natural or production environment.

Studies in this category are numerous. To provide some examples, there have been studies on cost-effectiveness of alternative prevention measures (De Vos et al., 2005), stamping out and vaccination alternatives (Schoenbaum and Disney, 2003; Mangen et al., 2002; Mangen et al., 2001; Saatkamp et al., 2000), surveillance methods (Prattley et al., 2007; Powell et al., 2008; Saatkamp et al., 1997; Klinkenberg et al., 2005) and different food safety procedures (Jensen et al., 1998; Goldbach and Alban, 2006). As there are many studies in this category, various methods and study targets exist. The methods utilised include, for instance, stochastic dynamic programming (Houben et al., 1994), stochastic state transition models (van der Gaag et al., 2004; Schoenbaum and Disney, 2003), portfolio theory (Prattley et al., 2007), the scenario tree model (De Vos et al., 2005), the gravity model (Otsuki et al., 2001) and generalised linear mixed models (Chriél et al., 1999).

As an example of category 3b research methodology, consider optimal control. Using a traditional cost-benefit analysis it is possible to analyse a set of possible policies; for instance, the cost-efficiency of using a depopulation ring of 1, 3 or 5 kilometres, but it is not possible to analyse what would be the optimal radius of the depopulation ring (Kobayashi et al., 2007). To answer this, some sort of optimisation method is needed. Optimal control is a traditional method in resource economics and it is therefore not surprising that there are several applications to biosecurity. The application may be directly to optimal pest or disease control (Dehnen-Schmutz et al., 2004; Christiaans et al., 2007) or to, for instance, optimal feeding policy under animal movement restrictions (Niemi and Pietola, 2004). The control strategies have also been studied by Hilje and Stansly (2008), Johansson et al. (2005); Buhle et al. (2005), and Ross and Pollett (2007). The optimal control policy has been found to depend on, for instance, the available annual budget (Taylor and Hastings, 2004), policy- and sector-specific factors (Breukers, 2007) and on the production function (Christiaans et al., 2007). It has also been found that the cost-effectiveness is reduced if all firms do not adopt the control procedure (van der Gaag et al., 2004) and that the adopted framework (for instance, expected utility) impacts on the optimal policy (Horan et al., 2002).

The study objects range from individual invasive species such as koalas (Ross and Pollett, 2007), *Bemisia tabaci* (Hilje and Stansly, 2008) and greenhouse whitefly (McKee et al., 2008) to marine invasive species (Fernandez, 2008; Jones and Corona, 2008), ornamental garden plants (Dehnen-Schmutz et al., 2004), and individual species in specific countries (Blignaut et al., 2007). In the context of animal diseases the applications vary from individual diseases such as classical swine fever (De Vos et al., 2005) or hypothetical foot and mouth disease (Schoenbaum and Disney, 2003) to assessments of management systems for multiple diseases such as the pig identification and recording system in Belgium (Saatkamp et al., 1997) or hog producer income under contagious animal disease quarantine (Niemi and Pietola, 2004). In the context of zoonotic diseases and human health the applications include, for instance, salmonella (Goldbach and Alban, 2006; Chriél et al., 1999; van der Gaag et al., 2004; Jensen et al., 1998) and aflatoxin regulation (Otsuki et al., 2001).

Similarly to studies on the choice of management strategy, here a convergence between privately and socially optimal methods has also been found in several studies. Hilje and Stansly (2008) find that the method that is overall most economic (plastic cover) was also too expensive for the resource-poor farmers. Thus, although, for instance, Goldbach and Alban (2006) argue that it is the relative profitabilities of alternatives rather than the absolute values that are important, they are of little relevance if the private agents have no incentives to pay for either method. De Vos et al. (2005) point out that in determining the optimal method, one should account for the cost effectiveness but also for: (1) ease of implementation (small or large investments, are significant adaptations required); (2) allocation of costs and benefits (some measures may be too costly for private producers); (3) the cost-benefit ratio; and (4) attributable costs (beneficial side effects, e.g. reduce the probability of other hazards).

3.4.3 Category 3c: Choice of Policy Instruments

In addition to considering the preferred control strategy (e.g. prevention) and preferred level of intervention, we are also interested in how that control strategy should be achieved. Should we, for instance, use command and control instruments to ban certain types of imports altogether, or should we perhaps tax the imports in a risk-based manner, or maybe we should resort to delivering information on the risks to all parties involved. Naturally, some policy instruments are complementary (e.g. information), whereas others can be seen as substitutes (e.g. trade ban and import tariffs cannot apply to the same products at the same time).

Economic analysis and development of policy instruments is one of the most promising areas in which economics can contribute to better biosecurity. For instance, Jones and Corona (2008) show that in the context of aquatic invasive species an ambient tax induces both short-run and long-run efficiency with minimal information requirements, and Blignaut et al. (2007) discuss the structure, size and distribution of a charge to eradicate invasive plants in South Africa over the next 25 years. Dehnen-Schmutz et al. (2004) show that a subsidy on control costs is the most effective and reliable method, and that increases in direct grants or in control volunteer numbers are less effective. In contrast, Hennessy (2007) finds that losses are smaller when production is concentrated, but that subsidies to small producers may exacerbate overall losses.

Overall, literature on this theme has been expanding vastly over the past few years, but is still relatively thinly dispersed. Topics that have recently been covered include the polluter pays approach (Jenkins, 2001), ambient taxes (Jones and Corona, 2008), user charges (Blignaut et al., 2007), tradable risk permits (Horan and Lupi, 2005), control subsidies (Dehnen-Schmutz et al., 2004; Hennessy, 2007) and Pigovian taxes (Knowler and Barbier, 2005), as well as discussion on economic incentives in general (Fernandez, 2008). Gren (2008) provides a discussion on policies available for the control of invasive species.

3.4.4 Example: Biological Hazards and Import of Goods

The economics of biological hazards in relation to import of goods has been a topic of much research (e.g. Anderson et al., 2001; Oude Lansink, 2008) so we use it as an example of the types of studies conducted. When a particular good is proposed for import, there can be a screening of whether the good should be allowed for import or not. There has been some discussion on whether we would be better off ignoring the advice of the screening process or not (Smith et al., 1999; Caley et al., 2006). An empirical application of a similar question is provided by Keller et al. (2007), who assess when risk assessment is worth it compared to letting all species in. They apply their model to the Australian ornamental industry, assuming that the decision to import a species is irreversible. Over relatively short periods there is not much difference in the net present value of the two policies (screen or do not screen), but for longer time periods screening creates large positive values and the fixed costs of undertaking the risk assessments have a negligible impact on the results. The authors point out that since the World Trade Organization requires that SPS-based risk reduction strategies must produce net economic gains, their model can be used to demonstrate that.

Import risk assessments (IRAs) can be used to evaluate whether some imports should be prevented altogether in order to protect the society from the risk of imported diseases and pests. Examples are provided by studies on the risks posed by import of animals vaccinated against foot and mouth disease and their products (Sutmoller and Casas Olascoaga, 2003), import of marker-vaccinated animals (Breidenbach et al., 2007), and the risk of BSE through imported animals and their products in Japan (Sugiura et al., 2003). Adamson and Cook (2007) point out that even in Australia, where relatively many import risk assessments have been undertaken, economic assessments are often limited to either estimating (1) how much consumers would benefit if free trade was allowed or (2) the economic impacts if a given pest or disease reaches the country. They argue that little work has been done to combine these two, which would be important in order to obtain a truthful estimate of the total impacts.

For instance, Cook (2008) studies the case where Chilean table grape producers were granted access to Australian markets, but not to Western Australia, which is free of many pests present elsewhere in the country. The outcome of the study is that the mean expected production damage from also allowing access to Western Australia would be around AU$10.3 million, whereas consumer benefits would be only AU$1.5 million. Hence there is no reason to allow market access. However, preventing trade in exotic species that also have beneficial impacts is not necessarily economically desirable. Knowler and Barbier (2005) studied saltcedar (*Tamarisk* spp.) in the United States, and their results indicate that to achieve the social optimum, the mere presence of risk associated with imports does not warrant prevention of commercial sales of exotic plant species with beneficial effects for consumers. Similarly, Otsuki et al. (2001) suggest that less stringent regulation may increase trade flows and income. Instead of bans, Knowler and Barbier (2005) suggest the use of market instruments, including a Pigovian tax, to regulate the industry and protect the society from the associated risks. Prevention of entry and the associated trade instruments are also studied by Horan et al. (2002), Costello and McAusland (2003), McAusland and Costello (2004), Horan and Lupi (2005), and Margolis et al. (2005).

In addition to being a question of choosing the right policy instrument, import risk assessments can be seen as a special case of hazard ranking (Sect. 3.3), because although not necessarily conducted in order to compare different biological hazards, the procedure is often so standardised that it allows the comparison of hazards for which an assessment has been conducted. This is especially so if the IRA follows consistent methodologies. In many cases IRAs are based on established risk assessment procedures; for instance, the OIE import risk analysis framework or the EPPO guidelines, which are accepted by the World Trade Organization as a justification for actions that could also be considered artificial barriers to trade. Naturally, even if the general framework is standard, the different economic components vary within the studies (e.g. which valuation methods have been used and what effects have been taken into account). Hence the different studies may still not be entirely comparable.

For goods and shipments accepted for import, regulation may still be necessary through, for instance, inspections or tariffs, which are meant to reduce the probability of harmful organisms entering the country. However, the inspection services are under pressure from the increasing import quantities as well as in many cases due to resource constraints. Further complications arise from the diverse range of imported goods and their origins. For instance, Costello et al. (2007) show that the risk varies by trading partner and that the cumulative number of introductions from a region is a concave function of imports. As for many countries it is vital to maintain an appropriate level of protection, a typical question in economic studies is how to establish efficient border protection measures.

The basic premise for many of the studies on optimal inspection strategies is that there is a number of shipments coming to a port, the probability that a crate within the shipment is infested is unknown, and the inspector has to decide how to allocate the scarce surveillance resources; for instance, how many crates to inspect and in which shipments, and how long a time should be used for the inspection. Research along these lines has been conducted by, for instance, Moffitt et al. (2008), Surkov et al. (2008), Batabyal and Beladi (2006), and Batabyal and Nijkamp (2006). An empirical assessment is provided by Work et al. (2005), who evaluate the effectiveness of monitoring the arrival of insect species in the United States through four cargo pathways.

As for tariffs, Paarlberg and Lee (1998) discuss the link between import tariffs and the level of health risk from imports with special reference to the foot and mouth disease. They show that the optimal level of tariffs is very sensitive to the risk of importing a contaminated product, as well as to the expected spread rate of the disease. More specifically, the infection rate of the imported goods has been shown to increase the optimal tariff level (McAusland and Costello, 2004). However, at very high levels of infection the optimal level of inspections decreases, possibly all the way to the point of no inspections. This occurs when most incoming goods are infected, and it is better to let them in without any inspections and instead charge a high tariff equal to the expected damage (McAusland and Costello, 2004).

However, too high a tariff is also socially suboptimal. Margolis et al. (2005) show that private actions result in the regulator choosing a higher tariff level than is socially optimal. Further, tariff escalation (the more processed the imported good, the higher the charged tariff) has been shown to increase the probability of hazard introduction (Tu et al., 2008). This is because it results in trade being biased towards primary commodities (with higher probability of harmful organisms) as opposed to processed products. Tu et al. (2008) show that a reduction in tariff escalation would increase allocative efficiency and reduce damage from invasive species.

In a case of unlimited research resources, the studies would consider the questions regarding whether to import or not, how to inspect and place tariffs optimally, how to control the species and disease if it arrived and escaped inspections, and so on, and then compare all possible combinations with each other to determine the optimal course of actions in the face of uncertainty. However, this is not practical in reality, and hence the questions are divided into smaller manageable questions. It is, as always, up to the researchers to place the research in the wider framework.

3.5 Category 4: Cost Estimates of Disease Outbreaks and Species Invasions

The final category discussed here includes the evaluations of individual invasion events or policies. There are hundreds of estimates of the damage caused by individual species or diseases, for both real invasions as well as for simulated ones. Some of these are related to specific countries or groups of countries, some to specific groups of species, and some to specific pests and diseases. They may be based on materialised damage from real invasions or on simulation of potential damage, and incorporate various and varying cost elements, making any comparison between the studies laborious or impossible. Some examples are provided in Table 1.

Figure 3 presents a collection of published empirical economic biosecurity studies by type and target country. Only countries for which more than one study was readily available are presented in the figure. The results were obtained with an internet search, as well as through the author's personal collection of papers. Only studies in which there is an empirical application to a real-life situation were included in the sample. Altogether, there are 231 studies in the sample. Although the literature search has not been exhaustive, general trends are easy to observe. The publications are divided such that 39% deal primarily with animal health, 25% with plant health, 11% with human health (only zoonotic diseases and biological food safety included in the sample) and 25% with environmental health (invasive alien species detrimental to the environment in general). Of course, sometimes it is difficult to precisely differentiate between plant health and environmental health.

The United States is the country in which most applications take place (35% of all studies in the sample), followed by Australia (13%), the United Kingdom (10%) and the Netherlands (9%). Together these four countries represent two-thirds of all studies. However, the domination of the US, European Union and Australia in these figures is not surprising considering that they are all industrialised countries who produce and export large quantities of agricultural products.

It also appears that many assessments in the United States (44% of all US studies) and South Africa (100%) relate to invasive species that pose a threat particularly to the environment. In contrast, in Australia (46% of Australian studies) the emphasis is

Table 1 Examples of individual invasion cost estimates

Level	Target	Annual cost estimate	Reference
Multi-country multi-hazard	Invasive alien species in 6 countries (USA, UK, Australia, South Africa, India and Brazil)	USD 314 billion	Pimentel et al., 2001
Single country multi-hazard	Invasive alien species in US	USD 120 billion	Pimentel et al., 1999, 2005
	Invasive alien species in China	USD 14.5 billion of which 83.4% indirect. Equivalent to 1.4% of Chinese GDP	Xu et al., 2006
	Invasive alien species in Canada	CAD 13–34 billion	Colautti et al., 2006
	Invasive alien species in Germany	100–265 million euros	Gebhardt, 1996; Reinhardt et al., 2003
	Weeds in Australia	AUD 4 billion	Martin, 2003
	35 animal diseases in UK	Economically most important are mastitis for cattle (£179.7 million), enzootic abortion for sheep (£23.8 million), swine influenza for pigs (£7.7 million) and salmonellosis for poultry (£104.7 million)	Bennett and Ijpelaar, 2003
	34 animal diseases in UK	£372-1061 million annually	Bennett and Ijpelaar, 2005
	Foodborne bacteria in US	USD 3.5–4.8 billion	Roberts, 1989
	Invasive pests in US	Quarter of agricultural gross national product	Schmitz and Simberloff, 1997; U.S. Congress, OTA, 1993
	South Africa fynbos	Value from R19 million under low valuation and poor management to R300 million under high valuation and good management. Cost of clearing alien plants 0.6–5% of the ecosystem value	Higgins et al., 1997
Multi-country single hazard	H5N1 avian influenza on international markets	First year market loss USD 1.4 billion to 14 billion, depending on scenario	FAO/CTD, 2006; Food Outlook, 2006
	Asian longhorn beetle in Europe	90% of wood in infested areas could lose half of its value	MacLeod et al., 2002
	Salmonellosis in England and Sweden	For cost of illness (control cost) 2.6 pence (2.0 pence) per chicken in England and Wales and 0.5 pence (10.8 pence) per chicken in Sweden	Persson and Jendteg, 1992
	EU aflatoxin standard in 9 African countries	USD 670 million	Otsuki et al., 2001
	Foot and mouth disease in South America	Estimates benefits from various disease control strategies	Rich and Winter-Nelson, 2007
	Mnemiopsis leidyi in the Black Sea	Decline in profit from USD 17 million to USD 0.3 million	Knowler and Barbier, 2000; Knowler, 2005
	HPAI avian influenza globally	Simulate the impact of supply reductions on export prices using 16 scenarios and assuming constant demand, but do not calculate total monetary impacts	Djunaidi and Djunaidi, 2007
Single country single hazard	Hundreds, 231 depicted in Fig. 3		

somewhat more on plant health and in the Netherlands (80%) and Denmark (67%) on animal health, which is not a big surprise, given the relative importance of animal production in these two countries. In the United Kingdom, plant health constitutes about 39% and animal health about 48% of all studies.

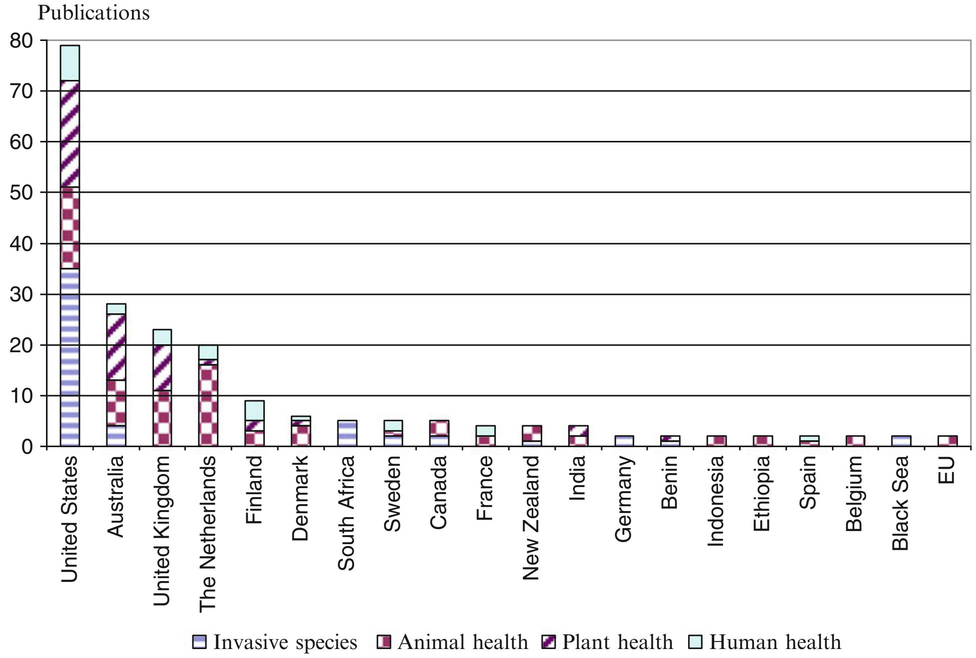

Fig. 3 Reviewed biosecurity studies by category and country of application

These trends are similar to those found in a meta-analysis of the ecological invasion literature. Pysek et al. (2008) analysed 2670 papers dealing with 892 invasive species, and showed that all major groups are well studied, but most information on invasion mechanisms is based on a limited number of invaders. They also show a strong geographical bias, with Africa and Asia understudied and America and Europe having the highest number of both species studied and studies published. Almost half of all the invasive species and more than half of all studies analysed by Pysek et al. (2008) relate to North America. They also note that the position of Africa is largely determined by South Africa, representing two-thirds of research on the continent. The fact that these trends reflect on the economic literature is understandable, given that empirical applications in biosecurity economics are to a large extent dependent on availability of biological data or expertise.

Pysek et al. (2008) also point out that only 49 species were subject to 10 studies or more, the most studied being zebra mussel and Argentine ant. A similar conclusion of most research concentrating only on a few species was reached in the context of plant health by the EUPHRESCO project in the European Union (Buzy et al., 2007).

The publications were also classified by journal and year (Figs. 4 and 5). Figure 4 demonstrates that for animal health economics Preventive Veterinary Medicine is the primary journal of publication. On the other hand, for invasive species economics Ecological Economics is the top choice, followed by the Journal of Environmental Management and Biological Invasions. For plant health economics the most popular choice is Crop Protection, again followed by the Journal of Environmental Management and Agricultural Economics. It is also remarkable how thinly the studies are distributed over a wide variety of journals. In the sample of 231 studies there were 48 journals that contributed one paper each.

Just over 20% of the papers were published in economic journals, the major economic journals being Ecological Economics (14 studies), the American Journal of Agricultural Economics (10), Agricultural Economics (3) and Environmental and Resource Economics, the Journal of Agricultural and Applied Economics and the European Review of Agricultural Economics (2 each). Some 10% were in primarily natural science journals, the main journals being Ecological Modelling (3 studies), and Vaccine, Veterinary Microbiology, Ecological Applications and the International Journal of Food Microbiology (2 each). This

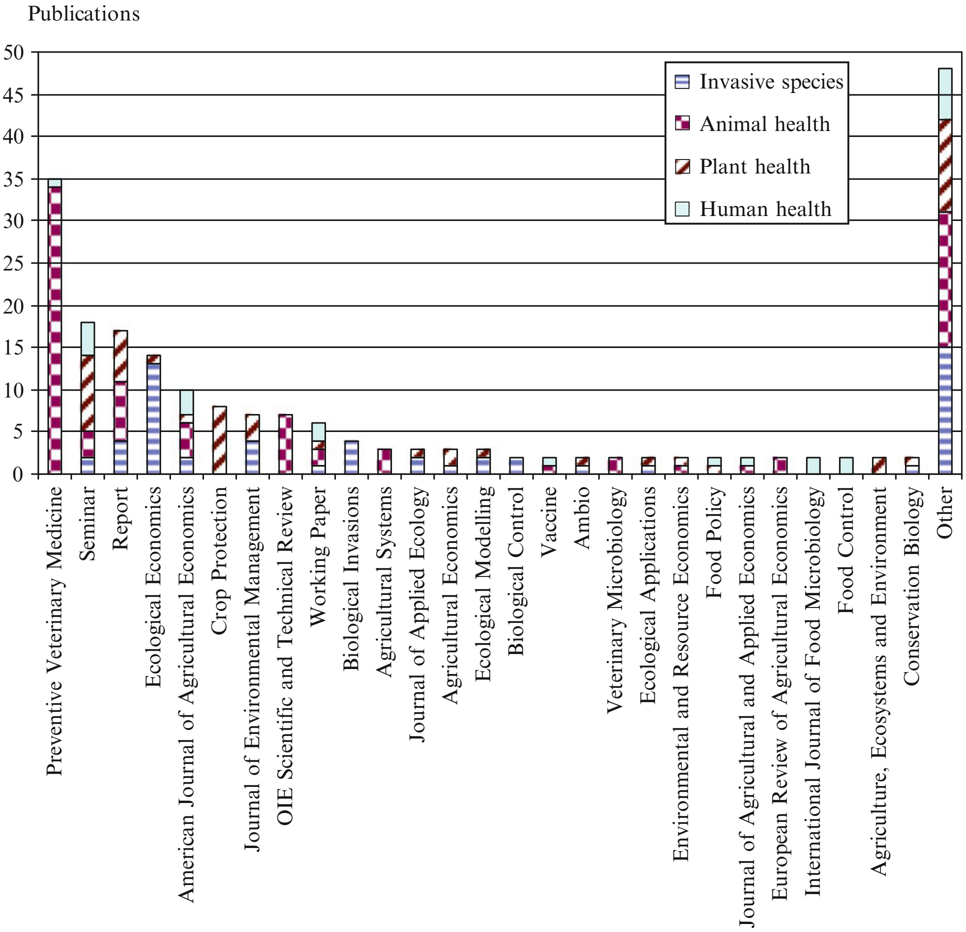

Fig. 4 Reviewed biosecurity studies by category and journal

also means that most papers were published in fairly interdisciplinary journals, as depicted in Fig. 4.

Figure 5 demonstrates that the volume of papers in the sample has increased gradually over the years and the field is growing steadily. It also seems that the number of papers on animal health economics has remained relatively stable over the past 15 years or so, while the number of papers on economics of invasive species has increased rapidly.

4 Discussion

It seems apparent that the broader the context (categories 1 and 2), the less economic research there is. Category 1 is virtually not studied at all, and category 2 is studied very little, with the exception of the risk ranking studies. These, however, would be likely to benefit from more rigorous economic considerations. In category 3 there are numerous studies concentrating on specific questions and employing a wide but relatively thin range of methodologies (see, e.g., Oude Lansink, 2008; Perrings et al., 2000). In category 4, a substantially greater number of studies exists, but these would benefit from some standardisation of approaches. The fact that in many assessments there are no clear common methods or frameworks (applying to both the methodologies used as well as to the types of costs included), cross-study comparison is relatively difficult. Yet, such comparison would be needed when deciding on how to allocate the available resources of the society. Of course, as mentioned earlier, the categories identified here are separated sections from the

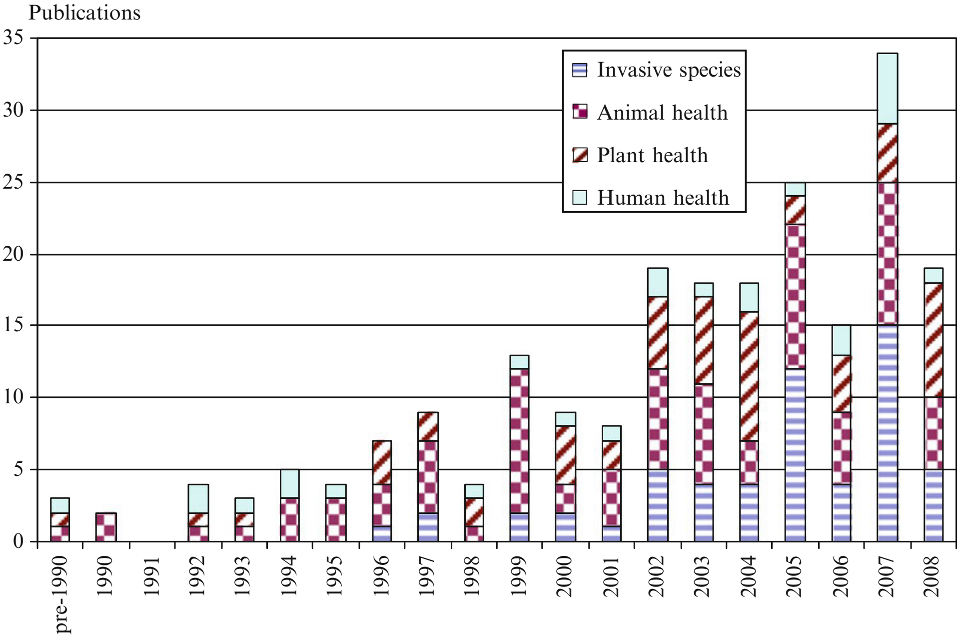

Fig. 5 Reviewed biosecurity studies by category and year of publication

larger framework, which is seldom evaluated in individual papers, as demonstrated by the example of studies on import of goods.

It has been pointed out that two major contributions of economics to biosecurity discussion are, on one hand, to provide estimates on the impacts of invasions (category 4 in this paper) and hence improve the cost effectiveness and efficiency of control (category 3b), and on the other hand, to develop economic sanitary and phytosanitary measures (category 3c) (Evans et al., 2002). Shortle (2007) emphasises the importance of economic research to the development of effective and efficient management strategies (category 3a) as well as to understanding the co-evolution of economic and ecological systems. It is worthwhile to emphasise the role of economics in resource allocation and its use to determine how to prioritise different hazards, their management strategies, methods and policies.

Environmental and health impacts are notoriously difficult to value. However, as pointed out by Adamson and Cook (2007), even quantification of the area of impact and a list of native species at risk (and the level of risk) would be informative. It has also been suggested that the value of modelling is the actual process of working through the problems and issues rather than the identification of a final solution (Rushton and Upton, 2006). Modelling helps in conceptualising and thinking through complex problems analytically. In principle, for valuation of the effects of invasions or biosecurity policies almost any valuation method available in the economic literature is available. However, given the fact that we are dealing with thousands or tens of thousands of individual biological hazards, it seems clear that no nation has enough resources to undertake thorough studies of them all. Hence development of general assessment protocols, frameworks and prioritisation schemes for assessing the risk associated with biological hazards seems like a good candidate for a top priority research area.

The distribution of costs and benefits is an important issue, which is already beginning to affect the current animal health policies in the European Union. Also, in other contexts the distribution of costs and benefits has been found to be very uneven, as discussed in Section 3.4. Yet having a clearer idea about this would help in understanding the private incentives and subsequently in designing appropriate policies to account for the convergence of social and private interests. As Hennessy (2007) notes, designing public policies in biosecurity requires a clear understanding of the failure in private incentives.

Another challenge, as highlighted by Gramig and Wolf (2007), is that many studies are done on a disease-by-disease basis, yet preventative actions often protect against multiple diseases. As they point out, preventative actions against multiple hazards can be thought of as either multi-product outputs of individual management practices, or as either positive or negative input externalities. Given that prevention is generally acknowledged to be a cost-effective strategy, it is somewhat surprising how few assessments there are of the ongoing costs and cost-efficiency of prevention at the farm level. The government-level costs are often known (although not necessarily published), but we often know very little about the farm-level costs and their impact on the level of prevention (Siekkinen et al., 2008). This is important, because these costs materialise every year, regardless of whether there is a pest or disease outbreak or not.

To conclude, we suggest the following top five priority areas (in no particular order) for study: (1) development of sophisticated economic policy instruments to mitigate biological hazards; (2) design of rigorous economic prioritisation frameworks to guide both academic research and government policies; (3) clearer understanding of the distribution of biosecurity impacts and the associated private incentives; (4) establishing a closer link between the costs and methods of prevention, and the economic risk; and (5) the role of human behaviour in biosecurity and how that behaviour can best be directed. We might also wish to find out to what extent lessons learned in some fields of biosecurity are applicable for other hazards, areas or circumstances. In general, it seems warranted to search for holistic approaches – for instance, determining simultaneously the optimal strategy, optimal management methods and optimal policies to achieve the targets – and doing so for the right hazards. No small task.

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Describing and Locating Cropping Systems on a Regional Scale

Delphine Leenhardt, Frédérique Angevin, Anne Biarnès, Nathalie Colbach, and Catherine Mignolet

Contents

Abstract At regional scale issues such as diffuse pollution, water scarcity and pollen transfer are closely related to the diversity and location of cropping systems because agriculture interacts with many other activities. Although sustainable land use solutions for territorial development and natural resource management are needed, very few agro-environmental studies account for both the coherence and the spatial variability of cropping systems. The originality of this article is to review methods that describe and locate cropping systems within large areas. We mainly based our analysis on four case studies using the concept of cropping systems on a regional scale, but differing in their objectives and extents. We found that describing and locating cropping systems in space meets not only decision-making stakes but also a scientific stake that allows multi-simulations over large areas when models require cropping system information. Simulation models are indeed necessary when the study aims at estimating cropping system externalities. Then, the involved process determines the extent, and the model determines the support unit, unless socio-economic considerations prevail. In this case, as well as when no model is involved, it is often considerations related to stakeholders that determine extent and support unit choices. On a regional scale, the cropping system must be described by only a few variables whose selection depends on the study objective and the involved processes. Collecting cropping system information for all support units is often simplified by identifying determining factors of cropping systems. However, obtaining deterministic relations between easily accessible factors and cropping system variables is not always possible, and sometime accessing modalities of determining factors for all support units is also difficult.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_6,

from Leenhardt et al., Agron. Sustain. Dev. 30 (2010) 131–138. DOI: 10.1051/agro:2009002

We found that describing and locating cropping systems relied very much on expertise and detailed survey data. The development of land management practice monitoring would facilitate this description work.

Keywords Agricultural practices • Spatial distribution • Modelling • Cropping system • Scale change • Environmental impact

1 Introduction

On a regional scale, where agriculture interacts with many other activities, and where various farmers and various cropping systems interact together, many management problems are closely related to the nature, diversity and location of cropping systems. For example, to adjust the quality of cereal lots to millers' requirements, the cooperative needs to know the distribution of the performance, e.g. yield and protein content, of the collected crop over its collect basin (Le Bail, 2005). This results from the characteristics of the cropping systems such as preceding crop, sowing dates, varieties and fertilisation. For agricultural planning of a country or to assist marketing decisions for farmers and grain traders, it is also necessary to predict crop or forage yield on a national or regional basis (e.g. Lal et al., 1993; Rosenthal et al., 1998; Chipanshi et al., 1999; Donet, 1999; Faivre et al., 2000; Yun, 2003). Calculating water requirements for agriculture within the area served by a water provider requires knowing where, in terms of soil and weather conditions, irrigated cropping systems are located, and to distinguish whether these cropping systems are intensively irrigated or not (e.g. Sousa and Santos Pereira, 1999; Heinemann et al., 2002; Leenhardt et al., 2004). Similarly, an accurate knowledge of cropping systems is needed to estimate nitrogen or pesticide pollution of streams or groundwater from agricultural land (Beaujouan et al., 2001; Gomez and Ledoux, 2001; Louchart et al., 2001; Biarnès et Colin, 2006) or to evaluate the risk of occurrence of genetically-modified (GM) seeds in non-GM harvests (Colbach, 2008; Angevin et al., 2008). The existence of dispersal processes such as water runoff and pollen dispersal may require, in addition, knowing the exact and relative locations of cropping systems. It may also require knowing the location of semi-natural areas such as grass strips and roadsides because a cropping system in one location can reduce or increase the effect of a neighbouring cropping system. For instance, herbicide runoff from a field may be reduced by a grass strip; GM propagation from a GM field will be increased if fields of similar GM cropping systems are surrounding it.

This rapid overview shows that information regarding crops and agricultural practices is required for solving many agri-environmental management problems. Accounting for the strong links that can exist between technical operations, between successive crops, and between crops and technical operations, can considerably improve decision-making by evoking more sustainable management decisions. Describing and locating cropping systems in space thus meets a public decision-making need.

This outlook article aims at presenting how cropping systems can be described and located within large and/or heterogeneous areas including a great number of fields, and therefore likely to present a great variability of cropping systems. For this purpose, it is based on four groups of studies covering a wide range of agri-environmental problems and extents (Table 1). They all use the concept of the cropping system but developed the necessary simplifications to account for the spatial variability of cropping systems on a regional scale. First, we present the concept of the cropping system and its classical use on a regional scale. Then, we present the objectives for which it is necessary to describe and locate cropping systems, which lead us to specify when environmental process models are used. In a second section, we highlight some scale issues. The third section states the problem of the description of cropping systems, in particular the choice of the variables to use. The collection of these variables at the required resolution is the subject of the last section, which distinguishes the direct collection of these variables from an indirect way that involves identifying and collecting determining factors of the cropping systems.

2 Using the Concept of Cropping Systems on a Regional Scale

The concept of the cropping system used in this article was developed in the framework of traditional French agronomic research. It was precisely defined

Table 1 Short description of the four case studies on which the analysis of the article is mainly based, with their references and, in italics, related references

Case study	Objective and thematic of the study	References	Extent	Support unit
A	Effect of cropping systems on regional gene flow	Angevin et al., 2002 Colbach, 2008	Group of fields <10 km^2	Field 1 m^2— <50 ha
B	Impact of weed control practices on water quality in a small catchment	Biarnès and Colin, 2006 Biarnès et al., 2004 *Louchart et al., 2001*	Water catchment 75 km^2	Sub-catchment area 1–5 km^2
C	Estimating regional irrigation demand	Leenhardt et al., 2004 Maton, 2006 Maton et al., 2007a, b Clavel and Leenhardt, 2008	Irrigated perimeter 500 km^2	Not a priori defined (Maton et al., 2007a) or Intersection of a small agricultural region and a water management region ∼100 km^2 (Clavel and Leenhardt, 2008)
D	Simulating nitrate flow in the Seine basin	*Gomez and Ledoux, 2001* *Ledoux et al., 2007* Le Ber et al., 2006 Mignolet et al., 2001, 2004, 2007	River basin 95000 km^2	Small agricultural region ∼400 km^2

by Sebillotte (1974), for an area managed homogeneously, as "the crops, their succession order and the crop management systems associated with each crop". The cropping system was thus considered as a subsystem of the complex land use system, its typical spatial scale being the field plot. On such a scale, Meynard et al. (2001) insisted on the temporal coherence of the cropping system: the crop management system corresponds to a logical and coordinated succession of actions; the various technical choices made within a given cropping system are not independent. Later, Veldkamp et al. (2001) showed that agronomic research could also be relevant at the landscape level, and, on such scale, Benoît and Papy (1998) and Jouve (2006) noticed that crop successions and crop management systems did not result from a random process but were the result of logic and decision processes. These authors highlighted the spatial coherence of cropping systems.

Nevertheless, the concept of the cropping system is rarely emphasised on scales greater than the field. The main reason is probably that information related to cropping systems is lacking or difficult to collect when a great number of fields is concerned. Therefore, the description of agricultural activities and land use is most often very simplified on such a "regional"[1] scale. Simplification often leads to neglecting the concept of the cropping system. A first simplification consists of restricting the concept of the cropping system to its primary characteristic, i.e. the knowledge of the cultivated species, as noted by Jouve (2006). This schematisation is common in the remote-sensing approaches used to describe land use data for environmental issues (Martin et al., 2006). The description of agricultural land cover is usually restricted to soil occupancy, e.g. cultivated areas, forests, grassland and built-up areas; and to main crop groups such as annual versus permanent crops and, at the most, crop species such as maize and wheat (Verburg and Veldkamp, 2001). Details on crop management systems and crop successions are usually omitted. A second level of simplification consists of describing the crop management system as a fixed and uniform sequence of technical operations. This sequence can either consist of average practices

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[1] We use here the word "region" to refer to any area so large or heterogeneous that it includes a great number of fields impossible to survey. In this article, a "region" could be a small area of less than 10 km^2 with many small fields, as well as a very big river catchment (∼100 000 km^2).

(Moen et al., 1994) or correspond to recommended management guidelines (Yun, 2003). The spatial variability in crop management systems is often neglected and the same recommended or averaged practices are used for a large part or the whole spatial extent. The temporal coherence of the management system is also often limited: crop management systems are expressed as a fixed calendar of technical operations. This second level of schematisation is frequently adopted in optimisation studies which aim at rationalising crop acreage over a region to reach defined targets. Each crop or crop rotation is associated with a fixed sequence of technical operations which allows the calculation of various indicators such as input costs or externalities (de Juan et al., 1999; Nordblom et al., 2006; Reca et al., 2001; Stoorvogel, 1995).

However, Barson and Lesslie, (2004) and Leteinturier et al., (2006) note that detailing crop management systems and crop successions can be of importance in many environmental perspectives and Stomph et al. (1994) specify that a quantitative description of land use practices is necessary. This is why some studies, e.g. those reported in Table 1, use the concept of the cropping system to describe and locate agricultural activities over large areas.

3 Objectives of Describing and Locating Cropping Systems on a Regional Scale

This description of cropping systems may directly provide the solution to the management problem, or may only be a means to obtain this solution. For instance, to estimate the impact of land use on water resource use, the key is to know the distribution of irrigations during summer, i.e. to know the area cultivated with irrigated crops and the amount of water applied during the season (Leenhardt et al., 2004). However, most agri-environmental problems are relative to the impact of cropping systems on their environment. They deal with externalities of cropping systems, e.g. nitrate loss and pollen dispersal. Externalities are usually simulated by models using cropping system information as input. Depending on the physical processes involved, two modelling approaches exist. The first consists of spatially-explicit modelling to represent flows within the area under consideration (Dunning et al., 1995). The second consists of running a 1-D model such as a crop model at every point, or a sample of points, of the area (Hartkamp et al., 2004). For spatially-explicit models the exact location of cropping systems is required, while for 1D models only the relative area grown with each crop × management combination for each soil × weather condition is necessary. Both modelling approaches require high-resolution land use data (Verburg et al., 2002). For instance, to estimate water pollution by nitrate leaching, it is necessary to model the nitrogen use by crops, nitrogen transformation in the soil, and vertical and lateral water flows (e.g. Beaujouan et al., 2001; Gomez and Ledoux, 2001). Such a modelling approach needs input data on the crops sown and their location, their sowing dates, that determine the periods of crop growth and water and nutrient uptake, and the periods of fertiliser and water supply. Describing and locating cropping systems in space thus meets a scientific need. It makes it possible to use on a regional scale models developed on a local scale (Faivre et al., 2004; Leenhardt et al., 2006).

We can distinguish three different types of issues where cropping system information is needed. First, diagnostic studies for evaluating the impact of land use and land cover on the environment or on natural resources require data on existing or past land use, and therefore, on existing or past cropping system distribution. Second, scenario simulation approaches also evaluate impacts, but those of proposed prospective rather than actual situations. Therefore, the required data not only concerns the current distribution of land use but also options and constraints for modifications in the current practices. Since prospective situations are usually compared with the present or a former situation, scenario studies are often associated with diagnostic studies. The last type aims at identifying optimal land use for a given economic, environmental or multicriteria objective. Optimal land use, or even optimal cropping system distribution, is determined by iterative choice and evaluation algorithms and two kinds of data are therefore required. First, a set of cropping systems is necessary to initiate the run. This set can correspond to the current situation, but this is not compulsory. Second, a set of alternative cropping systems, or a range of cropping system components to be combined during evaluation, must be specified. As in scenario simulation, the search for optimal solutions belongs to a planning approach and aims at determining the spatial organisation of cropping systems to reach desirable objectives.

To summarise, describing and locating cropping systems is used for diagnostic or scenario studies, either because cropping systems are directly targeted or because cropping system information is required as input for an environmental model. Describing and locating cropping systems in space allows multi-simulations on a regional scale. Describing and locating cropping systems can also serve to define the base situation and cropping system basket from which an optimal land use can be calculated to satisfy given objective functions.

4 Scale Issues

Up to this point the word "scale" has been used in its colloquial sense. It covers, in fact, both the "extent", i.e. the area of interest of the study, and the "support", comprising a finite number of smaller areas, the "support units", on which information is collected. Support units can cover integrally or not the extent; the ratio "support"/"extent" is called the coverage (Bierkens et al., 2000; Faivre et al., 2004).

The analysis of various case studies, and particularly those reported in Table 1, shows that the extent is most often determined by the processes involved, but also by the context and objectives of the study. For instance, when the studied environmental problem requires accounting for physical flows conditioned by topographical features, e.g. modelling water flows for calculating diffuse pollution, the extent corresponds to a space with natural landscape delimitations, e.g. a stream or a river catchment area (cases B & D, Table 1). In addition, the resulting pollution can be more easily measured at the outlet of the catchment, which also justifies choosing as the extent a whole catchment. In other situations, the process involved is not the only factor that determines the extent of the study. It is particularly the case when the study is conducted with a socio-economic partner who often influences the choice of the extent. For instance, in a study dealing with harvest purity (case A), the extent was partly determined by the expected impact of the studied process, here the area of pollen dissemination. Similarly, Gomez and Ledoux (2001) studied nitrate pollution for the whole Seine basin, rather than in a small catchment area, because it was the area of the water manager who was both partner and recipient of the study (case D). In the regional irrigation demand study (case C), no physical dispersal or diffusion process was involved. Therefore, the extent was entirely determined by management considerations and corresponded to a space delimitated by installations, the irrigated area, which is the decision unit for water allocation decisions.

Once the extent is determined, it must be broken down into support units, where data on the cropping system is provided. The choice of support units often depends on the process involved and the model used to simulate it.

When dispersal and other spatial processes have to be considered, their nature and impact distance are often the key factor for choosing the support units. For example, when output flows from the cropping systems convey water and other elements that may influence surrounding crops (e.g. gene flow, herbicide runoff), it is important to simulate these lateral flows with spatially-explicit models, and to account for the spatial arrangement of crops and cropping systems. The field, which can be considered as a homogeneous unit regarding the crop and its management, naturally becomes the support unit for providing data on the cropping system, as well as the simulation unit (case A) Some models require as a simulation unit a sub-catchment (1–5 km^2), which then becomes the support unit of cropping system information. However, when the extent is very large relative to the size of fields or small sub-catchments, spatial processes may be negligible compared with other processes. For instance, Ledoux et al. (2007) neglected lateral flows between fields because they assumed that these lateral flows did not change the overall diffuse pollution. Neglecting spatial processes led to using a 1-D model to simulate "vertical" nitrate leaching. The only constraint for running these 1-D models on the studied area is to define spatial simulation units that are homogeneous combinations of the variables used as model input (generally variables describing the soil, the weather and the cropping system). These homogeneous soil-climate-cropping system combinations should naturally become the support units on which information regarding the cropping system has to be provided. This is, though, not always possible when extents are very large (case D) because the number of simulation units may be excessive regarding the computation time (Ledoux et al., 2007). Support units were then chosen a priori because they retrieved correctly the spatial differentiations of cropping systems (Mignolet et al., 2004), and the 1-D model was run on homogeneous simulation units that were then not explicitly located within each support unit. Sometimes, no process model is required and conditions the choice of support units. For example, in case C, the objective of the study is to estimate the regional irrigation demand. This target can be reached by simply aggregating the irrigation demand estimated for each support unit, whatever its size and nature. Thus, in this case, the support unit can be determined a posteriori, with regard to the kinds of indicators used to estimate the spatial distribution of agricultural practices (Maton et al., 2007a), or a priori, with regard to their significance for the various stakeholders concerned with the study (Clavel and Leenhardt, 2008).

To conclude, we note that when the objective of the studies is to estimate externalities of cropping systems a process model is required. Then the process determines the extent and the support unit. When socio-economic considerations prevail, as well as when no model is involved, it is often considerations related to stakeholders which condition extent and support unit choices.

5 Describing Cropping Systems by Relevant Variables

Describing a cropping system is a difficult task, even on the field scale. Should all technical operations of the crop management system be characterised? How detailed should this description be? This problem is even greater on a regional scale. Can cropping systems from two different sites be considered as similar? The elements that constitute a cropping system are the crop rotation and the various technical operations of the management system of each crop of the rotation. Which then are the elements to survey in order to describe the main representative cropping systems of a region?

The necessary cropping system variables must therefore be carefully chosen in relation to the study objective and the analysed processes to optimise the compromise between key variables and data availability. For instance, Colbach (2008), working on spatio-temporal gene flow in oilseed crops and volunteers (case A), showed that it is sufficient to include those cropping system components that significantly influence the two major stages for gene flow, i.e. plant survival and pollen dispersal, and that the remaining techniques such as fungicides or fertiliser, which mostly influence seed production, can be neglected. Similarly, in a study dealing with regional irrigation demand in a region dominated by irrigated maize (case C), Maton (2006) simplified the various cropping systems in irrigated maize to the 3 main elements influencing the total irrigation applied on a maize field, i.e. irrigation management, sowing practice and earliness choice. Such approaches reduce the amount of data to be collected to characterise the cropping systems without neglecting their internal consistency. They also help to simplify the simulation models used in the studies, rendering them thus more robust.

6 Collecting Data

Data collection for characterising and locating cropping systems on a regional scale is a real challenge. As a consequence, exhaustive and systematic collection of information about land management practices on the regional scale is only at its early stage, and is most of the time partial and descriptive, with no understanding of the links between technical interventions within crop management systems (BRS, 2006; Mignolet et al., 2007).

Two ways of collecting data to describe cropping systems are possible and not exclusive: the direct way, which aims at collecting information regarding the relevant elements of the cropping systems, and the indirect way, which consists of collecting information about indicators, or determining factors, of the cropping systems. These two ways, and the techniques used, are more or less adequate, depending on whether the cropping system data collection aims at describing existing or past cropping system distributions for elaborating a diagnosis, at building scenarios of cropping systems, or at seeking an optimal cropping system distribution.

6.1 Direct Collection

Collecting directly the variables that characterise the cropping systems concerns mainly diagnosis studies. Direct data collection by survey requires long and arduous work because of the great number of farms on a regional scale and is thus generally unrealistic (Biarnès et al., 2004). Three main alternative solutions exist. The first is to use remote-sensing techniques to estimate the spatial variability of specific technical interventions, such as sowing dates (Launay and Guérif, 2005). However, some interventions and technical choices are not detectable by remote-sensing, or with difficulty or at high cost. This is the case, for example, for irrigation application dates and amounts or for the choice of cultivar or earliness for a given crop. The second is to make the best use of existing regional databases (e.g. Agricultural census, Ter-Uti-LUCAS database) to provide knowledge on past cropping systems, mostly by using statistical analysis. For example, Mari and Le Ber (2005) developed data-mining techniques to estimate the spatial variability of past crop rotations from such databases (Le Ber et al., 2006). This solution is, however, limited by the nature of variables collected systematically in administrative databases, and by the length and cost of specific surveys that may be conducted to get variables not systematically surveyed by administrations. If, in France, the number of variables collected by the many surveys conducted by the Department of Economical and Statistical Studies (SCEES[2]) is huge, the nature and size of the sample used differ from one survey to another, rendering multivariate analyses difficult if not impossible. The last option is to use expert knowledge, as Mignolet et al. (2004) did, to associate a management system with a crop or a crop rotation (case D). However, this option requires long surveys when the extent is large and if the spatial variability is to be taken into account, and the quality of expertise decreases as the survey goes further into the past. Experts can, however, inform not only on past and/or recommended practices, but also provide innovative practices that can be of interest for scenario studies (Lançon et al., 2008).

6.2 Indirect Collection: Use of Determining Factors

Despite the reduced number of variables to be collected for describing a cropping system, these variables may not be included in available databases, or not at a sufficient resolution for characterising past cropping systems on all support units. An indirect way to determine and locate the cropping systems within the extent is to identify the factors involved in their spatial organisation, e.g. soil depth and water availability for choosing crop species or soil type for choosing tillage strategies. These "determining factors" of the cropping system are often easier to collect on the support units and are thus used as indicators of current or past cropping systems for diagnosis studies. For scenario studies, the use of determining factors seems compulsory since it is not possible to collect cropping system data for future or hypothetical context changes. Knowing which factors determine the cropping system and estimating the evolution of such factors through modelling or expertise contributes to estimating and proposing cropping systems for future or hypothetical conditions. For instance, when cropping systems are determined by farming system or farm structure (case A – Angevin et al., 2002 – and C – Maton et al., 2007b), it is possible to deduce the distribution of cropping systems within a region from economic scenario analyses that produce changes in farm-type distributions (Zimmermann et al., 2006). Similarly, when cropping system elements can be related to climatic indicators (e.g. irrigation amounts and dates – Leenhardt et al., 2004, or sowing dates – Maton et al., 2007a), the use of climate change scenarios (e.g. long-term emission scenarios developed by the Intergovernmental Panel on Climate Change – IPCC, 2007) can provide indications on the way cropping systems over a region may change. Determining factors are also used in optimisation studies: they represent the constraints that should be satisfied when calculating the optimal crop distribution for a given objective function. For example, Rounsevell et al. (2003) consider that various variables determine (or constrain) the choice of cropping systems: the availability of machinery and labour on the farm, the existence of a sugar factory and regional sugar beet quotas, the previous crops, the soil and the climate, the latter two variables conditioning the workable hours.

Two kinds of "determining factors" may be used: external or internal factors. "External" factors include the characteristics of the physical environment, e.g. soil, topography or weather conditions; of the fields, e.g. shape and size; of the farm, e.g. farming systems, equipment and manpower; or of the socio-economic and administrative environment:

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[2] Service Central d'Études Économiques et Statistiques

professional networks, extension services and municipality. While maps, remote-sensing shots, land registers or interpolation procedures provide geo-referencing for most factors related to the physical environment or field characteristics (Faivre et al., 2004), the last two groups of factors, those related to farms and the socio-economic environment, are available in various administrative databases which provide a location indication, most often the municipality the farm belongs to. For instance, Biarnès and Colin (2006) used the municipality to predict practices over the whole study extent since they found, by analysing field data samples, that weed control practices were well correlated with this factor (case B). In case A (Colbach, 2008; Angevin et al., 2002) the type and location of crop successions was considered as determined by the type of farm, defined by intensive vs. organic management, and by the farm and field sizes. Grid weather data were used by Maton et al.(2007a) to predict the spatial variability of sowing dates and by Leenhardt et al. (2004) to predict the spatial variability of irrigation dates (case C). Using an "external" determining factor consists of using a simple "If-then" allocation rule:

If [external indicator(s)]
then [cropping system (or element of it)]

However, sometimes no deterministic relation can be identified between a set of potential explanatory variables and the cropping systems or elements of them. This can be illustrated by Maton et al. (2007b), and Biarnès and Colin (2006). Maton et al. investigated determining factors of maize sowing and cultivar choice from geo-referenced databases (geographical information systems containing environmental characteristics of fields, spatially interpolated weather data series, or administrative databases containing farm characteristics collected from censuses). However, the variability of cropping systems could not be completely explained by these geo-referenced indicators. A combination of indicators led only to a probability of occurrence of a given cropping system. Similarly, (Biarnès and Colin, 2006) linked each municipality of the study extent to a given distribution of weed control practices.

The rule (1) becomes then a stochastic allocation rule:

If [external indicator(s)]
then [probabilities of cropping systems (or elements of them)]

An "internal" factor corresponds to an element of the cropping system on which the choice of the other elements of this cropping system depends. For instance, Maton et al. (2007b) showed that the choice of cultivar earliness was partly determined by the sowing date, while Colbach (2008) and Angevin et al. (2002) used the crop succession to determine crop management choices. Internal factors are, by definition, not geo-referenced, and must be indirectly related to external indicators in order to predict the cropping system location. A second-order rule is thus needed:

If [external indicator(s)]
then [element(s) E of the cropping system]

And

If [element(s) E of the cropping system]
then [cropping system]

The use of determining factors is therefore a way to overcome the difficulty of accessing data on cropping system variables. Identifying variables that can be used as determining factors of cropping systems can be done either by expertise from extension services or farm surveys, or by statistical analysis of databases. However, to specify cropping systems on all support units of the study extent, it is also necessary to be able to determine the modalities of these determining factor variables for all support units. Although determining factors are chosen among variables more easily accessible than cropping system variables, this can be problematic. For instance, in case A, cropping systems were recognised as determined by farm types, but the fields (support units) were not associated with a farm type. The solution was then to allocate randomly farm types to fields, while respecting certain spatial constraints such as isolation distances between crops.

To conclude, identifying determining factors of cropping systems can simplify the collection of cropping system information, but it is not always possible to obtain deterministic relations between easily accessible factors and elements of the cropping systems. In some cases, the access to the modalities of determining factors for all support units can also be difficult.

7 Conclusion

Sustainable development of regions and sustainable management of resources often require actions concerning land use. Sustainable solutions need to account for the coherence of technical choices within cropping systems and with their environment. However, very few agro-environmental studies account for both the coherence and the spatial variability of cropping systems. It was the objective of this article to review methods to describe and locate cropping systems within large and/or heterogeneous areas. This article focused particularly on 4 case studies differing in their objectives, but also their extent and support. Describing and locating cropping systems is required for diagnostic or scenario needs, where environmental models are often used to simulate cropping system impact. We noticed that extents and support units were constrained by the objective of the study and, if a model was required, by the process to be modelled. Choosing the extent, and the nature and size of support units is crucial because it conditions not only the precision of the location of cropping systems but also the precision of their description. Indeed, on a regional scale, it is not possible to represent all aspects of a cropping system. A simplification is necessary. The variables strictly necessary to describe the cropping systems must be chosen in accordance with the study objective and with the analysed processes in order to optimise the compromise between key variables and data availability. Collecting information to fill all support units with the modalities of such variables may be done using administrative surveys, remote sensing or expertise. However, it appears that, very often, such sources of information are not sufficient to fill all support units or to get all necessary cropping system variables. The use of determining factors, that are easily geo-referenced, is a way to overcome the difficulty of accessing data on cropping systems. However, it is not always possible to obtain deterministic relations between easily accessible factors and elements of the cropping systems, and sometimes, the access to the modalities of determining factors for all support units can also be difficult. A common feature of the methods presented here is their reliance on expertise or on detailed survey data. Expertise regarding agricultural practices is available in most countries from extension services. However, its reliability decreases when the size of the support unit or the time period increase. Furthermore, detailed surveys that are necessary either to describe cropping systems directly or to relate agricultural practices to their potential determining factors are rare and difficult to carry out for a single study. This calls for the development of adequate and routine land management practice monitoring.

Acknowledgements The authors are grateful to Drs. V. Souchère, C. Thenail and B. Nicoullaud, and to the anonymous reviewers for their valuable comments, recommendations and corrections. We would like to thank M. R. Lesslie and D. Wallach for editorial advice in English. This study was conducted within the framework of the Cropping System network of the Environment and Agronomy Department of the French National Institute for Agronomical research (INRA).

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Part II
Food Security

Nutritional Quality and Safety of Organic Food

Denis Lairon

Contents

Abstract Food security, nutritional quality and safety vary widely around the world. Reaching these three goals is one of the major challenges for the near future. Up to now, industrialized production methods have clearly shown severe limitations such as a worldwide contamination of the food chain and water by persistent pesticide residues, and reduced nutrient and flavor contents through low-cost intensive food production and/or processing. In line with several published literature reviews, the French Agency for Food Safety (AFSSA) performed under my coordination an up-to-date exhaustive and critical evaluation of the nutritional and sanitary quality of organic food. This review is based on the AFSSA report issued and recently published studies. The major points are: 1/ organic plant products contain more dry matter and minerals (Fe, Mg); and contain more anti-oxidant micronutrients such as phenols and salicylic acid, 2/ organic animal products contain more polyunsaturated fatty acids, 3/ data on carbohydrate, protein and vitamin levels are insufficiently documented, 4/ 94–100% of organic food does not contain any pesticide residues, 5/ organic vegetables contain far less nitrates, about 50% less; and 6/ organic cereals contain overall similar levels of mycotoxins as conventional ones. Thus, organic agricultural systems have already proved able to produce food with high quality standards. I propose also improvements of organic production to achieve sustainable food production for humans in the near future.

Keywords Sustainable agriculture • Organic agriculture • Human food • Nutrition • Food safety • Contaminants • Health

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_7,

from Lairon, Agron. Sustain. Dev. 30 (2010) 33–41. DOI: 10.1051/agro:2009019

1 Introduction

While mass foodstuff production was achieved in industrialized countries during the twentieth century, the limitations of such an intensive production system have been highlighted for decades by ecologists and numerous agronomists, nutritionists and medical doctors. Briefly, great concern has been caused by high energy and chemical inputs, worldwide contamination of the food chain and water by persistent pesticide residues and nitrates, and the reduced nutrient and flavor contents through low-cost and intensive food production and extensive milling or processing. Only recently has the combined awareness for environment protection, food safety and security and well-being markedly raised public concern and demand for ecologically grown staple foods (El-Hage Scialabba, 2007; Niggli et al., 2007). For developing countries, concern is also focused on the appropriate way to ensure present and future food security, the number of malnourished and undernourished people approaching one billion worldwide, with no decreasing trend for the coming decade (FAO, http://www.fao.org). Worldwide, emphasis is increasingly being put on the relationship between food, nutrition and health (WHO, 2004; WCRF, 2007).

In fact, the nutritional and toxicological value of food produced under methods of ecological agriculture has long been a matter of interest and debate. Despite the potential importance of this topic for human well-being, only a limited number of studies have been specifically carried out due to the past general lack of consideration of alternative and sustainable methods of food production. During the last few decades, several literature reviews have already been performed and published in this field (Schuphan, 1974; Finesilver et al., 1989; Lairon et al., 1984a; Woëse et al., 1997; Worthington, 1998; Food Standards Agency, 2000; Soil Association, 2001; Brandt and Mölgaard, 2001; Bourn and Prescott, 2002). Our AFSSA report was issued in 2003 (AFSSA, 2003) and some new reviews have recently been published (Magkos et al., 2006; Winter and Davis, 2006; Rembialkowska, 2007). In most cases, these reviews have used data from original studies or previous reviews without true consideration of the quality of the data. The conclusions derived can somewhat differ but they generally highlight some benefits from organic agriculture.

In 2001, the French Agency for Food Safety (Agence française de sécurité sanitaire des aliments (AFSSA), http://www.afssa.fr) aimed to perform an exhaustive and critical evaluation of the nutritional and sanitary quality of organic food. To this aim, an expert working group was set up under my coordination. We aimed to reach the highest quality standard during our evaluation. We thus defined inclusion as well as exclusion criteria for use of original publications. Briefly, selected papers should refer to well-defined and certified organic agricultural practices, and have necessary information on design and follow-up, valid measured parameters and appropriate sampling and statistical analyses. After more than two years of work involving about 50 experts from all specific areas including organic agriculture, a final consensus report was issued in the French language in 2003 (AFSSA, 2003). The present review paper is a summary of this report including some updating and some more personal suggestions.

In all circumstances, organic agriculture is first defined as by the European Union regulation (CCE/2092/91 and CE/1804/99) and secondly by the French regulation for animal productions. To summarize, the main characteristics of the organic agriculture production system are respect for the environment and animals, promotion of sustainable cropping methods, use of non-chemical fertilizers and pest/disease/weed control means, production of high-quality foodstuffs and no use of genetically modified (GM) crops.

The limit of such an evaluation is the insufficient number of studies published in this area. For some aspects, the available studies allow one to reveal some trend or conclusion. In some others, too limited information of sufficient quality hinders any sound assessment.

In the first part of this review, the nutritional value of organic food will be described comparatively with that of conventional food. This includes the dry matter contents of fruit and vegetables, macronutrients, minerals and vitamins in various staple foods, and phyto-microconstituants, especially antioxidants. In the second part, sanitary properties of organic foodstuffs will be reported. Contaminations by pathogenic microorganisms, phytochemical contaminants or mycotoxins, and nitrate levels are reported. In the Conclusion, the main data obtained are discussed in the context of sustainable agriculture development, with some specific suggestions for further improving food quality.

2 Nutritional Quality of Organic Foodstuffs

2.1 *Dry Matter Content*

The available data mostly refer to vegetables and fruit. For leafy vegetables as well as root vegetables and tubers, a trend for higher dry matter contents in organic foodstuffs has been found while no significant difference has been identified for fruit vegetables and fruit (Woëse et al., 1997; Bourn and Prescott, 2002; AFSSA, 2003).

2.2 *Macronutrient Contents*

Data regarding the levels of starch and carbohydrates in organic food are too limited to allow any conclusion.

For protein levels, it has been reported that organically-grown cereals, especially wheat, can have comparable protein levels with conventional ones (Shier et al., 1984) but generally have somewhat lower levels of protein than the conventional ones (Woëse et al., 1997). Nonetheless, it is noteworthy that the cultivars selected by organic farmers are mostly high-protein ones (e.g. for bread-making) and that optimized fertilization practices can maintain reasonably high protein levels. Moreover, a 25–30% increase in lysine has been reported in organic wheat (Wolfson and Shearer, 1981; Brandt et al., 2000). Comparative studies performed on hen eggs (Kouba et al., 2002) and raw cow's milk (Toledo et al., 2002) did not show any noticeable difference in protein levels.

For lipids, few studies have compared the total lipid content of beef, pork or chicken meat (Pastsshenko et al., 2000; Hansson et al., 2000; Hönikel, 1998; Fischer, 2001; Castellini et al., 2002). It is acknowledged that meat from cows and sheep contains less fat when animals are fed with grass rather than concentrate. In that line, a study conducted in Sweden showed that organically-bred cows have more lean meat than their conventional counterparts (Hansson et al., 2000). This was not found in pigs (Sundrum et al., 2000). More qualitatively, feeding cows with grass compared with concentrate led to a four-fold higher muscle content of linolenic acid, a recommended essential fatty acid of the n–3 series, with a concomitant decrease in oleic and linoleic acids (Nuernberg et al., 2002). Meat from organically-grown cows has more polyunsaturated fatty acids (Pastsshenko et al., 2000). Castellini et al. (2002) showed that chickens of the same strain raised under an organic husbandry system have meat containing two- to three-fold less abdominal fat with 2–3 times less fat in the filet and 1.8 times less fat in the leg. Moreover, the n–3 fatty acid content in the filet was significantly higher with no difference for saturated fatty acids.

The total milk fat was not overall different, while some studies highlighted the higher levels of polyunsaturated fatty acids (Lund, 1991). Clearly, cow diet is a determinant of tissue and milk fatty acid levels, grazing or ingesting silages modulating the levels of polyunsaturated fatty acids as well as trans-fatty acids and conjugated linoleic acid (Ferlay et al., 2006).

A single study has shown that organic virgin olive oil has a higher oleic acid level (Gutierrez et al., 1999).

2.3 *Mineral Contents*

The most important mineral elements are calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se) and iodine (I). Phosphorus (P) and sodium (Na) are generally found in sufficient quantity.

Fruit and vegetables. 22 scientific publications were considered in the AFSSA report (2003). Regarding fruit, and especially apples, it is noteworthy that the mineral composition is generally not noticeably altered by the production system. Regarding vegetables (potato, carrot, beetroot, lettuce, kale, leek, turnip, onion, celeriac and tomato), a trend has been observed for higher levels of iron and magnesium expressed on a fresh matter basis in organic foodstuffs, with no other marked change.

Cereals. From two long-term fertilization trials, it appears that the mineral composition (P, K, Ca, Mg, Mn, Zn, Fe, Cu and Cr) of cereals is not markedly affected by the cropping regime (Miller and Dema, 1958; Morel et al., 1984). Another study did not show any marked difference but a trend for higher levels of Ca, Cu and Zn in organic barley (Alföldi et al., 1996).

In a recent review (Rembialkowska, 2007), it was estimated that organic crops overall contain 21% more iron and 29% more magnesium than their conventional counterparts.

Animal products. Very limited information is available. For milk, an evaluation of three different studies did not allow the identification of a difference due to the husbandry system. The same conclusion was reached by Woëse et al. (1997) from four comparative studies performed on meat. Nevertheless, it has been clearly shown that chickens grown in open fields compared with housing have somewhat higher iron levels (Castellini et al., 2002).

2.4 Vitamin Contents

The number of studies dedicated to vitamin contents is limited to some fruits and vegetables and eggs. Regarding water-soluble vitamins, the most studied one has been Vitamin C (ascorbic acid), a key vitamin for which higher daily intakes are recommended. Studies performed on potato (Fischer and Richter, 1986; Kolbe et al., 1995), tomato (Pither and Hall, 1990; Caris-Veyrat et al., 2004), celeriac (Leclerc et al., 1991) and kale showed higher vitamin C levels in organically-grown products. In contrast, no difference was found during studies in leek, carrot or beetroot. A study on apple did not show any difference either (Weibel et al., 2000). Very sparse and inconclusive data have been published on vitamin B1 and B2 levels.

Fat-soluble vitamin and carotenoid contents have been the subjects of some studies. A higher vitamin E level in organic olive oil has been found in one study (Gutierrez et al., 1999). A review by Woëse et al. (1997) related 27 studies reporting on β-carotene levels in vegetables and no noticeable differences were found overall between organic and conventional foodstuffs. Brandt and Mölgaard (2001) reported a positive relationship between N-fertilization and β-carotene levels in carrots, while a recent study on organic vs. conventional tomatoes showed higher contents of β-carotene (Caris-Veyrat et al., 2004). Another report (Martin et al., 2002) has shown that a grass-rich regimen compared with a maize silage or concentrate generates milk with a higher vitamin E and β-carotene content.

2.5 Other Phytomicronutrients

Fruit and vegetables contain a large variety of microcompounds which are secondary metabolites in plants such as polyphenols, resveratrol and some non-provitaminic carotenoids. These compounds have increasingly been shown to have drastic regulatory effects at cellular level and are thus involved in prevention of certain diseases such as cancers, chronic inflammation and other pathologies. Some of them are phytoalexins which are produced in plants as a response to external stress such as fungal disease. While several factors can modulate their plant level such as cultivar, maturity, light or temperature, some studies have compared the levels of some of these phytomicrocompounds in fruit or vegetables depending on the cropping system. For phenols and polyphenols, a majority of studies showed higher levels in organic foodstuffs such as apple (Lucarini et al., 1999), peach (Carbonaro et al., 2002), pear (Carbonaro et al., 2002), potatoes (Hamouz et al., 1999), onion (Ren et al., 2001), tomato (Mitchell et al., 2007), pepper (Pérez-López et al., 2007), orange (Tarozzi et al., 2006) and olive oil (Gutierrez et al., 1999), while some others did not show any difference. It has been estimated in a recent review (Rembialkowska, 2007) that organic plant foods overall contain double the amount of phenolic compounds. One study reported higher levels of resveratrol in organic wines (Levite et al., 2000).

The median contents of salicylic acid in organic vegetable soups were significantly higher (117 vs. 20) than in the compared non-organic ones (Baxter et al., 2001). Organically-grown tomatoes also have a higher salicylic acid content than conventional ones (Rossi et al., 2008). It is noteworthy that salicylic acid is the active anti-inflammatory compound of aspirin.

While some better anti-oxidant and anti-proliferative positive effects on cancer cells have been observed with organic vs. conventional extracts (Tarozzi et al., 2006; Olsson et al., 2006) the effects of chronic diets in humans have yet to be fully investigated (Grinder-Pedersen et al., 2003).

Table 1 Key items of nutritional and sanitary value of organic compared with conventional food

Increased contents	Reduced contents	Comparable contents
Dry matter in vegetables	Pesticide residues in all food (mostly absent)	Mycotoxins in cereals & milk
Some minerals (iron, magnesium) in vegetables	Nitrates in vegetables	Most minerals in fruit, vegetables & cereals
Anti-oxidants in crops: Vitamin C (potatoes) Polyphenols in fruit & vegetables, Salicylic acid in vegetables		Beta-carotene in fruit & vegetables
Polyunsaturated fatty acids in meat and milk	Saturated fatty acids in meat	
Most nutrients in wholegrain organic cereals and derivatives	Protein content in grains	

Table 1 highlights the key items of nutritional and sanitary value of organic compared with conventional food.

3 Sanitary Assessment of Organic Foodstuffs

Risk assessment is a scientific approach aiming at identifying known hazards and related risks. Contaminations by bacteria, viruses, worms, mycotoxins and agro-chemicals are mainly involved. Not all aspects have yet been comparatively studied such as hazards due to viruses or worms. We will therefore take some relevant examples on other aspects based on more reliable information.

3.1 Pathogenic Microorganisms

Plant products. Concern has been raised by the EU Scientific Committee on Food on the possible contamination of fruit and vegetables by sewage sludges, animal manures or irrigation waters (SCF, 2002). Indeed, they can provide pathogenic microorganisms such as bacteria pathogenic to humans (Strauch, 1991), *Listeria monocytogenes* (Van Renterghem et al., 1991) or *Salmonella sp*. (Warnick et al., 2001). In fact, sewage sludges are not allowed for use in organic agriculture and fresh manures are not used as such for fertilization but composted for variable periods of time. It has been shown that the aerobic composting process, including a high temperature phase, has the capability of markedly reducing or fully eliminating the pathogenic microorganisms initially present such as *Salmonella Enteritidis* or *E. coli* (Lung et al., 2001; Droffner and Brinton, 1995; Vuorinen and Saharinen, 1997; Tiquia et al., 1998). This could be less efficient in eliminating *Clostridium botulinum* (Bohnel and Lube, 2000). In addition, when added to the soil, the exogenous bacteria are quickly eliminated due to unfavorable conditions (Van Renterghem et al., 1991; Dowe et al., 1997). In conclusion, it appears that the systematic use of aerobic composting is a suitable way to maximize the hygienic properties of organic fertilizers, and thus to avoid significant contamination of organic foodstuffs by pathogenic microorganisms.

Animal products. A survey conducted in Austria (Zangerl et al., 2000) evaluated the degree of bacterial contamination of dairy products using two indicators (*E. coli* and *S. aureus*) and did not find different levels in organic or conventional foodstuffs. Another survey conducted in France (Echevarria, 2001) in 1997–99 in four different regions also found comparable levels for total bacteria count or butyric microorganisms in milks produced with the two husbandry systems. In fact, the limited use of silage in organic husbandry could be beneficial for reducing the possible contamination of ruminant feed by *Listeria monocytogene E. coli* O157*s* (Herriott et al., 1998). In contrast, a Danish study reported that about 100% of poultry samples were contaminated by *Campylobacter* sp in organic farms, whereas 36–49% of samples in conventional farms were (Heuer et al., 2001).

To summarize, there is no convincing information indicating that organic foodstuffs can be differently contaminated than conventional ones. This is likely the result of efficient preventive measures. Enteric viruses from five families (*Picornaviridae, Caliciviridae, Astroviridae, Reoviridae* and *Adenoviridae*) are known to be harmful to humans but there is a lack of comparative studies on this aspect.

3.2 Phytochemical Contaminants

The banned use of toxic chemical pesticides, fungicides and herbicides in organic agriculture systems is clearly a gold standard in terms of protection of land workers' health and environment biodiversity and well-being. Anyway, the question has repeatedly been raised of the level of contamination of organic foodstuffs by environmental pollution. Several surveys have been dedicated to this matter. The SETRABIO survey (SETRABIO, 2000) was conducted in France in 1993–99 on 15,772 samples of raw or processed foodstuffs, mostly cereals. 94% of organic samples were devoid of any contaminant residue while 3.3% only contained levels clearly above the detection level but far less than legal maximum levels. During the years, a trend has been observed for diminishing contamination levels (i.e. 1.7% in 1998–99 vs. 4.4% in 1993–97). The French DGAL/COOPAGRI/ESMISAB survey (2001) was then conducted in 1999–2001 on 1500 samples. No residue of 78 potential phytochemical contaminants was detected.

Another study performed on vegetables and strawberries in Sweden did not show any contamination of organic ones, while 17–50% of conventional ones contained residues (Bourn and Prescott, 2002). Results from the monitoring of pesticide residues in fruit and vegetables on the Danish market in 2000–01 found that only 2.8% of organic samples were contaminated by pesticide residues, all being below the MRL (Poulsen and Andersen, 2003). A recent survey conducted in Italy in the 2002–2005 period on 3500 samples of food of plant origin concluded that the vast majority (97.4%) of organic farming products do not contain detectable pesticide residues (Tasiopoulou et al., 2007).

In contrast, it is known that a large proportion of usual foodstuffs are contaminated by phytochemical residues. This has clearly been shown by the recent reports by the EU DG SANCO for 2001 and 2005. The 2005 report (DG SANCO, 2007) was based on 62 500 samples collected in EU member states and analyzed for 706 chemicals. 41% of samples were shown to be contaminated, with 4.7% of samples with levels above the legal maximum levels (MRL).

In fact, all these phytochemicals have been fully or permanently authorized for use and residue levels considered harmless have been set up on the basis of available toxicity data. Because these molecules have a high toxic capacity (including mutagenesis and carcinogenesis) and the long-term effects of chronic low-dose ingestion are not known in humans, the consumer and scientific concern about possible health damage is real. The EU REACH project is aimed at better evaluating their toxicity and deleting the most risky ones. In that context, organic products clearly show a real advantage.

It is worthwhile mentioning that some natural extracts are used in organic agriculture for pest and disease control such as pyrethrins, rotenone, copper salts and sulfur. The first two are quickly degraded in the fields and no contaminations of foodstuffs have been observed (Moore et al., 2000). Regarding copper and sulfur, their use is limited to surface spraying and copper amounts have been progressively reduced. Contamination levels have not been investigated.

3.3 Mycotoxins

Mycotoxins are a large family of toxic molecules synthetized by molds developing on plants such as *Aspergillus*, *Penicillium* and *Fusarium*. Most are highly toxic and heat-resistant and can be transferred along the alimentary chain from plants to animals then human food. The most recognized in terms of public health hazard are aflatoxins, Ochratoxin A (OTA), Fumonisins, Deoxynivalenol (DON), Patulin and Zearalenone. Overall, they display strong harmful effects such as immunotoxicity, teratogenesis, embryotoxicity, nephrotoxicity and liver cancerogenesis. They all have very low legal maximum levels in food (0.1–2 ppb/kg body weight).

Given the lack of use of chemicals (including chemical fungicides) in organic production, the question has been raised of a possible high level of contamination of organic productions. Several surveys have been conducted to test this hypothesis. A study in Germany was conducted on cereals in 1997–98 (Birzele et al., 2000), indicating that most organic samples were contaminated by DON and a minority by OTA, but that conventional samples were comparably contaminated. A French study searched for contamination of cereals by several mycotoxins and observed that conventional ones were frequently contaminated at low levels while organic ones were less frequently contaminated but at high levels in a few cases (Malmauret et al., 2002).

Wheat contamination has also been tested in a controlled DOC trial in Switzerland with mean DON levels of 74 ppb in organic and 109 ppb in conventional wheat (Kuhn, 1999). A study in Denmark (Jörgensen et al., 1996) showed a trend for higher mean OTA levels in organic cereals but with the highest levels recorded in conventional ones. A recent study showed that organic wheat was less contaminated by *Fusarium* and contained less ZEN and DON than conventional wheat: when fed to pigs, the bile samples from organically-fed pigs contained lower concentrations of ZEN (Schneweis et al., 2005).

Some other studies have been conducted on wheat flour, providing comparable or lower levels of mycotoxin contamination for organic flour. Finally, processed cereal products such as bread, muesli and biscuits have been tested (Parent-Massin et al., 2002). Overall, almost half of the organic products were found to be contaminated to variable degrees but no comparison was made with conventional ones. When a comparison was made, lower levels of contamination were found in organic ones (Usleber et al., 2000). No marked differences in OTA levels were found in cereal derivatives in Italy (Biffi et al., 2004) or in cereal-based baby food (Beretta et al., 2002). Two studies have observed lower levels of aflatoxin in organic milks compared with conventional ones (Gravert et al., 1989; Frank Hansen, 1990), whereas another one found some high levels in organic milk (Ghidini et al., 2005). Organic beers collected in Belgium during 2003–2004 were more frequently OTA-contaminated than their conventional counterparts but this difference was not found in 2005 (Anselme et al., 2006). Contamination of beers by DON was marginal.

In conclusion, contamination of foodstuffs, especially cereals, is widespread but at a low level and an organic or conventional mode of production do not lead to overall noticeable differences. In fact, the preventive measures used in organic systems, despite the non-use of fungicides, appears generally able to maintain contamination at a low level.

3.4 Nitrates

Nitrates are a matter of concern for public health due to their easy transformation into nitrites. Nitrites are highly reactive molecules capable of i/ competing with oxygen in blood circulation for binding to hemoglobin, thus leading to methemoglobinemia and possible anoxia and, ii/ binding to secondary amines to generate nitrosamines which are among the most powerful natural cancer-promoting moities. For that reason, maximum daily intakes for nitrates (3.7 mg/kg body weight) and nitrites (0.07 mg/kg body weight) have been set up by the FAO/OMS JECFA, along with a maximum nitrate level in drinking water (50 mg/L).

In the human diet, about 80% of nitrates are provided by vegetables, while nitrate levels in fruits, cereals and legumes are very low (French Inventaire National de la Qualité Alimentaire, 1982; Stopes et al., 1988; Cornée et al., 1992). Animal products contain very low levels of nitrates while processed meat can contain added nitrites as a preservative.

Nitrates are naturally present in plants; they are absorbed through the roots and further used for amino-acid synthesis. They can accumulate in plant tissues, especially in vegetables. Several comparative studies have been performed on nitrate levels in vegetables.

At the level of a retail shop, we performed a study on five vegetables in spring and observed significantly lower nitrate contents (–28 to –85%) in organic potato, leek, turnip and salad but not in organic kale (Lairon et al., 1982). A comparable study performed in Austria on 17 vegetables found lower nitrate contents (–40% to –86%) in organic ones except in spinach (Rauter and Wolkerstorfer, 1982). In Germany, a comparison on carrots showed 61% less nitrates in organic ones (Pommer and Lepschy, 1985).

At farm level, by comparing designated crops on matched farms, three studies provided interesting data. We performed one in Provence (Lairon et al., 1985) and found in the organically-grown samples –39% nitrates in lettuce, –46% in potato, –22% in carrot and a higher content in one sample for leek. In Switzerland, organic lettuces grown over two years contained 2.5 times less nitrates than their conventional counterparts in May–June, 1.2 times less in October and comparable high levels in November (Temperli et al., 1982; Vogtmann et al., 1984). In contrast, two other studies performed on tomato in Israel (Basker, 1992) and carrot in Norway (Hogstad et al., 1997) did not show noticeable differences.

Fertilization trials have also been designed to compare the effects of fertilization regimes on nitrate contents in vegetables. Overall, composts compared with chemical fertilizers lead to lower nitrate accumulations in most vegetables such as lettuce, potato, carrot, turnip, leek, beetroot and spinach (Lairon et al., 1984a, 1984b; Mäder et al., 1993) while an absence of difference can also be found on a few occasions (Vogtmann et al., 1984). Nitrogen-rich organic fertilizers can also generate lower nitrate contents, but when mineralization conditions are very favorable they can also lead to high nitrate accumulations (Lairon et al., 1985; Termine et al., 1987). Finally, it is noteworthy that some natural nitrogen-rich fertilizers such as Chili sodium nitrate can stimulate nitrate accumulation in sensitive vegetables (Lairon et al., 1984b). Indeed, it is clear that the level of nitrate in vegetables results from nitrogen availability for roots, temperature, light exposure, and cultivars and species. The use of organic fertilization with slowly or moderately available nitrogen (especially composts) is key to explaining the generally observed lower nitrate accumulation in organic vegetables.

From the above data, it appears that depending on seasons, organic vegetables can overall contain at least 30–50% less nitrates than conventional ones. Because the habitual average level of nitrate intake is in the range 120–280 mg/d (French Inventaire National de la Qualité Alimentaire, 1982; Cornée et al., 1992; Stopes et al., 1988) and close to the maximum daily dose defined by the FAO/OMS JECFA (i.e. 220 mg/60 kg person), and a marked increase in vegetable and fruit consumption is widely recommended (WHO, 2004; PNNS, 2001), organic vegetables can make this recommended increase safer regarding the nitrate issue.

4 Conclusion

The present review based on available scientific literature highlights that organic plant products tend to have more dry matter, some minerals (Fe, Mg) and anti-oxidant micronutrients (phenols, resveratrol) while animal organic products have more polyunsaturated fatty acids. Regarding safety issues, the vast majority (94–100%) of organic food does not contain any pesticide residues, organic vegetables contain markedly less nitrates (about half) and organic cereals contain overall comparable levels of mycotoxins with conventional ones. The conclusions of this new review are in line with those of most reviews published on this matter.

This critical literature review indicates that organic agriculture, as developed until now, has the potential to produce high-quality products with some relevant improvements in terms of contents of anti-oxidant phytomicronutrients, nitrate accumulation in vegetables and toxic phytochemical residue levels.

After decades of smooth increase in organic production, a sharp rise in consumer demand and producer awareness is occurring now. The nutritional and toxicological data I reported here should encourage such a new trend. Indeed, I think that organic agricultural systems, recently recognized as highly efficient and sustainable ones (El-Hage Scialabba, 2007), are now facing the challenge of turning from a "niche" into a potential long-term worldwide impact. This implies a global development strategy for a sustainable organic food chain as summarized in Table 2. To successfully

Table 2 Key aspects of a sustainable organic food chain

Agricultural management	Food production	Food access and security	Food quality	Consumer attitude
Soil fertility management	Reliable certification	Fair trade	High sensory value	Confidence
Preventive crop protection	Sustained optimal yields	Local food access	High nutrient content	Appropriate awareness and demand
Animal welfare			Controlled pathogens	
Environment and biodiversity protection	Low inputs	Optimized supply chain	No pesticide residues	Health protection
Farmer well-being	Sufficient income	Affordable retail price		Sustained well-being

achieve this goal new and significant support for the development of these sustainable systems should be raised. For instance, raising new cultivars and crops suited to low-input systems and more resistant to diseases, along with optimized nutrition value, is one of the biggest challenges for future sustainable agriculture worldwide. This may be performed in the context of sustained biodiversity rather than GM productions, which are not allowed in organic/ecological farming systems. Regarding nutritional aspects, I suggest that several research lines are developed in the near future to improve sustainable food production systems such as increasing the levels of dry matter in foodstuffs to optimize nutrient density and intakes, increasing the levels of recommended n–3 fatty acids and lower saturated ones, increasing levels of limiting minerals, vitamins and anti-oxidants, improving the taste and flavor of fruit and vegetables to stimulate awareness, developing the use of wholemeal/partly-refined cereal flours rich in fibers and nutrients and the sourdough fermentation process for optimized nutritional efficiency, further limiting accumulation of nitrates in vegetables and mycotoxins in cereal products, and improving producers', consumers' and stakeholders' knowledge in the food production-diet-health chain.

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Minerals in Plant Food: Effect of Agricultural Practices and Role in Human Health

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Contents

Abstract Interest in nutrient absorption and accumulation is derived from the need to increase crop productivity by better nutrition and also to improve the nutritional quality of plants as foods and feeds. This review focuses on contrasting data on the importance for human health of food mineral nutrients (Ca, Mg, K, Na and P) and also the trace elements considered essential or beneficial for human health (Cr, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn). In addition, environmental stresses such as salinity, drought, extreme temperatures and light conditions that affect mineral content were revised in the light that the effect of these factors depends on the species or cultivar, and the specific plant organ, as well as the intensity and duration of the stress. Differences between inorganic and organic fertilisation practices on the mineral levels were also analysed to evaluate the influence of external factors on the quality of plant-based foods.

Keywords Environmental stress • Human health • Mineral fertilisation • Mineral nutrition

1 Introduction

One of the most important challenges for agriculture, besides enhancing food production, is to provide almost all the essential minerals and organic nutrients to humans for maintenance of health and proper organ function. Humans need more than 22 mineral elements; some of them are required in large amounts, but others, such as Fe, Zn, Cu, I and Se, are required in trace amounts because higher concentrations can be harmful (Welch and Graham et al., 2004; Grusak

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_8,

from Martínez-Ballesta et al., Agron. Sustain. Dev. 30 (2010) 295–309. DOI: 10.1051/agro:2009022

and Cakmak, 2005). Although vegetables constitute the main source of minerals in the human diet, crops do not always contain sufficient amounts of these essential nutrients to meet dietary requirements (Welch et al., 1997). Elements that might enhance growth or that have a function in some plants (not in all plants) are referred to as beneficial elements. Concerning mineral nutrients, deficiencies, including those of Ca, Zn, Se, Fe and I, are almost certainly impairing the health and productivity of a large number of people in the developing world, especially poor women, infants and children (Graham et al., 2001). However, an excessive intake of minerals may also have a deleterious effect on the systemic physiology, that has led researchers in the last few years to acquire accurate data on the minimum requirements and toxic dosages of the minerals present in food. The level of minerals in vegetables depends on a number of factors including genetic properties of the crop species, climatic conditions, soil characteristics and the degree of maturity of the plant at the moment of harvesting.

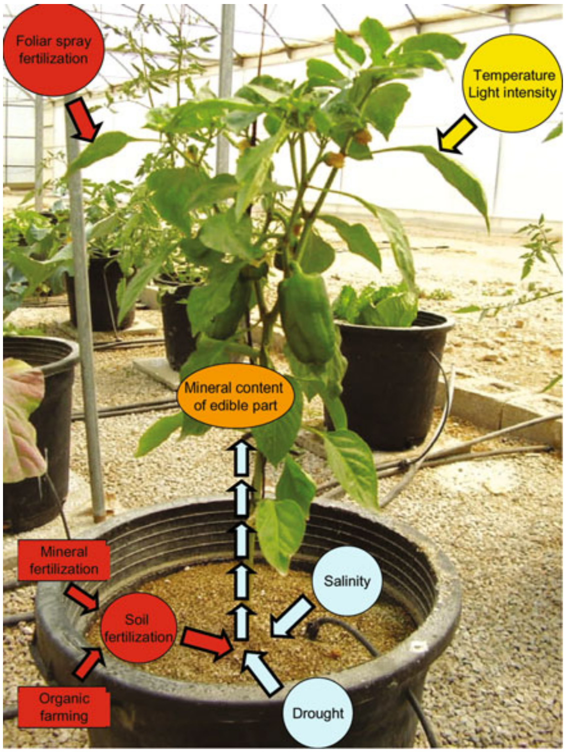

Fig. 1 Agricultural practices and environmental stresses affecting mineral composition in vegetables and fruits

Consideration of the environmental consequences and soil fertility practices are an essential component of the research in plant nutrition. Thus, some plant nutrients, such as potassium and sodium, are involved in plant responses to salt and water stress. Also, recommendations for amounts and application of fertilisers are continually modified to optimise the quality of the food production (Fig. 1).

2 Minerals in Foods of Plant Origin: Their Role in Human Health

Before attempting to modify the nutritional components in plants destined for human foods, careful consideration must be made in selection of minerals, their efficacy, and whether low or high dietary intake could have unintended negative health consequences. For selected mineral targets, the clinical and epidemiological evidence clearly plays a significant role in maintenance of optimal health, and they are limited in the diet worldwide (Lachance, 1998). In the following, we summarise the effect that essential or beneficial mineral nutrients have on human health.

2.1 Calcium

The concentration of calcium (Ca) in foods of plant origin shows a wide range of variation. The lower values belong to apples (*Malus domestica*), green pepper (*Capsicum annuum*) and potatoes (*Solanum tuberosum*) (<8.7 mg/100 g) and higher values are present in broccoli (*Brassica oleracea* L. var.italica) (100 mg/100 g) and spinach (*Spinacia oleracea*) (600 mg/100 g). Data on the mineral content of foods are important and should be considered when recommending the daily intake of minerals, as the Recommended Daily Allowance (RDA) for these nutrients is set out in the wide range of 800–1300 mg/day (http://www.anyvitamins.com/rda.htm). Calcium is an essential mineral for human health, participating in the biological functions of several tissues (musculoskeletal, nervous and cardiac system, bones and teeth, and parathyroid gland). In addition, Ca may act as a cofactor in enzyme reactions (fatty acid oxidation, mitochondrial carrier for ATP, etc.) and it is involved in the maintenance of the mineral homeostasis and physiological performance in general (Theobald, 2005; Huskisson et al., 2007; Morgan, 2008; Williams, 2008). Recent reports showed the unequivocal role of Ca as a second messenger (Morgan, 2008). With respect to disease prevention, Ca intake moderately reduces the risk of colon cancer (Pele et al., 2007; Peters et al., 2004). An increase in Ca intake during pregnancy is recommended to prevent risk of pre-eclampsia (Peters et al., 2004). Several studies have shown an association between suboptimal Ca intake and osteoporosis, hypercholesterolemia and high blood pressure (Unal et al., 2007). Although Ca levels undergo homeostatic controls to avoid an excessive accumulation in blood or tissues, there are a number of conditions that result in an excess of Ca within the body because of a failure in the control mechanisms: hypercalcaemia may occur as a result of either increased mobilisation of Ca from bone, or increased tubular reabsorption or decreased glomerular filtration in the kidneys, and less frequently, as the result of an increase in the dietary intake (Theobald, 2005).

2.2 Magnesium

Magnesium (Mg) has a strong presence in vegetable foods and also shows a critical role in the maintenance of human health through the diet. Vegetables and fruits contain, in general, Mg^{2+} in the range of 5.5–191 mg/100 g fresh weight; and the recommended daily intake is 200–400 mg (http://www.anyvitamins.com/rda.htm). This essential mineral acts as a Ca antagonist on vascular smooth muscle tone and on post-receptor insulin signalling. It has also been related to energy metabolism, release of neurotransmitters and endothelial cell functions (Bo and Pisu, 2008). In addition, Mg participates with muscle and nerve excitability, as a cofactor of up to 300 enzymes (Huskisson et al., 2007). Magnesium deficiency is related to ageing and age-related disorders, mainly as a consequence of deficient intake in the diet (Durlach et al., 1998; Killilea and Maier, 2008). Recent findings showed that an increase in the intake of this mineral helps to protect people from the incidence of chronic diseases such as diabetes, metabolic syndrome, hypertension and several cardiovascular conditions (Bo and Pisu, 2008), where a low-Mg diet may contribute to insulin resistance, especially when this deficiency is combined with a high-fructose diet. Moreover, reduced Mg intake is linked to inflammatory response as a result of modulation of the intracellular-Ca concentration (Ahokas et al., 2005; Rayssiguier et al., 2006). Magnesium toxic effects are not frequent, the most common side effects of an excessive intake of this mineral being headache, nausea, hypotension and unspecific bone and abdominal pain (Guerrero-Romero and Rodríguez-Morán, 2005).

2.3 Potassium

Foods of plant origin have potassium (K) contents of 20 to 730 mg/100 g fresh weight, although some plants such as 'Idaho' potatoes (*S. tuberosum*), banana (*Musa* spp.) and avocado (*Persea americana*) may all present high K contents (>700 mg/100 g fresh weight). Seeds and nuts are rich in K, showing values significantly higher than those mentioned above, up to 2240 mg/100 g. The recommended intake for this mineral is 3500 mg per day (http://www.anyvitamins.com/rda.htm). Potassium plays a role in the maintenance of the balance of the physical fluid system and assisting nerve functions through its role in the transmittance of nerve impulses. It is also related to heart activity muscle contraction (Rosenthal and Gilly, 2003; Schwarz and Bauer, 2004; Ko et al., 2008; Lambert et al., 2008; Sobotka et al., 2008). However, K requirements are also dependent on the physiological or pathological moment. A deficiency may result in fatigue, cramping legs, muscle weakness, slow reflexes, acne, dry skin, mood changes and irregular heartbeat. Moreover, a reduced level of K produces alkalosis, which makes the kidney less able to retain this mineral. Excessive K can be toxic systemically when associated with hyperkaelemia in a catabolic state accompanied by oliguria (secondary to kidney failure) (Sobotka et al., 2008).

2.4 Sodium

Raw vegetables and fruit juices contain relatively low levels of sodium (Na) in the range of 2.28 to 94.0 mg/100 g and from 0.04 to 277 mg/100 g, respectively (Szefer and Grembecka, 2007). The role of Na in human physiology is related to the maintenance of the balance of physiological fluids (blood pressure, kidney function, nerve and muscle functions) (Sobotka et al., 2008; Hall, 2003; Hall et al., 1999; French and Zamponi, 2005). The recommended daily intake for Na is 2400 mg (http://www.anyvitamins.com/rda.htm). A deficiency is rare, but it can happen in cases of diarrhoea, vomiting or excessive sweating, and a shortage may lead to nausea, dizziness, poor concentration and muscle weakness, etc. (Smith et al., 2000; Soupart and Decaux, 1996). Excessive Na may be due to an increase in absorption or a secondary condition to kidney alteration, causing high blood pressure and neurological complications (Hall, 2003; Hall et al., 1999; Agrawal et al., 2008; Kahn, 2008). Excessive long-term use of Na may also cause a secondary loss of Ca.

2.5 Phosphorus

Phosphorus (P) is present in vegetables in the range of 16.2–437 mg/100 g. The lowest content of P is shown in fruits, which are in the range 9.9–94.3 mg/100 g (Szefer and Grembecka, 2007). The phosphorus daily recommended intake is 800–1300 mg (http://www.anyvitamins.com/rda.htm). Phosphate (PO_4^{3-}) is required to produce ATP, GTP and CP as energetic substances and to regulate the activity of a number of proteins by means of phosphorylation reactions (Sobotka et al., 2008). Phosphorus is closely related to Ca homeostasis and also related to bone and teeth formation and the majority of the metabolic actions in the body, including kidney functioning, cell growth and the contraction of the heart muscle (Theobald, 2005; Szefer and Grembecka, 2007; Renkema et al., 2008). Deficiency of this element is unusual but symptoms are described as painful bones, irregular breathing, fatigue, anxiety, numbness, skin sensitivity and changes in body weight. If Ca supply is also deficient, then the condition may become severe because of increased risks of high blood pressure and bowel cancer. Ingesting dosages of P exceeding 3–4 g/day may be harmful as it can interfere with Ca absorption (Ghosh and Joshi, 2008; Moe, 2008).

2.6 Chromium

The concentration of chromium (Cr) generally ranges from 4×10^{-5} to 6×10^{-3} mg/100 g in vegetables and 0.005 to 0.018 mg/100 g in fruits (Szefer and Grembecka, 2007). A RDA for Cr is not well defined, but it is considered to be between 25–35 μg/day, fruits and vegetables being the major dietary contributors of Cr intake (http://www.anyvitamins.com/rda.htm). Because of its ‘micronutrient’ characteristics, it is difficult to differentiate its content in foods from improper food contaminations (Lukaski, 2004). It is well accepted that Cr is essential for normal blood glucose and lipid metabolism and an insulin-coadjuvant (Huskisson et al., 2007; Lukaski, 2004; Shenkin, 2008). Other biochemical actions for Cr such as involvement in gene expression, energy production, lipoprotein or lipid synthesis and metabolism regulation have been also described (Shenkin, 2008). Deficiencies in Cr are accompanied by glucose intolerance, weight loss and peripheral neuropathy (Shenkin, 2008). Moreover, low Cr levels may increase the risk of cardiovascular diseases (Thomas and Gropper, 1996). Chromium is not easily absorbed and shows low levels in the organism, explaining the absence of data on its toxicity. However, high doses of Cr have been related to chromosomal damage, alterations in the kidney and liver, and metallic-mineral disorders (Guerrero-Romero and Rodríguez-Morán, 2005).

2.7 Cobalt

There are not many data on levels of cobalt (Co) in foods of plant origin in the scientific literature. The available data showed low levels of this micronutrient, often under 0.001 mg/100 g, with the lowest levels observed in vegetables (Szefer and Grembecka, 2007). The RDA for Co has been defined at around 300 micrograms (http://www.anyvitamins.com/rda.htm). Cobalt is required in the haematopoiesis of red blood cells and in preventing anaemia (Narasinga Rao, 2003). Its function is closely related to the physiological role of vitamin B12 in the production and maintenance of red blood cells. Moreover, Co stimulates appetite, and promotes growth and energy release (Kräutler, 2005; Mertz, 1981). Excessive intake of Co may damage the heart muscles, elevate the haemoglobin concentration, cause congestive heart failure and may cause damage to the thyroid gland, reducing its activity (Barceloux et al., 1999).

2.8 Copper

Low levels of copper (Cu) have been described in vegetables, ranging from 0.004 to 0.24 mg/100 g, except legumes, that can be up to 0.5 mg/100 g. Fruits contain small amounts of Cu, ranging from 0.01 to 0.24 mg/100 g (Szefer and Grembecka, 2007). The RDA of Cu ranges between 1.0 and 1.6 mg per day (http://www.anyvitamins.com/rda.htm). Copper primary functions are related to enzyme function including Phase-I detoxifying enzymes (i.e., the cytochrome C oxidase family of enzymes) (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008). In addition, Cu is also necessary for the development of connective tissue and nerve coverings (myelin sheath) (Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008) and also participates in the Fe metabolism (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005). Copper may be accumulated in the adult body (liver and brain) up to a limit of 80 mg (Guerrero-Romero and Rodríguez-Morán, 2005), supporting deficient dietary intake, without inducing clinical symptoms of toxicity for a short period of time. Cu deficiency is not frequent in humans, although it can cause several haematological symptoms such as normocytic, hypochromic anaemia, leucopenia and neuropenia, and skeletal disturbances (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005). Toxic levels of Cu have been related to liver damage in chronic intoxication and gastrointestinal effects with cramps, nausea, diarrhoea and vomiting in acute episodes (Guerrero-Romero and Rodríguez-Morán, 2005).

2.9 Iron

Iron (Fe) contents in vegetables and fruits are low, varying from 0.13 to 3.01 mg/100 g. The iron in foods of plant origin is mostly present in the form of insoluble complexes of Fe^{3+} with phytic acid, phosphates, oxalates and carbonates. However, the bioavailability of the Fe present in foods is less than 8%. Nuts and cocoa powder may be a good source of Fe (16.1 and 25.8 mg/100 g, respectively; Szefer and Grembecka, 2007; http://www.anyvitamins.com/rda.htm). The recommended intake of iron is 8–18 mg per day (http://www.anyvitamins.com/rda.htm). The major function of Fe is related to the synthesis of haemoglobin and myoglobin (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008). It is also required for energy production. The first reason for Fe deficiency is inadequate Fe intake (Lukaski, 2004). Severe Fe deficiency results in hypochromic anaemia (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005). Toxic levels of Fe in the body may be a consequence of genetic or metabolic disorders, frequent blood transfusions or excessive intake. An excess of Fe over a long period could result in liver and heart damage, diabetes, and skin changes (Fraga and Oteiza, 2002).

2.10 Manganese

Fruits and vegetables are also characterised by a low content of manganese (Mn). Vegetables contain Mn in the range 0.01–0.078 mgh/100 g and fruits 0.01–0.66 mg/100 g (Szefer and Grembecka, 2007). The recommended intake of Mn is 2 mg/day (http://www.anyvitamins.com/rda.htm), and its main physiological function is being an enzyme cofactor involved in antioxidant reactions related to the glucose metabolism (metabolism of carbohydrates and gluconeogenesis; Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008). Deficiencies in Mn are extremely rare but have shown a reduction in cholesterol, red blood cells and mucopolysaccharide abnormalities. Under experimental conditions signs of a scaly rash and low levels of plasma cholesterol have been observed (Shenkin, 2008). An excess of Mn produces a toxic effect in the brain, causing a Parkinson-like syndrome (Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008).

2.11 Molybdenum

Molybdenum (Mo) is present in plant-based foods, normally at low levels. However, certain foods may concentrate extremely high levels of Mo. The range of variation between foods is very wide (from 1×10^{-6} mg/100 g in wine to 0.15 mg/100 g in peas).

Canned vegetables contain up to 0.03 mg/100 g (Szefer and Grembecka, 2007). Doses <250 µg are considered safe (http://www.anyvitamins.com/rda.htm). Molybdenum function is related to the turnover of amino acids and purine metabolism, assisting in the elimination of secondary dangerous compounds (nitrosamines). Furthermore, Mo is a cofactor for oxidant enzymes, especially sulphite oxidase and xanthine oxidase (Shenkin, 2008). A Mo deficiency constitutes a hereditary metabolic disorder characterised by severe neurodegeneration, resulting in early childhood death (Schwartz, 2005). Toxic quantities and excess of Mo may interfere with the metabolism of Co and might give symptoms of anaemia and slow growth (Xiao-Yun et al., 2006).

2.12 Nickel

Vegetables usually present nickel (Ni) levels in the range of 5×10^{-4} to 0.28 mg/100 g, and fruits between <0.004 and 0.05 mg/100 g (Szefer and Grembecka, 2007). The recommended daily intake of Ni is in the range of 302–735 µg (Roychowdhury et al., 2003).

2.13 Selenium

The concentration of selenium (Se) ranges from 10^{-4} to 0.06 mg/100 g in foods of plant origin (Szefer and Grembecka, 2007). Seventy micrograms per day is taken as the required dosage for this micronutrient (http://www.anyvitamins.com/rda.htm). Selenium is an essential component of selenoproteins, which are implicated in antioxidant reactions (Guerrero-Romero and Rodríguez-Morán, 2005). In addition, although Se functions are not fully known, it seems that it also presents activity related to thyroid and immune system functions through its intervention (Shenkin, 2008). Selenium is associated with marked reductions in risks of several types of cancer (Combs, 2004) and its deficiency may contribute to heart disease, hypothyroidism and deficiencies in the immune system (Guerrero-Romero and Rodríguez-Morán, 2005; Combs, 2000; Zimmermann and Köhrle, 2002). An excess of Se has been related to several symptoms including: gastrointestinal upset, hair loss, fatigue and mild nerve damage. However, Se toxicity is not frequent and is related to accidental exposures (Guerrero-Romero and Rodríguez-Morán, 2005).

2.14 Zinc

The concentration of zinc (Zn) in plant-based foods generally varies from 0.05 to 11.8 mg/100 g. The lower levels of Zn are found in fresh fruits (0.02–0.61 mg/100 g). Fruit juices and beverages are characterised by low levels of Zn ranging from 0.01–0.27 mg/100 g (Szefer and Grembecka, 2007). Recommended daily Zn consumption ranges from 8–11 mg (http://www.anyvitamins.com/rda.htm; Lukaski, 2004). It is required for the structure and activity of more than 100 enzymes (Huskisson et al., 2007; Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008), for the synthesis of nucleic acids and proteins, for cellular differentiation, and for glucose use and insulin secretion (Lukaski, 2004). This mineral takes part in the Zn fingers associated with DNA, haemoglobin, myoglobin and cytochromes (Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008). The bioavailability of Zn is reduced by the presence of large amounts of other elements such as Fe or Cu (Shenkin, 2008). Zn deficiency is relatively frequent and well characterised, and the absence of Zn negatively affects the immune system efficacy, and the sensibility of taste and smell senses, and impairs DNA synthesis (Guerrero-Romero and Rodríguez-Morán, 2005; Shenkin, 2008). It has also been described that Zn deficiency produces hair loss and hypochromic anaemia (Shenkin, 2008). Zn toxicity shows both acute and chronic effects. Intakes of 150–450 mg per day over an extended period of time have been associated with poor Cu levels, altered Fe and immune functions, and reduced levels of HDL (Guerrero-Romero and Rodríguez-Morán, 2005; Hamilton et al., 2001).

3 Environmental Stress Affecting Plant Mineral Content

Abiotic stresses such as high salt levels, low water availability and extreme temperatures can severely modify the mineral and nutritive quality of the crops for human consumption. These types of external stresses are becoming increasingly important because of the global reduction in the availability of water resources of good quality for irrigation, which indeed is affecting the plant mineral status and consequently, the nutritional quality of a given cultivar. To date, the reports have mainly studied the influence of environmental stresses on the carbohydrates (sugars), amino acids or antioxidant production of vegetables and fruits, and most investigations have been focused on salinity as the main abiotic stress. However, there is limited information about the influence of general abiotic factors on the mineral content of plant-based foods and food products as a bioindication of the food nutritive value and quality. In general, the mineral nutrient contents change when external conditions affect the plant growth (i.e., environmental stress) and there is a reduced plant growth and reduced biomass at harvest, accompanied by less dilution of nutrients on a dry mass basis (Fig. 2).

3.1 Salinity

The use of saline water for irrigation may affect the mineral composition of plants and, therefore, the fruit quality. In a saline environment, ion homeostasis can be disturbed by excessive uptake of Na^+ and Cl^-. Competition between these and further anions and cations has been well documented over the last 20 years (Sharpley et al., 1992; Lopez and Satti, 1996; De Pascale et al., 2005). Thus, in general, salinity reduces phosphate uptake and accumulation in crops as well as Ca^{2+} soil bioavailability and transport, which affects the quality of both vegetative and reproductive organs including fruits and edible parts of the plants. In addition, Na^+ and Cl^- ions may reduce K^+ and NO_3^- uptake, respectively (Grattan and Grieve, 1999). Under saline stress, a reduction in NO_3^- content has been observed in edible florets of broccoli (*Brassica oleracea* var. italica) and in tomato (*Solanum lycopersicon)* (Lopez-Berenguer et al., 2009) and although the reports on the effect of nitrate on human health are still conflicting, its reduction in foods could add a nutritional value to the cultivar of interest (Anjana and Iqbal, 2007). Also, in these reports, concentrations of Na^+ and Cl^- were higher in the leaves than in the florets, in agreement with the fact that under saline stress plants attempt to minimise the concentration of toxic ions in their reproductive organs (Hachicha et al., 2000). However, Del Amor et al. (2001) found that in tomato fruits, total anion Cl^- and NO_3^- concentrations increased by 11% as the salinity level increased from 2 to 8 $dS{\cdot}m^{-1}$ but fruit K^+, Na^+, Ca^{2+} and Mg^{2+} contents were reduced significantly by salinity levels. Interactions between salinity and fertilisation have been described and concentrations of P, K^+, Mg^{2+}, Cu and Zn significantly decreased at high salinity and when urea was used as a nitrogen source. In this case, the total nitrogen concentration was not affected. Other studies on tomato and salinity showed that fruit Ca^{2+} was also decreased by salinity or NH_4^+, with the negative effect of NH_4^+ being higher than the effect of salinity (Flores et al., 2003).

On the other hand, saline stress is a condition that may cause a combination of complex interactions affecting the plant metabolism or the inner nutritional

Fig. 2 Greenhouse experiment evaluating environmental stresses (salinity and drought) in horticultural crops

requirements, but little information on the distribution of essential minerals in plants for foods grown under salinity has yet been published. Moreover, the effects of salinity on mineral contents are often equivocal depending on the species or cultivar and the specific plant organ (De Pascale et al., 2005). On this subject, it has been reported that salinity can originate stimulatory as well as inhibitory effects on the uptake of some micronutrients by crop plants. Thus, as recently observed in two strawberry cultivars under salt stress, the mineral status of the berries was improved (increased Na^+ and Cl^- , as well as N and P contents), but a different response was detected for K^+ and Zn, which remained unaffected in the less-sensitive cultivar, and rose in the sensitive cultivar (Keutgen and Pawelzik, 2008).

The uptake of Fe, Mn, Zn and Cu generally increases in crop plants under salinity stress (Alam, 1994). However, the detrimental effects of NaCl stress on the nutrition of bean plants reflected differences in distinct plant organs and showed higher concentrations of Cl^- and Mn in roots, Cl^-, Fe and Mn in leaves, and Cl^- and Fe in fruits (Carbonell-Barrachina et al., 1998). Therefore, when applying moderate salinity levels for quality improvement, it is necessary to consider changes in the pool of mineral nutrients depending on the sensitivity of the cultivar and differences in mineral accumulation in the plant organs in order to avoid negative effects of the treatment.

In the fruits of courgette plants (*Cucurbita pepo* L. var. Moschata) an 80 mM NaCl treatment improved yield and fruit quality (Víllora et al., 1999) and significantly increased the concentrations of micronutrients (Fe and Zn mainly) in the edible part of this crop (Víllora et al., 2000). In addition, the concentrations of total Mn and total extractable Fe, Cu and Zn followed no linear pattern in response to the increased NaCl concentrations. These results for courgette contrast with findings for tomato, squash and green beans, in which the level of each microelement reportedly fluctuates with salinity, perhaps due in part not only to the salt treatments but also largely to the type of crop and the cultivar used in each experiment (Grattan and Grieve, 1999). In a recent report, Ca^{2+}, Mg^{2+}, K^+ and Mn contents decreased in the hot pepper fruits of a sensitive cultivar of *Capsicum annuum* L. as NaCl concentration increased (Ramirez-Serrano et al., 2008). Some studies on salinity in grafted plants of a “Star Ruby” grapefruit scion on two rootstocks, “Cleopatra” mandarin and “Carrizo” citrange, showed that fruits from saline treatment on “Carrizo” had Cl^- and Na^+ concentrations (2,87 and 1,6 times higher, respectively) than fruits from no saline treatments. Moreover, in the first harvesting, salinity increased K^+ concentration in the juice of fruits from trees grafted on “Carrizo” and treatments with 30 mM of NaCl decreased Ca^{2+} concentration in fruits from trees grafted with both rootstocks. However, salinity had no major effect on juice K^+ concentration on the second harvesting date or on juice Mg^{2+} concentration at both sampling times (García-Sánchez et al., 2003).

Plants respond to environmental stress by synthesising signalling molecules that activate a range of signal transduction pathways. Several such signalling molecules have been identified in plants such as Ca, jasmonic acid (JA), ethylene (C_2H_4) and salicylic acid (SA) derivatives. However, the effect of signalling molecules applied externally under stressful conditions (saline treatments) on the plant mineral uptake is not fully understood or well documented. Positive effects of SA on the ion uptake, and inhibitory effects on Na^+ and Cl^- uptake have been described for maize plants under salinity (Gunes et al., 2007). Similar effects of SA on the Na^+, K^+, Ca^{2+} and Mg^{2+} contents in wheat plants grown under salinity have been described (Al-Hakimi and Hamada, 2001).

In addition to its role as a cellular messenger, effects of Ca^{2+} on integrity of membranes, rigidity of the cell wall, and maintenance of cell-to-cell contact are reported. Supplemental Ca^{2+} has been successful in improving crop quality due to the correction of Ca^{2+} deficiencies induced by Na^+. Under osmotic stress, the distribution of Ca^{2+} to the distal end of fruits is decreased, leading to a local deficiency of Ca^{2+} that causes rotting at the distal end of fruits known as blossom end rot (BER) (Ho et al., 1993; Saure, 2001; Guichard et al., 2001). However, BER is known to be affected not only by one factor, but also by interactions between water availability, salinity and nutrient ratios in the root zone, and the product of average daily solar radiation and air temperature, root temperature and air humidity (Adams and Ho, 1993; Ehret and Ho, 1986). In apples, it has also been observed that low Ca contents are associated with bitter pit disease (Fukumoto et al., 1987) or pit breakdown (Tomala and Dilley, 1990).

In general, salinity influences the uptake and transport of other ions by the plant and such antagonism could occur between Na^+ and Ca^{2+}, K^+ or Mg^{2+}

and between Cl^- and NO_3^-. These effects may be involved in the occurrence of nutritional disorders in plant tissues, affecting food quality.

3.2 Drought

Limited water supply in many areas of the world, especially in arid and semiarid regions, is a major problem in irrigated agriculture. In recent years, it has become clear that the maintenance of a slight water deficit can improve the partitioning of carbohydrates to reproductive structures such as fruit and also control excessive vegetative growth (Chalmers et al., 1981). This is called "regulated deficit irrigation", consisting of irrigation input being removed or reduced for specific periods during the growth cycle of crops (Chalmers et al., 1986). This technique results in more efficient use of irrigation water and often improves product quality (Turner, 2001). Rouphael et al. (2008) showed that using three different levels of irrigation based on evapotranspiration (ET) rates (1.0, 0.75 and 0.5 ET) and two grafting treatments on watermelon plants no significant differences among treatments were observed for P and Ca^{2+} concentrations, whereas K^+ and Mg^{2+} concentrations were significantly improved by both the irrigation rate and grafting combination with no significant differences between irrigation and grafting interaction.

Drought and salinity can differentially affect the mineral nutrition of plants. While salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with other nutrients such as K^+, Ca^{2+} and NO_3^-, drought can affect nutrient uptake and impair translocation of some nutrients.

It has been recently reported that in banana, the main effect of drought was to reduce K^+ levels, which is the major mineral nutrient in this fruit. By contrast, the content of certain elements increased (i.e., Ca^{2+}, Na^+, Fe and Zn), or remained stable (i.e., N, P, Mg^{2+}, Mn and Cu) under the drought treatment, which also generated a positive effect on the organoleptic properties of the fruit (Mahouachi, 2007). After rehydration, the mineral content of the bananas was similar between stressed and non-stressed plants. These data illustrate the ability of this cultivar to maintain relatively normal levels of minerals and functional fruit tissues after dehydration despite the long period of water stress. Nonetheless, the fruits lost their commercial value to a certain degree (reduced size and biomass) after the period of water stress.

Wild plants play an important role in the diet of inhabitants in different parts of the world. These plants tend to be drought-resistant and are gathered both in times of abundance and times of need, and for this reason numerous reports have been focused on wild edible plants. In a study of dietary practices in Northeastern Nigeria, it was observed that the edible wild species available during the wet season generally were inferior in micronutrient mineral contents compared with the dry season plants (Lockett et al., 2000). Commonly consumed species of edible wild barks, fruits, leaves, nuts, seeds, and tubers were analysed and Kuka bark (*Adansonia digitata*), given to infants, was high in Ca^{2+}, Cu, Fe and Zn. Cediya (*Ficus thonningii*), dorowa (*Parkia biglobosa*) and zogale (*Moringa oleifera*) were also good sources of Ca^{2+}, Fe, Cu and Zn. Fruits, leaves and nuts of aduwa (*Balanites aegyptiaca*) are widely used during the dry and drought seasons. Tsamiya seeds (*Tamarindus indica*), consumed commonly during pregnancy, were good sources of Zn, and Kirya seeds (*Prosopis africana*) contained the highest Zn concentrations. Shiwaka leaves (*Veronia colorate*), consumed by pregnant women to increase breastmilk production, were high in P, Mg^{2+} and Ca^{2+}.

In another report it has been illustrated that the mineral content of some edible wild leaves contained higher N, K^+, Ca^{2+} and Mg^{2+} concentrations than those of some commonly used vegetables such as spinach (*Spinacia oleracea*), pepper (*Capsicum annum*), lettuce (*Lactuca* spp.) and cabbages (*Brassica oleracea*). However, P, S and Na^+ contents were lower, and Fe, Mn, Zn and Cu levels were equal (Turan et al., 2003).

The time of application as well as the duration of water stress during the fruit development can influence the mineral content. In 'Williams' pears a reduced water supply at the end of the fruit development (late water stress) caused smaller reductions in the uptake of Ca^{2+}, K^+ and B within the fruits, while at the beginning of fruit development (early water stress) only a lower content of Ca^{2+} was observed in the fruits. Reduced water supply either at the beginning or the end of the productive cycle induced a higher N content in the fruits. In the treatment of early water stress the K contents were higher than in the untreated controls (Hudina and Stampar, 2000).

The potential of roots to absorb nutrients generally declines in water-stressed plants, presumably due to a decline in nutrient element demand, but the ability to take up and transport the mineral nutrients differs in distinct crops and depends on the plant's tolerance to drought.

3.3 Extreme Temperatures

It is evident that the roots play a principal role in the absorption of the mineral elements that will be translocated to the aerial parts of the plant. In general, a consistent decrease in the nutrient concentrations in the plant shoots is parallel to the growth suppression at low root temperatures. Growth and mineral composition of fruits in potted trees were studied at two temperatures (19 and 24°C) in 'Golden Delicious' and in 'Cox's Orange Pippin' apples (*Malus domestica*) (Tromp, 1975). In this report the levels of K, N, Mg and P were increased at the higher temperature. With respect to Ca, the high temperature regime reduced its influx in 'Golden Delicious' but favoured the Ca influx in the 'Cox's Orange Pippin' apples. Similarly, in two cultivars of tomato with contrasting response to elevated temperature, Ca was poorly transported to the fruits but in fruit explants, the elevated temperature (40°C) increased the Ca import into the fruits in both cultivars. This permanent flux of Ca to the fruits may have a pivotal role in maintaining an optimal level of Ca^{2+} in the cytoplasm of fruit cells, as a factor for increasing the tolerance to high temperatures (Starck et al., 1994). On the other hand, only a few studies have examined the effect of differences in temperature regimes between day and night on the mineral status of fruits or vegetable foods, but low concentration of nitrate was observed as a consequence of variations in temperature in root and shoot Ca^{2+} due to lower night air temperatures (Gent and Ma, 2000).

3.4 Light Intensity

A certain influence of the light on the transport of nutrients from shoots to the fruit through the transpiration stream has been suggested in different studies. Caruso et al. (2004) reported that shading caused a reduction in the content of the main mineral elements except for nitrates in strawberry fruits. Also, the effects of sunlight on the mineral contents of apples were investigated (Iwane and Bessho, 2006). The treatment of sunlight from East and West directions on 75 apples from the inside and outside of the crown of the trees revealed a significant negative correlation between the amount of solar radiation received by the fruit and its mineral content for K^{+}, Ca^{2+} and Zn. The concentration of minerals in apples grown on the inside was higher than in the fruits grown outside of the crown. The concentration of minerals was higher in apples grown on the shaded side than in the apples grown on the sunny side. No significant differences in mineral concentrations were observed between fruit grown on the East or West orientation.

Effects of exposure to light and air movement on the accumulation of some mineral elements in fruits of kiwifruit (*Actinidia deliciosa* var. deliciosa) have been presented (Montanaro et al., 2006), where the main differences were found for Ca^{2+}, exhibiting twice the content in exposed fruits (>40% full sunlight) than in shaded fruit (<20% full sunlight).

Light is one of the main external factors influencing the nitrate concentrations in vegetables. Several human health hazards due to nitrate toxicity have been identified. The accumulation of nitrate in the plant tissues is more frequent under poor light conditions in leafy vegetables such as lettuce, spinach or kohlrabi (*Brassica oleracea* var. gongylodes L.) (Blom-Zandra and Lampe, 1985; Steingröver et al., 1986; Sritharan and Lenz, 1992). A controlled nutritional regime is then needed to reduce the leaf nitrate in the leaves (i.e., lettuce) under such unfavourable light conditions (Demsar et al., 2004).

Anjana et al. (2006) have reported that nitrate concentration was lowest at noon on a sunny day in spinach leaves. Thus, the time at which plants contain the lowest nitrate concentration may vary with the environmental conditions in different geographical regions of the world, and also depends on the interaction with other environmental factors. Santamaria et al. (1999) observed in different fresh vegetables that under conditions of low light availability, an increase in temperature increases the nitrate accumulation.

4 Fertilisation Practices and Mineral Content in Food Crops

The supply of essential nutrients for the health of consumers by improving the fertilising practices in the productive sector has awakened great interest in recent years. Here, we will summarise the available information on the effects of fertiliser applications on the mineral content of crops in relation to food quality for human consumption.

4.1 Nitrogen Fertilisation

The effect of N fertilisers on the mineral content of edible parts or fruits and vegetables is variable depending on the doses applied, the nutrient analysed, the species under study, and the organ to be consumed. Thus, in tomato grown under different N doses (0, 60, 120 and 180 hg ha^{-1} N), only the higher N doses increased total fruit N levels but, antagonistically, K^+ levels decreased continuously with the increased N (Cserni et al., 2008). In tuber and root crops such as potato (*Solanum tuberosum* L.) and sweet potato (*Ipomoea batatas* L.), which have enlarged underground stems and roots as edible parts, the application of N fertilisers usually led to increased tuber N concentration (Eppendorfer and Eggum, 1994). Also, the content of elements such as K^+, P, Ca^{2+} and Mg^{2+} in mature tubers of potato was not significantly different to the untreated tubers after N fertilisation (0–200 kg.ha^{-1}) (Ilin et al., 2002). In contrast, in broccoli sprouts (*Brassica oleracea* var. Italica), higher S concentrations were found when increasing N and S fertilisation rates up to a determined dose of N and S, but higher N rates did not yield higher S uptake (Aires et al., 2007).

In experiments studying the influence of the NH_4^+/NO_3^-ratio in the nutrient solution and its effects on mineral status (in *Brassica oleracea* var. Italica), when this ratio was 0.5:0.5, the concentrations of P, K^+, Ca^{2+} and Mg^{2+} were all higher in cabbage roots and leaves than those in plants grown in nutrient solutions with different ratios of decreased NH_4^+ supply (Zhang et al., 2007).

It is well known that the application of N improves plant growth and yield. However, the application of high concentrations of N not only contaminates the environment, but also causes NO_3^- accumulation in the leaves of vegetable crops, which have been found to be the major source of toxic NO_3^- uptake by humans. In general, vegetables that are consumed with their roots, stems and leaves have a high NO_3^- accumulation, whereas those with only fruits as the edible part have low NO_3^- accumulation rates (Zhou et al., 2000). In addition, NO_3^- contents vary depending on the organ of the plant (Santamaria et al., 1999; Anjana et al., 2006) and the physiological age of the plant (Maynard et al., 1976; Anjana et al., 2006). Field experiments have shown that NO_3^- concentrations in leafy vegetables were positively correlated with N rates, and N fertiliser added to the soil was the major cause of NO_3^- accumulation in vegetables (Wang et al., 2001). In the same way, Zhang et al. (2007), and Staugaitis et al. (2008) found a linear increase in NO_3^- concentration with increasing N fertilisation in cabbage (*Brassica campestris*) leaves and roots, and heads, respectively.

4.2 Phosphorus Fertilisation

Phosphorus promotes root growth, enhances nutrient and water-use efficiency, and increases yield. Therefore, since absorption and reduction of NO_3^- is a process which consumes ATP, the metabolism of NO_3^- is related to P supply. In pot experiments, it has been observed that a high soil N:P ratio was one of the key causes of NO_3^- accumulation in vegetables (Gao et al.,1989). In contrast, P fertilisation decreased NO_3^- concentration in cabbage (*Brassica campestris*) and canola (*Brassica napus*), and had no significant effect in spinach (*Spinacia oleracea*) (Wang and Li, 2004).

Togay et al. (2008) studied the effect of different doses of P (10, 40 and 80 kg ha^{-1}) on the P content in grain of dry bean (*Phaseolus vulgaris*) and observed increased P levels when 80 kg ha^{-1} P was applied. When looking at the micronutrients, Moreno et al. (2003) found that Fe and Mn concentrations in cucumber were higher in P-fertilised treatments compared with the unfertilised control. This effect is similar to what was found with edible fruits such as apple (*Malus sylvestris* Mill var. *domestica* (Borkh.)) where increased fruit P was obtained with P treatments. On the contrary, in cereals Komljenovic et al.

(2006) revealed that maize grain (*Zea mays*) was less dependent on P fertilisation compared with the leaf; and it has also been shown in pear (*Pyrus communis*) that foliar P fertilisation decreased the content of B and Zn in fruits (Hudina and Stampar, 2002).

4.3 Potassium Fertilisation

Potassium is closely related to N assimilation in plants and can accelerate transport of NO_3^- from roots to aboveground plant parts. Thus, Zhou et al. (1989) showed that compared with the control, NO_3^- concentration in cabbage (*Brassica campestris*) decreased with the application of K^+, whereas it increased in spinach (*Spinacia oleracea*) (Gao et al., 1989).

In relation to other minerals, K^+ fertilisation has different effects. In fact, Hudina and Stampar (2002) showed that foliar fertilisation with K^+ increased the content of K in pears (*Pyrus communis* L.), whereas K^+ concentrations in broccoli heads (*Brassica oleracea* var. Italica) showed no differences among four levels of K^+ fertilisation (Vidal-Martínez et al., 2006). In other studies, the concentrations of B and Zn in pears (Hudina and Stampar, 2002) as well as N concentrations in potato (*Solanum tuberosum*) and sweet cabbage (Eppendorfer and Eggum, 1994) decreased with increased K^+ fertilisation.

4.4 Sulphur Fertilisation

Sulphur fertilisation may be recommended for certain crops to reduce the undesirable NO_3^- contained in their edible parts. In fact, an increased soil S level significantly reduced NO_3^- concentrations in tubers and leaves of kohlrabi (Losak et al., 2008), and turnip tops (*Brassica rapa* L.) (De Pascale et al., 2007). In S-deficient soils, the application of S fertilisers can decrease the tuber N concentration in potato (*Solanum tuberosum*) due to increased dry mass yield (Eppendorfer and Eggum, 1994). Nevertheless, in a greenhouse pot experiment using 'Luna' kohlrabi (*Brassica oleracea*), the effect of S fertilisation on N content in tubers and leaves was insignificant (Losak et al., 2008).

4.5 Calcium Fertilisation

Leafy vegetables can be an excellent dietary source of calcium, and are a good alternative for individuals with a diet low in dairy products. Increasing the calcium content in leafy vegetables through fertilisation management could further improve their nutritional benefits. Thus, it has been observed that in lettuce produced in a hydroponic system an increase in Ca^{2+} concentration in the nutrient solution increased the Ca^{2+} levels in the leaves (Neeser et al., 2007).

It has also been shown that Ca^{2+} application increased Ca^{2+} concentrations in peripheral layers of apple fruits and reduced K^+ concentrations (Grimm-Wetzel and Schonherr, 2007). Similar results were reported by Val et al. (2008), where Ca^{2+} treatments increased the concentration of Ca^{2+} in the skin, but not in the flesh of fruit, and several sprays were needed to promote a prolonged increase in the concentration of Ca^{2+} in the skin. However, Ca^{2+} sprays did not influence the concentrations of Mg^{2+} and K^+. In a recent study the application of $CaCl_2$ increased the Ca^{2+} content in litchi fruit; firmness and skin colour were affected, and some positive correlations with leaf and fruit K^+ were detected (Cronje et al., 2009).

In kiwifruit, fruit quality is associated with the correct Ca^{2+} level; however, the application of a biostimulant such as Ca^{2+} fertiliser, which is recommended to prevent calcium deficiency resulting from lack of uptake into fruit, did not affect fruit yield (Otero et al., 2007).

4.6 Microelement Fertilisation

Although the majority of experiments have been performed using the widely distributed N, P and K^+ fertilisers, there are also a few pieces of information about the effects of microelement fertilisers on the edible parts of plants for human consumption (Baize et al., 2009). Graham et al. (2001) showed that application of Zn fertiliser to Zn-deficient soil at sowing significantly increased the Zn concentration in wheat grain. Also, the content of Zn and several other micronutrients, such as I, Se, Cu and Ni, was usually enhanced by application of the appropriate mineral forms (Wang et al., 2008). Micronutrient foliar fertilisation seems to be a cheap and effective method, depending on the nutrient supplied and the time of application. In this way, Wojcik and Wojcik (2003) showed that foliar B sprays before full bloom or after harvest increased B concentrations in fruitlets of pear (*Pyrus communis* L.) at 40 days after flowering.

Selenium deficiency is a very serious nutritional and health problem. That is why the effect of selenite and selenate fertilisation on Se content has been widely studied (Chen et al., 2002; Fang et al., 2008; Ducsay et al., 2009). In reported experiments, selected mineral contents were higher with application of selenate than selenite to certain species. Thus, a higher content of Se in rice (*Oryza sativa*) (Chen et al., 2002) and S in lettuce plants (*Lactuca sativa* cv. Philipus) (Ríos et al., 2008) were found. Other studies showed that differentiated doses of selenite in soil caused a significant increase in Se content in dry matter of wheat grain (Ducsay et al., 2009), whereas non-significant effects were observed in lettuce (Ríos et al., 2008). In addition, Fang et al. (2008) indicated that Zn and Se were the main variables increasing the Zn, Se and Fe contents of rice. Therefore, the application of Zn, Se and Fe mixed fertiliser as foliar spray could alleviate the physiological deficiency of these micronutrients in rice.

4.7 Organic Farming Versus Mineral Fertilisation

The massive use of chemical fertilisers in intensive agriculture has greatly increased concern for the declining fertility of soils. Soil nutrient depletion is the result of increasing pressure on agricultural land (Wopereis et al., 2006; Lal, 2009). That is why organic inputs are required to ensure that intensive systems do not threaten the sustainability of land use. However, small farmers are reluctant to use organic wastes or composts because organic fertilisers do not release nutrients as fast as mineral fertilisers and they do not supply a balanced ratio of nutrients at the right time (Bath, 2000; Kirchmann et al., 2002; Gunnarsson, 2003). However, demand for organically-grown products has risen steadily and the number of growers adopting organic farming systems has also increased, because organic foods are believed to be more nutritious than conventionally-grown foods, with a better balance of vitamins and minerals. Nevertheless, the scientific community has not conclusively shown that organic products are more nutritious than conventionally-grown foods (Winter and Davis, 2006). Thus, it seems to be important to see how organic and inorganic fertilisers affect food quality in terms of mineral content. Also, the negative effects that fertilisers have on food quality must be considered, since the anthropogenic activities aimed at enhancing food production may facilitate the accumulation of undesirable substances.

Several experiments have been performed in order to compare the effects of organic and conventional (mineral) fertilisers on the crop yield and nutritional status of plants, since organic yields are often lower compared with conventional production (Mäder et al., 2002; Dumas et al., 2003; Gopinath et al., 2008).

Usually, the organic-amended soils showed significantly higher soil mineral content (Edmeades, 2003). However, other authors indicated lower mineral contents for organically fertilised soils (Gosling and Shepherd, 2005).

The influence of organic soil fertilisation on nutrient content in crops has been studied and different results have been recorded. Some authors showed that the application of organic amendments improved the soil nutrient content, but did not always increase the plant nutrient concentration (Roe, 1998; Warman, 2005) since it depends on the crop type, the nutrient used, the climate parameters and the year of the study (Warman and Havard, 1997, 1998; Maqueda et al., 2001). Furthermore, the available scientific literature shows that some of the comparisons are not experimentally valid due to variation in crop varieties, timing in fertilisation, and handling and storage after harvesting (Warman and Havard, 1997). However, there are certain results that support that higher P and K^+ contents in wheat grain were obtained by applying organic amendments than the elemental contents using mineral fertilisers instead (Colla et al., 2002; Wszelaki et al., 2005; Gopinath et al., 2008; Basu et al., 2008).

The long-term use of organic composts (vegetal compost and green residue of previous crops) on greenhouse soils induced few differences in the macronutrient concentrations in the edible parts of food crops compared with the experiments using mineral fertilisation, although there was a trend of showing higher N concentration in minerally-grown crops and higher K^+ concentration in organically-grown crops (Herencia et al., 2007). Moreover, the NO_3^-

concentrations in the edible parts of organically-grown crops were significantly lower than in the minerally-fertilised plots (Vogtmann et al., 1993; Williams, 2002; Malmauret et al., 2002; Hajslova et al., 2005). This can provide a clear benefit for human health. Nevertheless, the results were variable depending on the crop, season cycle and year, and these factors must be considered carefully in the conclusions and potential recommendation to producers and consumers.

5 Conclusion

The quality of edible fruits concerning mineral contents may vary depending on interactions between cultivars, environmental factors such as light and temperature, composition of the nutrient solution, crop management practices, and the interaction of all these factors. This is the reason why all of them must be taken into account in order to characterise the nutritional value (mineral status) of fruits and vegetables, as well as the factors influencing the content of a specific element in a given cultivar. In addition, the physiological parameters of the fruit (stage of development, ripening, marketable maturity, physiological maturity, senescence) and the plant or tree as a whole are also of interest.

The influence that fertilisation practices may have on the mineral status and nutritive value of fruits and vegetables also depends on the fertiliser used, the macro- (i.e., N, P, K, S, etc.) or micronutrient (i.e., Fe, Mn, Zn, Cu, Ni, Co, Se, etc.) studied, and the plant part of interest for consumption (i.e., leaf, root, tuber, fruit). Similarly, a determined or programmed pattern cannot be established for the use of irrigation regimes with waters of different qualities. Nonetheless, fertilisation seems to remain one of the most practical and effective ways to control and improve the nutritional value of crops to meet the needs of the population, as well as proper water management integrating practices for food quality and safety. A large body of research results has been performed in the past decades on the effects of distinct agronomical practices on specific crops of human interest, but more concise and precise studies are needed to improve the load of essential microelements in foods and to prevent or avoid the accumulation of toxic or undesirable contaminants.

Acknowledgements This work was funded by the CICYT (AGL2006-06499) and the Fundación Seneca-Comunidad Autónoma de la Región de Murcia (08753/PI/08). This work was funded partly by a Convenio de Desempeño-UTA-MECESUP2 (Arica-Chile). E. Bastías received a fellowship from the Fundacion Séneca, 09769/IV2/08.

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Fertiliser Trees for Sustainable Food Security in the Maize-Based Production Systems of East and Southern Africa

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Contents

Abstract The negative effects of soil fertility depletion on food security, especially among smallholder farmers in Africa, is of economic importance, and may be worsened by climate change and rising global fertiliser prices. Substantial efforts and investment have gone into development of alternative soil fertility management options. These include vigorous research and development of N-fixing plants or "fertiliser trees", that has been on-going in the last two decades in East and Southern Africa. In this paper, we review several studies conducted both on-station and on-farm and synthesise the results in terms of improvements in soil physical, chemical and biological properties, and crop yield in response to fertiliser trees. Our major findings are that (1) fertiliser trees add more than 60 kg N ha^{-1} per year through biological nitrogen fixation (BNF); (2) nutrient contributions from fertiliser tree biomass can reduce the requirement for mineral N fertiliser by 75%, translating to huge savings on mineral fertilisers; (3) fertiliser trees were also shown to substantially increase crop yield. A meta-analysis has further provided conclusive evidence that with good management, fertiliser trees can double maize yields compared with local farmer practices of maize cultivation without addition of external fertilisation. (4) Financial analyses showed that fertiliser tree systems are profitable and also have higher net returns than the farmers' de facto practice, i.e. continuous maize cropping without fertiliser. We conclude that widespread adoption and scaling up of fertiliser trees can reduce the amount of mineral fertiliser needed, maintain the soil ecosystem, and positively impact on the livelihoods of farm households in southern Africa.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_9,

from Akinnifesi et al., Agron. Sustain. Dev. 30 (2010) 615–629. DOI: 10.1051/agro:2009058

Keywords Agroforestry • Integrated soil fertility management • N-fixing legumes • Scaling up

1 Introduction

Sub-Saharan Africa is often described as food-insecure (Conway and Toenniessen, 2003). The 2007–2008 food price crisis has not only increased food insecurity around the globe, but also exposed long-term failures in the functioning of the world food system (von Braun, 2009). Food shortage has nearly reached challenging dimensions and may become more crippling in the near future than anything the world has ever seen, unless efforts are geared toward improving productivity. At the centre of the imminent food catastrophe is maize, one of the main inputs in biofuel production as well as a staple food in most parts of Africa. Driven by a rapid rise in petroleum prices and, in response, a massive global expansion of biofuel production from maize (Cassman, 2007), the price of maize rose by over 50% from 2001–2007 (FAO, 2008). The rise in maize price not only impacts on the price of food products made from grains, but also the price of meat due to increased prices of livestock feed. Both food crops and feed demand are estimated to double in the next half century (Gowing and Palmer, 2008). The trends in southern Africa are worse than those in other parts of sub-Saharan Africa as population growth, dietary change and land-use pressures have driven prices of food and agricultural inputs to new heights. The prospects for meeting food demand in sub-Saharan Africa, which depends mainly on rain-fed and smallholder agriculture (Conway and Toenniessen, 2003), will likely remain bleak without major efforts to reverse current trends.

The focus of this work is the maize-based mixed farming system, which is the most important food production system in East and Southern Africa. It extends across plateau and highland areas at altitudes of 800–1500 metres, from Kenya and Tanzania to Zambia, Malawi, Zimbabwe, South Africa, Swaziland and Lesotho (Dixon et al., 2001). Maize accounts for 60% of the cropped area in some countries such as Malawi, Zimbabwe and Zambia, and it is almost a dominant crop in other countries including Kenya and Tanzania. In Malawi, maize is estimated to be grown on over 70% of the arable land and nearly 90% of the cereal area, making Malawi the world's highest consumer of maize at 148 kg per capita per year (Smale and Jayne, 2003). Thus, maize will remain a central crop in the food security equation even if the agricultural economy is diversified (Sauer et al., 2007). Crop-livestock integration is strong in the maize mixed farming system, where cattle are the most important livestock species. This farming system accounts for 10% of the land area and 19% of the cultivated area (Dixon et al., 2001). The climate varies from dry sub-humid to moist sub-humid. The most typical areas have unimodal rainfall, but some areas experience bimodal rainfall. The maize mixed farming system is currently in crisis (Dixon et al., 2001). Average farm sizes have fallen to under 0.5 ha in several areas (Dixon et al., 2001), while opportunities for expansion of cultivated land are limited as rapid population growth has led to progressive encroachment upon marginal lands (Bojo, 1996). Most farmers in the maize-based farming systems are crowded out of the agricultural input market and can hardly afford optimal quantities of inorganic fertiliser (Sauer et al., 2007).

The rapid deterioration of soils in this farming system directly affects productivity and it perpetuates rural poverty. Malawi alone loses US$350 million worth of nitrogen and phosphorus through erosion each year, which translates to a gross annual loss of income equivalent to 3% of the agricultural Gross Domestic Product of Malawi (Bojo, 1996). If the situation is to be improved, agricultural production needs to be intensified through the application of agro-ecological technologies that do not require large amounts of capital and labour; a development paradigm termed the "Doubly Green Revolution" (Conway and Toenniessen, 2003). The fertiliser tree system is one of such innovations. A range of fertiliser tree options have been developed and several publications have documented individual studies (Kwesiga et al., 2003; Akinnifesi et al., 2008). There is need for an updated evidence-based review on the lessons learnt from about two decades of Research for Development on fertiliser tree technologies in terms of the science and their adoption and impact. Therefore, the objective of this review is to synthesise experiences in the development, scaling up and impact of fertiliser tree systems in the last two decades in southern Africa.

2 Fertiliser Tree Systems

Fertiliser tree systems involve soil fertility replenishment through on-farm management of nitrogen-fixing trees (Mafongoya et al., 2006). They represent a new paradigm because they use a completely different approach to land-use management by smallholder farmers. First, fertiliser tree systems capitalise on biological N fixation by legumes to capture atmospheric N and make it available to crops. Secondly, they permit growing of trees in association with crops in space or time to benefit from complementarity in resource use (Gathumbi et al., 2002). Thirdly, they address most of the biophysical and socioeconomic limitations identified with the earlier technologies based on using N-fixing tree legumes such as green manures (Kwesiga et al., 2003; Akinnifesi et al., 2006, 2008). The different fertiliser tree systems that have been developed and promoted in East and Southern Africa in the last two decades are briefly discussed below.

2.1 The *Faidherbia Albida* System

The potential of faidherbia (*Faidherbia albida*) for improvement of soil fertility and crop yields has been demonstrated in many parts of Africa (Saka et al., 1994; Kang and Akinnifesi, 2000). This species has a unique phenology in that it sheds its leaves during the wet season and resumes leaf growth during the dry season. This makes it possible to grow crops under its canopy with minimum shading on the companion crop. About 20 to 30 mature trees are needed to completely cover one hectare of land and maintain optimum crop response (Kang and Akinnifesi, 2000). Several studies in Africa showed yield benefits when crops were grown under the canopy of Faidherbia. Saka et al. (1994) reported 100–400% increase in maize yield in the Lakeshore plain o Malawi.

However, it takes a long waiting period (up to 20 years) for the tree to reach maturity and have an impact on the under-storey crop (Kang and Akinnifesi, 2000). Recent development has shown that with closer spacing, 10 × 10 m, earlier impact can be achieved at 12–15 years (Dutch Gibson, pers. comm.). A major improvement of this system is integration with other sustainable land management options, such as use of short rotation fallow species in the first 10–15 years.

2.2 *Sequential Tree Fallow*

Sequential tree fallow, often known as ‘improved fallow’, is a practice whereby a piece of land is planted with fast-growing nitrogen-fixing trees or shrubs for 2–3 years’ fallow (Mafongoya et al., 2006). Tree fallows have distinct advantage over herbaceous fallows, particularly in seasonally dry climates, because they have the ability to tap nutrients from deeper soil layers and are capable of accumulating large quantities of biomass through which nutrients are recycled back for crop use. Nitrogen-fixing trees also add large quantities of N through biological nitrogen fixation and improve crop yield.

Improved fallows have been widely tested on farmers’ fields in Zambia and this technology has now spread to other parts of southern Africa (Kwesiga et al., 2003). Several studies reviewed by Akinnifesi et al., (2008) showed that planted fallows of sesbania (*Sesbania sesban*) in Zambia, Malawi and Zimbabwe had doubled or tripled maize yield compared with control plots.

2.3 *Annual Relay Intercropping*

In relay intercropping, fast-growing nitrogen-fixing legumes are planted in a crop field at a time when annual crops such as maize have already been well established, usually within 2–4 weeks of crop sowing (Phiri et al., 1999). The legumes continue to grow after the crop harvest throughout the off-season. Legumes such as sesbania, tephrosia and pigeon pea (*Cajanus cajan*) are recommended. As farmers prepare land for the next season, they clear-cut the legume and incorporate the biomass into the soil. Although the yield levels are usually less than those of intercropping and 2-year improved fallow systems, it works well on small farms, and the benefit of trees can be seen immediately after one season of tree growth. Additionally, farmers do not lose any cropping year of maize. The main limitation of this technology is that the legumes need to be replanted every year.

2.4 Gliricidia Intercropping

The intercropping of gliricidia (*gliricidia sepium*) with crops is an improvement building on the characteristics and advantages of alley cropping but minimising its biophysical limitations such as the "hedge effect", "competition" and tree management (Akinnifesi et al., 2006). A detailed description of this innovation has been published elsewhere (Akinnifesi et al., 2008). Gliricidia-maize intercropping has formed an important part of on-station and on-farm research in Malawi since the early 1990s (Akinnifesi et al., 2006). The socioeconomic and biophysical conditions in southern Malawi seem to meet most of the broadly defined criteria for the success of intercropping of crops with trees (Akinnifesi et al., 2006). The fact that land is scarce, labour is relatively cheap, fertiliser is costly in Malawi and the country is highly nitrogen-deficient, coupled with the fact that maize is a high nitrogen-demander, creates the prospect for adoption of gliricidia-maize intercropping in southern Malawi.

The main advantage of gliricidia intercropping is that once established it can be managed to continuously supply nutrients to crops year in, year out. Although gliricidia requires labour to establish seedlings and tree management, this is not yet a bottleneck as land holdings are less than a hectare and less than a quarter of a hectare is put to gliricidia-maize intercropping in the southern region of Malawi. Additionally, labour is cheap in Malawi due to high population density. Farmers appreciate that coppicing trees need to be established only once and can then be used for many years, despite low initial returns.

2.5 Biomass Transfer

Biomass transfer is essentially moving green leaves and twigs of fertiliser trees or shrubs from one location to another, usually in the wetlands to be used as green manure. Recent studies (Kuntashula et al., 2004) have shown that biomass transfer using fertiliser tree species is a more sustainable means for maintaining nutrient balances in maize and vegetable-based production systems. The advantage is that synchrony between nutrient release and crop uptake can be achieved with well-timed biomass transfer. The management factors that can be manipulated to achieve this are litter quality, rate of litter application, and method and time of litter application (Mafongoya et al., 1998).

Although it has been argued that biomass transfer technologies require a lot of labour for managing and incorporating biomass, economic analyses have concluded that it is unprofitable to invest in biomass transfer when labour is scarce and its cost is thus high (Kuntashula et al., 2004, 2006). In addition to increasing yields of vegetables such as cabbage, rape, onion and tomato, and maize grown after vegetable harvests, biomass transfer has shown potential to increase yields of other high-value crops such as garlic (Kuntashula et al., 2004, 2006).

3 Rationale for Promoting Fertiliser Trees

What is the evidence base for promoting fertiliser tree systems? The benefits of fertiliser tree adoption include significant increase in crop yield, improvement in soil health, and savings on mineral fertiliser costs and labour. Significant benefits are also derived from fertiliser trees in terms of other ecosystem services, including provision of fuelwood and fodder, reduction of erosion and carbon sequestration. The state of knowledge on the various ecosystem services of agroforestry has been reviewed by Sileshi et al., 2008. In the following sections we will briefly describe improvement in crop yields and soil health.

3.1 Improvement in Crop Yield

One of the direct benefits of fertiliser trees is the maize yield response, as discussed in the next section. In an effort to fill the long-standing knowledge gap and conundrum of "marginal versus high impact" arguments regarding the effect of fertiliser trees on crop yield, a meta-analysis was undertaken using 94 peer-reviewed publications across sub-Saharan Africa (Sileshi et al., 2008). The results of the analysis provided a solid perspective for making recommendations about fertiliser trees to policy-makers, investors and scientists. Table 1 presents maize yields achieved using fertiliser trees across a range of sites in Malawi, Zambia, Zimbabwe

Table 1 Average maize yield and yield increase (t ha^{-1}) with fertiliser trees relative to the control (unfertilised maize grown continuously) in southern Africa

Species	Country	Number of sites	Yield (t ha^{-1})	Yield increase (t ha^{-1})	Percentage increase
Gliricidia	Malawi	5	3.9	2.9	345.6
	Tanzania	2	2.3	0.8	55.8
	Zambia	4	2.8	1.8	349.7
Sesbania	Malawi	7	2.5	1.3	161.4
	Tanzania	2	1.2	0.7	171.4
	Zambia	9	3.2	2.2	480.0
	Zimbabwe	4	3.0	1.9	583.1
Tephrosia	Malawi	9	2.0	1.1	232.7
	Tanzania	2	2.0	0.9	80.1
	Zambia	8	1.7	0.8	198.4
	Zimbabwe	5	3.6	0.2	17.7

Note: yield increase is the yield difference between the treatment (T) plot and the unfertilised control (C) plot, which is farmers' de facto practice. Percentage increase (%I) was calculated as follows: %I = 100((T-C)/C).

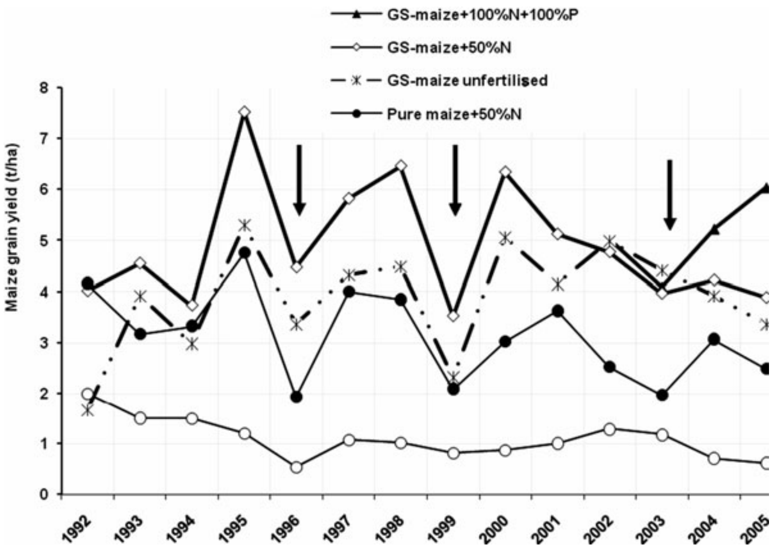

Fig. 1 Long-term maize grain yield as affected by fertiliser and pruning incorporations in a gliricidia-maize intercropping in Makoka, Malawi. Arrows indicate flood due to excessive rainfall in 1996/97, and droughts in the 1999/00 and 2003/04 seasons (Akinnifesi F.K., unpublished). Gs = *Gliricidia sepium*; N = nitrogen, P = phosphorus

and Tanzania. On average, gliricidia gave 55–350% yield increase over the control, while sesbania gave 160–583% increase. Yield increases with tephrosia spp were modest, and ranged from 180% to 233% (Table 1).

In a long-term trial in Makoka, gliricidia intercropping with maize increased maize yield in the range of 100 to 500%, averaging 315% over a ten-year period (Akinnifesi et al., 2006). Increase in yield is more apparent from the third year after tree establishment and onwards (Akinnifesi et al., 2006). The unfertilised plots not amended with gliricidia had steadily declining yield, and amendment with N and P could not sustain high maize yield over time (Fig. 1). Continuously cropped maize plots without gliricidia or fertiliser declined steadily from 2 t ha^{-1} at the start of the experiment in 1992 to half a tonne in 2006. Unfertilised maize under gliricidia maintained yield at 3 to 4 t ha^{-1}. When the intercrop plots were amended with 46 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1} (representing 50% N and 100% P, respectively), there was a 79% increase in grain yield over the recommended practice, indicating complementarity between the applied fertiliser and organic inputs from gliricidia (Akinnifesi et al., 2007).

Similarly, in an on-farm experiment, 30% of the 40 on-farm type II farmers (farmer-managed trials) experienced increase in yield in the first two years, and 90% of these experienced yield increases in the subsequent two years (Akinnifesi et al., 2008). Yield increases in the third and fourth years averaged 69%. The authors observed that farmers with low yields in these early years were associated with poor field management conditions. Similarly, Makumba and Maghembe, (1999) reported yield increase in Makoka of 126% over three years for type I farmers' fields (researcher-managed), and an increase of 37% in on-farm type II averaged over five years. They attributed low response to erratic rainfall during the period.

3.2 Soil Health

Soil health has been broadly defined as the capacity of a living soil to function, within natural or managed ecosystem boundaries, to sustain biological productivity and diversity, maintain or enhance water and air quality, and promote plant and animal health (Doran, 2002; Sileshi et al., 2006a, b, 2008). In a global context, soil quality affects not only soil productivity but is also a significant factor governing environmental quality, and human and animal health and food safety and quality. Soil health is enhanced by management and land-use decisions that weigh the multiple functions of soil, and is impaired by decisions which focus only on single functions, such as crop productivity. Trees have been known to contribute to soil health in a number of ways: (i) enhancing soil physical structure and water regimes, (ii) improving soil chemical properties and nutrient input, (iii) increasing biological (microbial and faunal) communities, and (iv) suppressing soil pests. Several of these aspects of fertiliser tree management on soil health have been addressed in various studies in southern Africa. In the following section we will discuss these in detail.

3.2.1 Improvement in Soil Physical Properties

Among the commonly used indicators of soil physical health are soil depth and rooting, infiltration, bulk density, water-holding capacity, aggregate stability, and penetration resistance. Fertiliser trees improve soil physical properties due to the addition of large quantities of litter fall, root biomass, root activity, biological activities, and roots leaving macropores in the soil following their decomposition (Rao et al., 1997). In studies conducted in eastern Zambia, sesbania fallows significantly increased the percentage of water-stable aggregates (>2 mm) compared with continuous maize cultivation without fertiliser (Sileshi and Mafongoya, 2006a). In the same experiment after two years of cropping, significantly lower bulk density and higher porosity ($P < 0.05$) was recorded in pigeon pea and sesbania fallows than a monoculture maize (Fig. 3). Similarly, bulk density was higher under monoculture maize compared with maize grown in association with gliricidia and *L. leucocephala* (Sileshi and Mafongoya, 2006a). The fact that fertiliser trees consistently improve soil physical properties is seen from measured increases in infiltration rates (Fig. 2), soil penetration resistance (Fig. 3), and reduced runoff and soil losses (Nyamadzawo et al., 2007; Phiri et al., 2003). Treatments involving fertiliser trees (leucaena, gliricidia, sesbania) have consistently shown significantly higher infiltration rates than monoculture maize (Fig. 2). Increased water infiltration implies reduced water runoff and thus low soil erosion. Generally, plots under fertiliser trees had lower resistance compared with continuously cropped maize plots (Chirwa et al., 2003; Fig. 3).

In a study conducted in Kagoro in eastern Zambia, the soil in maize planted following improved fallows had lower penetration resistance compared with monoculture maize at all soil depths (Fig. 3). The lower values following planted fallows could be attributed to the high amounts of litter biomass left on the surface by the fallow species. The lower infiltration and high penetrometer resistance in the monoculture maize indicate soil compaction as a result of degradation of soil structure. The improvement in soil structure under fertiliser trees was evident, as reflected by the results from time-to-runoff studies (Phiri et al., 2003). Rainfall simulation studies (Nyamadzawo et al., 2007) also indicated that sesbania and gliricidia mixed with Dolichos increased infiltration rates significantly compared with continuously fertilised maize plots.

In another study, Chirwa et al., 2007 reported that gliricidia did not compete with maize in a gliricidia-maize intercropping system in Makoka. The water-use efficiency (WUE) was higher in the agroforestry system than sole maize or pigeon pea.

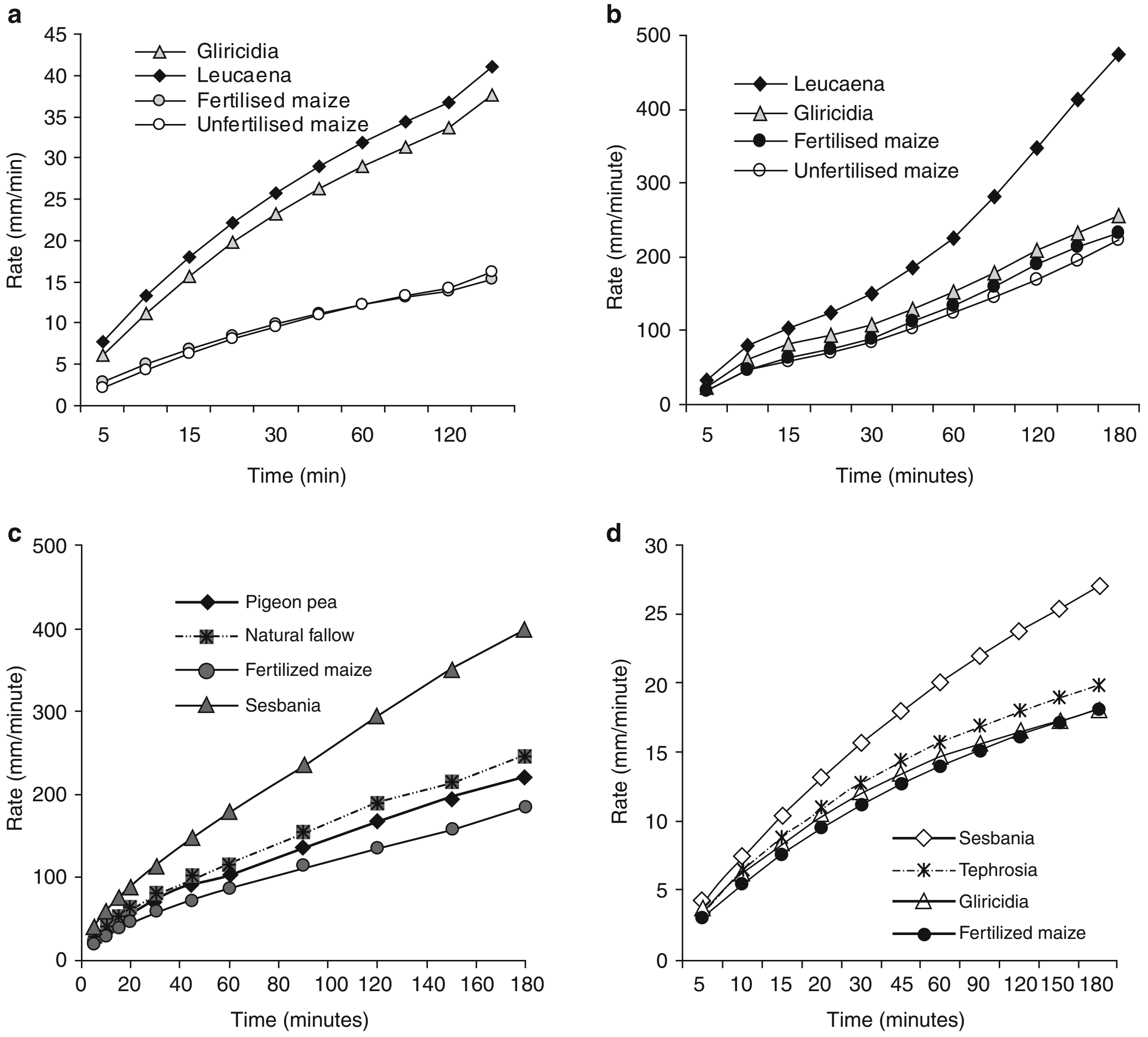

Fig. 2 Cumulative water intake in different treatments: (**a**) experiment 99-2, (**b**) experiment 2000-3, (**c**) experiment 92-2 in Masekera and (**d**) experiment in Kagoro (adapted from Sileshi and Mafongoya, 2006a and b; Chirwa et al., 2003)

3.3 *Improvement in Soil Chemical Properties*

Among the chemical indicators of soil health, total soil organic matter, the carbon to nitrogen ratio, carbon and nitrogen mineralisation rates, pH, electrical conductivity, and extractable N, P and K are commonly used. A recent study (Beedy, pers. comm., 2008) indicates that soil organic matter balance under gliricidia intercropping is positive following 14 years of continuous cropping. The study concluded that after 14 years, predictors of soil fertility and supporting soil organic matter fractions were significantly greater under the gliricidia-maize intercrop than under sole maize (Beedy, pers. comm., 2008). Both gliricidia intercrop and N fertiliser had a significant positive effect on dry season available N (Ikerra et al., 1999).

The legumes used in the sequential (e.g. fallow, relay) and simultaneous (e.g. intercrop) systems described above contribute to soil N through BNF and capture of subsoil N (otherwise unutilised by crops). Estimates of the amounts of N accumulated by fertiliser trees are given in Table 2. Out of the N accumulated, 55–84% is N derived from the atmosphere (Table 2). A series of multi-location trials were set up to measure the amount of N_2 fixed by different

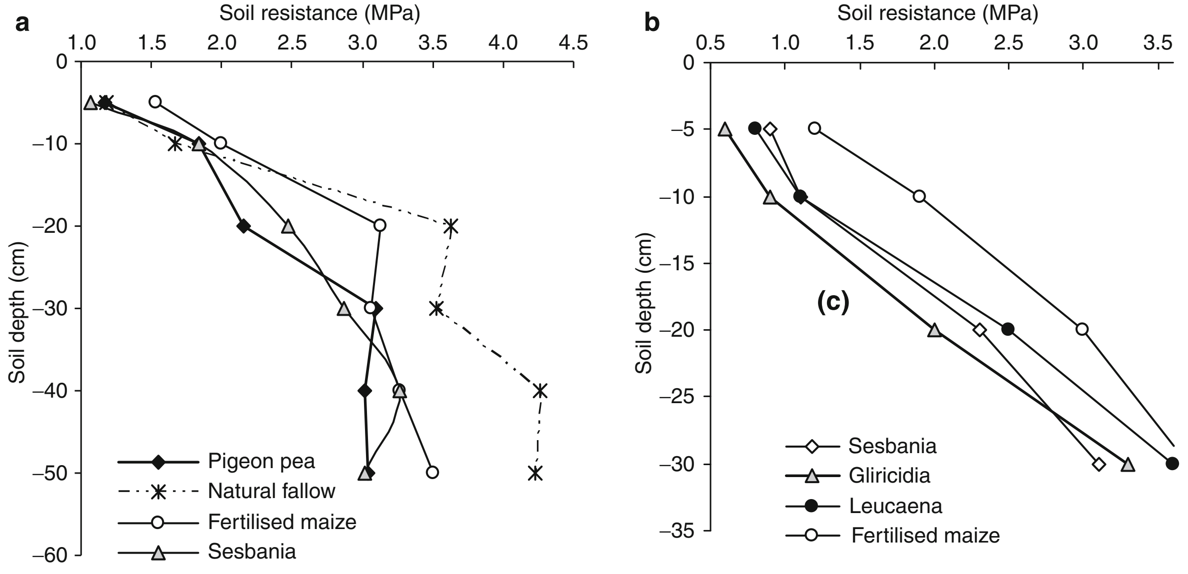

Fig. 3 Soil penetrometer resistance in different treatments: (**a**) in the 1999 cropping season in experiment 92-2 in Msekera (adapted from Sileshi and Mafongoya, 2006a) and (**b**) in Kagoro (adapted from Chirwa et al., 2003). Treatments significantly differed in 1998 ($P = 0.004$) and 1999 ($P = 0.018$)

Table 2 Amount of N fixed (kg/ha) and the percentage of nitrogen derived from the atmosphere (% Ndfa range) by fertiliser trees in southern Africa

Species	N fixed	%Ndfa	Site (Country)	References*
Acacia anguistissima	122	56–79	Chikwaka (Zimbabwe)	1
	210		Chipata (Zambia)	2
Pigeon pea	NA	65–84	Chikwaka (Zimbabwe)	1
	64	96–99	Nyambi (Malawi)	3
	85	94–97	Ntonda (Malawi	3
	34	66–96	Gairo (Tanzania)	3
	54	95–99	Babati (Tanzania)	3
Gliricidia sepium	212	NA	Chipata (Zambia)	2
Leucaena collinsii	300	NA	Chipata (Zambia)	2
Sesbania sesban	84	55–84	Chikwaka (Zimbabwe)	1
Tephrosia candida	280	NA	Chipata (Zambia)	2
Tephrosia vogelii	157	NA	Chipata (Zambia)	2

* References: 1. Chikwko et al. (2004); 2. Mafongoya et al. (2006); 3. Adu-Gyamfi et al. (2007).

tree genera and provenances using the ^{15}N natural abundance method in Zambia. The data shows high variability among species and varieties of the same species in percent N derived from the atmosphere (Ndfa). So the measurement task is still a challenging one (Mafongoya et al., 2006). Two-year tree fallows of the non-coppicing species sesbania and tephrosia are able to replenish soil N to levels sufficient to grow three subsequent high-yielding maize crops in southern Africa (Kwesiga and Coe, 1994). Unlike non-coppicing species, coppicing trees such as gliricidia and *Leucaena* spp. cause increases in residual soil fertility beyond 2–3 years because of the additional organic inputs that are derived each year from coppice re-growth that is cut and applied to the soil. The fertiliser value of total N was estimated to exceed 60–75 kg N ha^{-1} (Akinnifesi et al., 2008), which can replace the current need for mineral N. Some legumes were more effective in improving soil productivity and maize yield than others, probably due to differences in biomass production, N_2 fixation and recovery of leached nutrients.

Legumes can also have other beneficial effects on crop yield as they can improve availability and uptake of nutrients such as phosphorus. In small-scale farming systems in Africa, crop harvesting removes almost all of the P accumulated by cereal crops (Sanchez et al., 1997). Application of plant biomass from fertiliser trees as green manure can contribute to P availability, either directly by releasing tissue P during decomposition and mineralisation or indirectly by acting on chemical processes that regulate P adsorption-desorption reactions (Mweta et al., 2007). Table 3 presents the P and K input from biomass for different fertiliser tree species. Soil organic matter contributes indirectly to raising P in soil solution by complexing certain ions such as Al and Fe that would otherwise constrain P availability (Li et al., 2003; Mweta et al., 2007). Decomposing organic matter also releases anions that can compete with P for fixation sites, thus reducing P adsorption. The more extensive root systems that trees and shrubs have compared to crops increase the exploration of larger soil volumes, which results in enhanced uptake of P and other nutrients (Schroth, 1999).

Rotation of maize with legume fallows can result in more effective subsoil nitrate and water utilisation than maize monoculture (Chirwa et al., 2007; Nyamadzawo et al., 2007; Phiri et al., 2003). Where both soil organic matter and phosphorus are very poor, legumes may not accumulate a significant amount of biomass and will fix little N. To maintain positive nutrient balances for N and P in these environments, organic resources need to be combined with low rates of mineral fertiliser amendment (Ajayi et al., 2009).

The retrieval and cycling of nutrients from soil below the zone exploited by crop roots is referred to as nutrient pumping. Deep capture is favoured when perennials have a deep rooting system and a high demand for nutrients, when water or nutrient stress occurs in the surface soils, and/or when extractable nutrients occur in the subsoil (Buresh and Tian, 1997). These conditions were observed in eastern Zambia where nitrate accumulated in the subsoil during periods of maize growth. Fertiliser trees grown in rotation with maize could effectively retrieve the nitrate in the subsoil that is not accessible to maize (Mafongoya et al., 2006). Intercropping rather than rotating fertiliser trees with crops appears to improve the long-term efficiency of nutrient use in deep soils. The nutrient balance has been shown to be positive after 8–12 years of continuous cultivation with fertiliser trees such as gliricidia in Malawi and Zambia (Akinnifesi et al., 2007; Mafongoya et al.,

Table 3 Annual inputs of the major nutrient (kg/ha) from biomass‡ from fertiliser trees added to the soil

		Nutrient input				
Tree species	Tree management	N	P	K	Site	Reference
G. sepium	Coppicing	33.7	2.0	21.4	Muheza (Tanzania)	1
	Pollarding	71.9	4.4	45.8	Muheza (Tanzania)	1
L. leucocephala	Coppicing	65.6*	3.6	30.9*	Msekera 1 (Zambia)	2
	Coppicing	44.3†	2.5†	20.6†	Msekera 2 (Zambia)	2
G. sepium	Coppicing	69.9*	4.6*	26.2*	Msekera 1 (Zambia)	2
	Coppicing	69.2†	4.6†	25.9†	Msekera 2 (Zambia)	2
	Coppicing	72.1	-	-	Kagoro (Zambia)	3
	Coppicing	67.3	-	-	Kalunga (Zambia)	4
	Coppicing	74.4	5.2	42.5	Makoka (Malawi)	5
S. sesban	Non-coppicing	38.0	-	-	Chikwaka (Zimbabwe)	6
Pigeon pea	Non-coppicing	82.0	-		Chikwaka (Zimbabwe)	6
G. sepium	Coppicing	-	2.2	13.2	Msekera 3 (Zambia)	4
	Coppicing	-	4.3	25.3	Kalunga (Zambia)	4

‡ In the case of coppicing species this represents only coppice biomass, while in non-coppicing species both litter and standing leaf biomass are considered.

* Averaged over 9 years.

† Averaged over 5 years. Msekera 1 and 2 represent experiments 92-3 and 97-3, respectively.

†† References: 1. Meliyo et al., (2007); 2. Sileshi and Mafongoya, (2006a); 3. Chirwa et al., (2003); 4. Sileshi and Mafongoya, (2006b); 5. Akinnifesi et al., (2006); 6. Chikowo et al., (2004).

2006). Intercropping with fertiliser trees such as gliricidia may be more effective for pumping of soil nutrients than a fallow legume-maize rotation. The introduction of gliricidia with maize rotation has a great potential for deep capture of Ca and Mg compared with continuously fertilised monoculture maize.

3.3.1 Soil Biological Processes and Functions

Soil biological processes, mediated by roots, flora and fauna, are an integral part of the functioning of natural and managed ecosystems. Soil biota have been identified as potential indicators of soil health and sustainability at the farm level (Sanginga et al., 1992). These include microflora numbers, microbial biomass, enzyme activity and respiration, and soil fauna (abundance, diversity and community structure of soil arthropods, earthworms, etc.), as they respond sensitively to land management practices and correlate well with beneficial soil functions including water storage, decomposition and nutrient cycling, and suppression of pestiferous organisms.

Soil microflora such as fungi and bacteria are responsible for the breakdown of plant litter and most soil activities. Very few studies have examined the effect of fertiliser trees on soil biological properties. In a study conducted in Zimbabwe using leaf biomass of various fertiliser trees, microbial biomass carbon and nitrogen did not differ among treatments. However, fungal Actinomycetes populations differed with the biomass of legume species used as well as the method of biomass application (Mafongoya et al., 1997).

Among the macrofauna essential in soil processes in agro-ecosystems, probably the most important ones are the so-called ecosystem engineers (termites, earthworms and some ants), and the litter transformers including millipedes, some beetles and many other soil-dwelling invertebrates. Earthworms can be used as an integrative measure of soil health, assuming their importance in regulating soil processes which are vital to the continued formation of soil and as protection against soil degradation. These have been used to monitor changes in soil quality and to provide early warning of adverse trends and identify problem areas. In five separate experiments conducted in eastern Zambia, the number of invertebrate orders per sample and the total macrofauna (all individuals per square metre) recorded were higher when maize was grown in association with tree legumes than under fertilised monoculture maize. Similarly, densities of earthworm and millipede were also higher than under monoculture maize (Sileshi and Mafongoya, 2006a, b). Cumulative litter fall, tree leaf biomass, and re-sprouted biomass under legume species appeared to explain the variation in macrofauna densities (Sileshi and Mafongoya, 2007). Litter transformer populations were higher under gliricidia, which produced good quality organic inputs, than among the other fallow species. On the other hand, a higher population of ecosystem engineers was found under trees that produce poor quality organic inputs (Sileshi and Mafongoya, 2006a, b).

3.3.2 Reduction in Weed Problems

Declining soil fertility, along with the concomitant problems of weeds, pests and diseases is now a significant constraint to Africa's aspiration for sustainable development and food security (Sanchez, 2002). The declining soil resource base has also contributed to loss of biodiversity and persistent soil pest problems and weeds such as *Striga* spp. (witchweed). The effect of fertiliser trees on weeds and soil insects has been studied in eastern Zambia (Sileshi and Mafongoya, 2003; Sileshi et al., 2005, 2006). Abundance of *Striga asiatica* was significantly influenced by the quantity and the interaction effect of quantity and quality of biomass. Species that produce low to medium quantities of slow-decomposing biomass tended to reduce *striga* abundance in maize, while fast-decomposing ones did not (Sileshi et al., 2006). Similarly, in East Africa, reduction of another witchweed (*Striga hermontica*) by legume fallows depended on the rate of decomposition and nitrogen mineralisation of organic residues, which in turn was determined by quality in terms of carbon to nitrogen + polyphenol ratios (Gacheru and Rao, 2001). This indicates that the mechanism by which legume fallows influence striga is much more complicated than just by soil fertility improvement. Among the legumes tested, sesbania appeared to be the best in reducing striga infestation in maize in eastern Zambia (Sileshi et al., 2006).

Fertiliser trees have also reduced arable weed problems (Sileshi and Mafongoya, 2003; Sileshi et al., 2006). The mechanism by which legumes suppress arable weeds varies. Rotational fallows can modify the chemical ecology of the soil by releasing a range of volatile and water-soluble compounds that may act as germination stimulants or inhibitors. Chemicals such as nitrate and ethylene stimulate germination of numerous agricultural weeds. These compounds also sensitise weed seeds to other environmental factors such as changes in soil temperature and exposure of weed seeds to light (Sileshi et al., 2006).

3.3.3 Reduction in Soil Insects

Although termites are generally essential ecosystem engineers, some are also crop pests. Few, if any effective methods exist to control pestiferous species. Fertiliser tree systems generally reduce insect pests such as termites (Sileshi and Mafongoya, 2003; Sileshi et al., 2005). In a study conducted in eastern Zambia, Sileshi and Mafongoya, (2003) recorded lower termite damage (% lodged plants) on maize planted after tephrosia + pigeon pea, sesbania + pigeon pea and pure sesbania compared with maize grown after traditional grass fallow. Monoculture maize grown after traditional grass fallow had about 11 and 5 times more termite damage compared with maize grown after tephrosia + pigeon pea and sesbania + pigeon pea, respectively. In another set of experiments, Sileshi et al., (2005) monitored termite damage on maize grown in coppicing fallows. Those studies showed that fully-fertilised monoculture maize suffered higher termite damage compared with maize grown in gliricidia and *L. leucocephala*.

4 Adoption, Scaling Up and Impact

Given the biophysical performance and relevance of fertiliser trees in southern Africa, since the mid-1990s, emphasis on the system has shifted from purely on-station field trials to on-farm research, which allows incorporation of socioeconomic studies of adoption, profitability, labour, farmer perception and acceptability of different fertiliser tree systems under farmers' field conditions (Ajayi, 2007). The research for development efforts has therefore been expanded to address questions on farmer uptake, determinants of adoption and factors influencing farmers' decisions to adopt fertiliser trees, impacts of the technological innovations, and constraints and obstacles against adoption.

4.1 Adoption

A number of empirical studies have been carried out to gain insights into the factors influencing farmers' decisions to adopt fertiliser trees and the impacts that the technology has made on livelihoods and the environment in southern Africa. Using a logistic regression approach, Thangata and Alavalapati, (2003) investigated the adoption of mixed inter-cropping of *Gliricidia sepium* and maize in Malawi. Their results suggest that age of the farmer, frequency of contact with extension, and the effective number of household members who contribute to farm work are important variables determining the adoption of agroforestry. A study in Zambia (Keil et al., 2005) found that 75% of farmers who initially tested fertiliser trees eventually adopted the technology. Their study shows that scarcity of capital, inadequate access to markets for fertiliser and relatively low population density are the conditions that enhance the adoption of the technology. Studies on the use of labour in agricultural field plots in Zambia show that over a five-year period, the total quantity of labour used in fertiliser tree plots was 13% lower than unfertilised maize, and far less compared with fertilised monoculture maize plots (Franzel et al., 2002; Franzel, 2004). A study by Ajayi et al., (2007) found that aggregated over a five-year cycle, the total quantity of labour input used in fertiliser tree plots (improved fallows) was lower than in fertilised continuously cropped maize fields, but higher than in non-fertilised maize. These results do not lend credence to the notion that fertiliser trees are more labour-intensive given that the quantity of labour inputs used per unit of fertiliser tree plot area is not higher than in fertilised maize. Given the small plot sizes of fertiliser trees, estimated at an average of 0.2 ha only, farmers' decision to test fertiliser tree systems or not may not be attributed to the quantity of labour requirements. Rather, the popular perception regarding labour constraints and adoption of fertiliser trees in fertiliser trees may be due to the fact that some field operations may coincide with operations in other fields (especially cash crops) that are managed by the same households, and which depend on the same labour supply drawn from household members (Ajayi et al., 2009; Ajayi, 2007). This suggests that both the quantity and temporal distribution of labour input requirements are important factors in farmers' decision to adopt fertiliser trees. It is expected that as the land area that farmers cultivate to agroforestry increases, the temporal distribution of labour requirement for tree establishment and management may become more significant than it is presently. A modification to the agronomic practices of the technology to shift some of the labour inputs away from the main cropping season to the "off peak" labour demand season is expected to enhance the acceptability of fertiliser trees among farmers. Based on these and several other studies, the main factors that affect the adoption of fertiliser trees have been identified. These can be grouped into four categories: household-specific, technology-specific, institution and policy, and geospatial factors (Ajayi et al., 2007), further elaborated below.

Household-specific factors: These include farmer perceptions, resource endowment, household size (a proxy for household labour supply), risk, and access to information on inputs and output prices. These factors vary widely across households, resulting in different levels of uptake of fertiliser trees by different typologies of farm households. Those households who have access to a larger pool of labour supply, e.g. higher household size or land and other production inputs tend to have higher levels of adoption (Ajayi et al., 2006; Keil et al., 2005). While economic performance and short-term profitability of fertiliser trees enhance the probability of farmers' uptake, these alone do not provide an exclusive explanation for farmers' adoption patterns. Key attitudinal issues such as farmers' perceived usefulness of the technology (Ajayi, 2007), and household resource endowment are important for adoption. Although most options of fertiliser trees have positive net present values over time, some of them attain break-even point only after two years, implying that farmers make an upfront investment for a couple of years before receiving returns to their investment in the technology. This poses challenges to some types of farm households in southern Africa, who may not be sufficiently well off to absorb the initial investment and/or who may want to derive immediate benefits from the technology (Ajayi et al., 2007). For some farmers, a long "waiting period" can forestall the adoption of certain fertiliser tree technologies that guarantee high net returns in future.

Technology-specific factors: The technology-specific factors that affect farmers' uptake of fertiliser trees include the management regime required under some options as well as characteristics of particular fertiliser tree technology. Smallholder farmers more readily adopt specific options of fertiliser trees if such options produce grain that could be consumed or sold for cash income, in addition to replenishing their soils (Ajayi, 2007). Different types of fertiliser trees require varying amounts of labour and this plays an important role in their acceptability to farm households, depending on their internal labour endowment or ability to command additional labour from outside the household. In general, species that can be directly sown are much more preferred by farmers than those which require nursery establishment, transplanting, and other operations that add to the complexity of the options. Apart from the quantity of labour required to manage fertiliser trees, the temporal distribution of the same is also important for adoption (Ajayi et al., 2007). Fertiliser trees are an emerging technology relative to conventional agricultural practices that farmers have known, been used to, and have received training on for a much longer period. Unlike annual crop production technologies and conventional soil fertility management options, fertiliser tree systems require skills in terms of management of the trees.

In terms of profitability, fertiliser tree systems are profitable and have positive net benefits (Franzel et al., 2002; Franzel, 2004). A field study in Zambia (Ajayi et al., 2009) found that the net present value of maize plots amended with only fertiliser tree systems (US$233–309) compared well with a full fertiliser dose (US$349), and performed better than a continuous unfertilised maize plot; US$130 (Table 4). In addition, the return to labour in fertiliser trees is two times higher than in unfertilised fields (Franzel, 2004). Improved fallows require 13% less labour inputs per hectare than unfertilised maize and 33% less labour inputs than fertilised maize (Franzel, 2004).

Policy and institution factors: The policy and institution context within which fertiliser trees are disseminated plays an important role in affecting decision-making regarding the technology. Such factors include input and output prices, customary land-use practices, land tenure and property rights. Policy and institutions are cross-cutting and affect several farmers because the adoption of a relatively long-term technology such as fertiliser trees depends on incentives created by market and non-market institutions (Ajayi et al., 2007). National policies may modify the profitability of fertiliser trees, thereby altering their

Table 4 Financial profitability of maize production systems using tree fallows, fertiliser and farmers' practices in Zambia[†††]

Description of system	Benefit-cost ratio	Net present value (US$ /ha)	% increase in net profit over unfertilised maize
Continuous maize – non-fertilised	2.01	130	0
Continuous maize – subsidised fertiliser[†]	2.65	499	284
Continuous maize – fertiliser priced at market rate[††]	1.77	349	168
2-yr *Gliricidia sepium* fallow	2.91	269	107
2-yr sesbania fallow	3.13	309	138
2-yr tephrosia fallow	2.77	233	79

[†] Fertiliser subsidised by government at 50%.
[††] Fertiliser at market rates.
[†††] Figures are on a one hectare basis, at prevailing costs & prices and annual discount of 30%.

attractiveness and potential adoptability by farmers. Lack of access to quality seeds is one of the greatest constraints to fertiliser trees. Private sector organisations have not yet engaged in the multiplication and distribution of fertiliser tree seeds as done for the seeds of food crops such as maize. One of the reasons is that the market size and potential returns on investment in the latter is expected to be more rewarding for private entrepreneurs because more farmers currently grow maize than fertiliser trees. A profitability analysis conducted on fertiliser trees in Zambia showed that the four factors that most influenced the financial attractiveness and potential adoptability of the technology are external to the household, and most smallholder farm households have very little or no control over them (Ajayi et al., 2009).

Some local customary practices affect the nature of risk and potential adoptability of fertiliser trees. Field studies in Zambia show that bush fires and browsing constrain widespread adoption of certain fertiliser technologies (Ajayi and Kwesiga, 2003). Extensive browsing by livestock led to the discontinuation of the promotion of pigeon pea-based fertiliser trees in Zambia (Franzel et al., 2002). In addition, local customary practices and institutions (especially incidence of bush fires and browsing by livestock during the dry season, and absence of perennial private rights over land) prevailing in southern Africa limit widespread uptake of some agroforestry technologies (Ajayi and Kwesiga, 2003). Collaborative efforts initiated by traditional rulers, and research and development organisations to respond to these challenges have contributed to solving some of the constraints posed by these customary practices, e.g. through the enactment of bye-laws against the practices, but have not completely resolved them. Short-term customary land tenure creates a disincentive to longer-term investment in tree-based technologies.

Geo-spatial factors: There is a spatial dimension to the adoption of fertiliser trees in southern Africa, as the performance of the technologies varies with location, across crops and with time. Geo-spatial factors focus on the performance of species across different bio-physical conditions and site or village location. They include the type and characteristics of soils, which determine the bio-physical limits of technologies, access to roads and markets, and location of a village relative to institutions promoting fertiliser trees. The choice of species used for fertiliser trees is critical as the bio-physical performance and social-economic needs of different communities vary from one region to another. The establishment of proper targeting of fertiliser trees to geographic and social niches is an important factor that affects the relevance of the technology to farmers and that they create the desired impact among smallholder farmers.

The fertiliser tree system is financially profitable, but its widespread uptake by smallholder farmers may be constrained by challenges posed at the farm, household and policy levels as enumerated above. One of the important lessons learnt is that scaling up of fertiliser trees requires both vertical processes (to influence policies and institutions that are conducive for farmer adoption) and horizontal processes (to quicken the spread of the technology across communities and geographic boundaries). In addition, there is the need for appropriate structures that support the uptake of fertiliser trees. Such structures include the existence of strategic partnerships with several research, education and development institutions, and viable seed and output markets.

4.2 Biophysical and Socioeconomic Considerations for Proper Targeting

After two decades of research, it is known that the technical performance of fertiliser trees is important but not an exclusively sufficient condition to guarantee their adoption by smallholder farmers. A substantially large volume of new knowledge has been generated on where these legume-based technologies fit best within spatially heterogeneous landscapes. The most important considerations include:

1. Landholding size: In areas where landholding is a problem such as in the southern region of Malawi, Gliricidia is best suited as a permanent system where fallowing and cropping are concurrent. Improved fallows are not appropriate where landholding is small, such as southern Malawi where average holding is less than one hectare. The two- to three-year waiting period may also be a disincentive in land-pressured areas as farmers may be unable to allocate a separate field for fallows for such long periods. A well-designed simultaneous intercropping or relay fallow cropping system is ideal for such situations.
2. Waiting period: Where landholding size is bigger, many fertiliser trees and shrubs can be practised. For instance, faidherbia is recommended where a farmer can afford to wait for at least 12–15 years before getting soil fertility benefit. To reduce this period, we recommend that short-duration species be used as fertiliser during the first 10–12 years. Gliricidia and faidherbia are not recommended for farmers without permanent lands. On the other hand, farmers without permanent ownership of land, but access for a few years, would prefer short-duration species.
3. Land and tree tenures: Where the small landholding problem is also coupled with land tenure due to tree tenure, especially in matrilineal matrilocal systems, annual relay fallow intercropping with short-duration fallow species such as sesbania, *Tephrosia* spp. and pigeon pea become more attractive. In such matrilineal systems men are less motivated to plant permanent trees. Faidherbia and gliricidia are not recommended where land and tree tenures are a problem.
4. Nursery investment: *Tephrosia* spp. has the advantage that it could be sown directly without nursery investment. Because gliricidia is planted once, farmers who are not interested in annual or bi-annual nursery establishment will prefer gliricidia. However, because of the shortage of seeds and need for nursery establishment, many farmers are encouraged to use *T. candida*. This also means that planting of gliricidia, and especially faidherbia, must be started early before the season, as these require 6–8 weeks for gliricidia and sesbania and 9–12 weeks for faidherbia before transplanting. Where the season has already begun, only pigeon pea or *Tephrosia* spp. are feasible as they could be sown in the field directly.
5. Germplasm availability: Availability of tree seeds may limit the type of fertiliser tree technology to embrace. Gliricidia is more expensive and difficult to obtain in large amounts. On the other hand, *Tephrosia* spp., faidherbia, pigeon pea and sesbania are prolific seed producers. However, gliricidia can be established from stem cuttings.
6. Soil type and catena positions: The fertiliser trees have specific niches they perform best. The survival of gliricidia on wetlands is generally poor. Well-drained soils are better for gliricidia. Sesbania is well suited to both well-drained and wetlands. However, it does not perform well on sandy soils. *Tephrosia* spp. performs well on flat to upper slopes, like gliricidia.
7. The results from the meta-analysis indicate that they are generally best-performing in low to medium potential sites in terms of rainfall and fertility.
8. Pests and disease: Some species (e.g. sesbania) are susceptible to pests during the seedling stage.
9. Grazing problem: where livestock grazing is a chronic problem, sesbania, pigeon pea and gliricidia may not be successful. In that case, *Tephrosia* spp. is ideal. Also, communities could formulate bye-laws to deal with animal encroachment during and after cropping seasons.
10. In some cases, the biomass produced by fallows could be constrained by low soil fertility and a supplement with micro-doses of inorganic fertilisers, especially P, is worthwhile. The use of P fertilisers from inorganic fertiliser or rock phosphate has been recommended for poor P-deficient soils (Sanchez, 2002).

4.3 Impact of Fertiliser Trees on Livelihoods

A large body of literature has been generated since the 1990s, and new results, innovations and challenges have emerged. Conclusions from these clearly indicate that agroforestry is making a positive impact on the livelihoods of people who adopt the technologies, although much remains to be done in quantifying the impact. One of the major impacts of fertiliser trees is on food security through the increase in maize yield (see Sect. 3.3). For example, an extra increase in yield equivalent to between 54 and 114 extra-person days of maize consumption reduced the hunger period by 2–3 months per household in Zambia (Ajayi et al., 2007).

A monitoring and evaluation framework used with partners in five countries showed that the number of farmers benefiting from the technology increased from a few hundreds in the mid-1990s to more than 400000 by 2007 in the region (Schuller et al., 2005). An impact assessment in the region also indicated that farmers have generally increased the land under agroforestry, and appreciated that the fertiliser tree technologies have improved soil fertility for 59–84% of farmers, increased maize yield for 70–90% of practising farmers, improved food security and reduced hunger months by at least 2 months for 54–94% of farmers, and contributed to fuel wood availability for 54–90% of farmers, income generation for 53–68% of farmers, and other livelihood indicators (Table 5) as shown below.

Empirical studies in Zambia show that farmers appreciate fertiliser tree technology for its ability to respond to the critical problems of poor soil fertility, its effects on food security, and the additional benefits obtainable from fertiliser trees to households (Ajayi, 2007). They mentioned, however, challenges to the widespread uptake of the technology to include land constraints, land tenure rights, lack of tree seeds, and the knowledge-intensive nature of the technology.

5 Conclusion

From the discussion above it can be concluded that the fertiliser trees can sustain crop yield and deliver a range of other benefits that enable farmers to produce adequate food to feed their families. A variety of fertiliser tree systems have been developed to fit into different farming systems and farmers' socioeconomic circumstances. Although the technical performance of fertiliser trees is important, it is not exclusively sufficient to guarantee their adoption by smallholder farmers. Compared with technologies based on annual crops, the adoption of fertiliser trees will be slower because the technology involves multiple components and the multi-years through which testing, modification and uptake of the technology by farmers take place. Moving fertiliser trees to the mainstream requires approaches that overcome the major adoption hurdles discussed above: robust technology that fits farming systems, clear economic benefits from farmers' perspective, establishment of a sustainable germplasm base and input supply system, and a supportive macro-economic policy environment.

Table 5 Assessment of the impact of agroforestry adoption on livelihoods of farmers in Malawi, Mozambique and Zambia (Schueller et al., 2005)

Impact indicator	Malawi (n = 31)	Zambia (n = 184)	Mozambique (n = 57)
Increase in area under Agroforestry	55	87	65
Yield increases (>quarter to tripled)	70	90	71
Significant food security (>2 months of hunger reduction)	94	84	54
Increase in income	58	68	53
Firewood availability	90	nd[†]	59
Increased savings	87	94	71
Change in wealth	77	84	77
Strong reduction in *Striga* spp.	90	93	88
Soil improvement	84	82	59
Other benefits	65[††]	nd	24

[†] nd, not determined.

[††] Malawi, seed sale; Mozambique, tree stakes.

* Figures in table represent % of respondents.

Acknowledgements The authors wish to acknowledge with gratitude the financial support for the Southern Africa Agroforestry Regional Programme by various investors including the Canadian International Development Agency (CIDA), Irish Aid, Ireland, DFID, European Commission, Rockefeller Foundation, Swedish International Development Agency (Sida), USAID and NORAD.

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Cereal Landraces for Sustainable Agriculture

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Contents

Abstract Modern agriculture and conventional breeding and the liberal use of high inputs has resulted in the loss of genetic diversity and the stagnation of yields in cereals in less favourable areas. Increasingly landraces are being replaced by modern cultivars which are less resilient to pests, diseases and abiotic stresses and thereby losing a valuable source of germplasm for meeting the future needs of sustainable agriculture in the context of climate change. Where landraces persist there is concern that their potential is not fully realised. Much effort has gone into collecting, organising, studying and analysing landraces recently and we review the current status and potential for their improved deployment and exploitation, and incorporation of their positive qualities into new cultivars or populations for more sustainable agricultural production. In particular their potential as sources of novel disease and abiotic stress resistance genes or combination of genes if deployed appropriately, of phytonutrients accompanied with optimal micronutrient concentrations which can help alleviate aging-related and chronic diseases, and of nutrient use efficiency traits. We discuss the place of landraces in the origin of modern cereal crops and breeding of elite cereal cultivars, the importance of on-farm and ex situ diversity conservation; how modern genotyping approaches can help both conservation and exploitation; the importance of different phenotyping approaches; and whether legal issues associated with landrace marketing and utilisation need addressing. In this review of the current status and prospects for landraces of cereals in the context

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_10,

from Newton et al., Agron. Sustain. Dev. 30 (2010) 237–269. DOI: 10.1051/agro:2009032

of sustainable agriculture, the major points are the following: (1) Landraces have very rich and complex ancestry representing variation in response to many diverse stresses and are vast resources for the development of future crops deriving many sustainable traits from their heritage. (2) There are many germplasm collections of landraces of the major cereals worldwide exhibiting much variation in valuable morphological, agronomic and biochemical traits. The germplasm has been characterised to variable degrees and in many different ways including molecular markers which can assist selection. (3) Much of this germplasm is being maintained both in long-term storage and on farm where it continues to evolve, both of which have their merits and problems. There is much concern about loss of variation, identification, description and accessibility of accessions despite international strategies for addressing these issues. (4) Developments in genotyping technologies are making the variation available in landraces ever more accessible. However, high quality, extensive and detailed, relevant and appropriate phenotyping needs to be associated with the genotyping to enable it to be exploited successfully. We also need to understand the complexity of the genetics of these desirable traits in order to develop new germplasm. (5) Nutrient use efficiency is a very important criterion for sustainability. Landrace material offers a potential source for crop improvement although these traits are highly interactive with their environment, particularly developmental stage, soil conditions and other organisms affecting roots and their environment. (6) Landraces are also a potential source of traits for improved nutrition of cereal crops, particularly antioxidants, phenolics in general, carotenoids and tocol in particular. They also have the potential to improve mineral content, particularly iron and zinc, if these traits can be successfully transferred to improved varieties. (7) Landraces have been shown to be valuable sources of resistance to pathogens and there is more to be gained from such sources. There is also potential, largely unrealised, for disease tolerance and resistance or tolerance of pest and various abiotic stresses too including to toxic environments. (8) Single gene traits are generally easily transferred from landrace germplasm to modern cultivars, but most of the desirable traits characteristic of landraces are complex and difficult to express in different genetic backgrounds. Maintaining these characteristics in heterogeneous landraces is also problematic. Breeding, selection and deployment methods appropriate to these objectives should be used rather than those used for high input intensive agriculture plant breeding. (9) Participatory plant breeding and variety selection has proven more successful than the approach used in high input breeding programmes for landrace improvement in stress-prone environments where sustainable approaches are a high priority. Despite being more complex to carry out, it not only delivers improved germplasm, but also aids uptake and communication between farmers, researchers and advisors for the benefit of all. (10) Previous seed trade legislation was designed primarily to protect trade and return royalty income to modern plant breeders with expensive programmes to fund. As the desirability of using landraces becomes more apparent to achieve greater sustainability, legislation changes are being made to facilitate this trade too. However, more changes are needed to promote the exploitation of diversity in landraces and encourage their use.

Keywords Diversity • Disease • Yield • Quality • Nutrition • Breeding • Genotyping • Competition • Cultivar degeneration • Whole-plant field phenotyping • Non-stop selection • Adaptive variation

1 Introduction

Elite cereal cultivars are derived from a relatively narrow germplasm pool and are predominantly well adapted to high input agriculture. However, climate change will bring ever greater challenges in response to both biotic and abiotic stresses. Together with pressures to move towards more sustainable agriculture, there is clearly a need to access and exploit a broader germplasm resource. Cereal landraces are just such a resource which could be very valuable and yet apparently underutilised in contemporary agriculture. Landraces have closer affinity with modern cultivars than wild species and can more easily be used as a foundation material in breeding programmes. During the course of the EU COST Action 860 "Sustainable variety development for low-input and organic agriculture" (2004–2008), a working group on cereal landraces was formed comprising a variety of scientific expertise, linked by common agreement on the potential value of landraces as a resource for contemporary agriculture. In this paper we therefore bring together a review of recent literature on multiple aspects of cereal landraces, offering insights and future direction for their more efficient incorporation and utilisation in agriculture for low-input as well for more favourable environments.

Various definitions of a landrace have evolved since the end of the 19th century. Owing to their complex nature, Zeven (1998) concluded that an all-embracing definition cannot be given. More recently, Camacho Villa et al. (2005) faced with the challenges this creates for inventory and conservation purposes, proposed the following working definition: "a landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems". They additionally recognised that although the above characteristics are commonly present, they are not always present for any individual landrace.

A distinction is made between landraces and modern, or so called elite, cultivars, the latter being the result of formal crop breeding programmes. It is useful to consider the implications of this from the genetic and breeding points of view. Modern cereal cultivars are almost always bred to be mono-genotypic as inbred or pure lines for self-pollinating species, or one-way hybrids in maize, and thus are genetically homogeneous. They are bred to exploit high-input environments with increased yield levels and with an emphasis on broad or wide adaptation. In the European Union (EU) and other International Union for the Protection of New Varieties of Plants (or UPOV (French: *Uion internationale pour la protection des obtentions vtégétales*)) countries, modern cultivars are accepted for commercial cultivation after they have passed through the Distinctiveness, Uniformity, and Stability (DUS) and Value of Cultivation and Use (VCU) systems of evaluation trials. In contrast, cereal landraces are genetically heterogeneous. They comprise a large number of distinct homozygous lines in the case of self-pollinating crops or, in the case of cross-pollinators like maize, are populations with more heterozygous components. An important difference between genetic heterogeneity and genetic homogeneity is that the former involves genetic competition among plants, whereas the latter lack such competition (Fasoula and Fasoula, 1997).

Landraces were the principal focus of agricultural production until the end of the nineteenth century with the arrival of formal plant breeding (Harlan, 1975). Then, in more favourable environments, gradual replacement during the early decades of the 20th century by selected component inbred lines and modern cultivars led to their virtual disappearance. However, their cultivation persisted in less favourable environments and despite earlier predictions about their imminent disappearance (Zeven, 1998), landraces still support subsistence farming worldwide. This persistence is not due to increased productivity levels (Almekinders et al., 1994), but because of their increased stability, accomplished through generations of natural and deliberate selection for valuable genes for resistance to biotic and abiotic stresses and inter-genotypic competition and compensation. The components of a genetically diverse population such as a landrace, have a disease buffering effect in reducing the pathogen spread (Frankel et al., 1995). Several important resistance genes were first identified in cereal landraces such as the durable *mlo* mildew resistance gene in Ethiopian barley landraces (Piffanelli et al., 2004) and later introduced into elite germplasm. In general however, elite cereal germplasm has very few durable resistance genes and little resistance to emerging or increasingly important diseases such as *Ramularia collo-cygni*, Fusarium disease complexes and rusts in general, a good example being the new race of stem rust (*Puccinia graminis*) Ug99. Therefore exploitation of landrace diversity to identify relevant resistance genes is an important goal. In addition, many cereal landraces are known to possess broad natural variation in valuable neutraceuticals, which has been inadvertently impoverished during the breeding of modern cultivars (Monasterio and Graham, 2000; Murphy et al., 2008).

The important issue of increased yield stability attributed to the inherent heterogeneity of landraces (e.g., Simmonds, 1979) is nicely described by Zeven (1998) as: "Yield stability of landraces under traditional low input agricultural systems is due to the fact that whatever the varying biotic and abiotic stress for each plant, one or more genotypes within the landrace population will yield satisfactorily". This emphasises the urgency for solutions to combat production problems encountered through lack of spatial and temporal uniformity, particularly in stress-prone or marginal environments. A longer-term breeding goal, described below, would include the incorporation of those desirable genes found in different landrace genotypes into one or few superior plants with elevated stability levels (Fasoula, 2008). However, a single genotype must express greater trait plasticity to compensate for its inability to exploit heterogeneity in spatial and temporal dimensions as flexibly as multiple genotypes (Newton et al., 2009).

Awareness that future increases in productivity may depend on improved yields in the high-stress environments, has focussed breeding on specific or narrow adaptation and on the need for conservation of genetic diversity (Cleveland et al., 1999). Conventional plant breeding has been successful in favourable environments and those which can be made favourable through interventions, but it is less successful in low-input environments, characterized by increased G × E interactions, or in organic farming systems. In these cases, participatory breeding approaches with the involvement of local farmers can have a significant and positive influence, as will be discussed. A renewed focus on cereal landraces for breeding purposes could ameliorate some negative consequences of modern agriculture and conventional breeding, such as the liberal use of high inputs, the loss of genetic diversity (Tilman, 1996), and the stagnation of yields in less favourable areas (Annichiarico and Pecetti, 1998).

The role of genebanks and on-farm conservation practices in preserving landrace genetic diversity is increasingly important, particularly since the pivotal involvement of local farmers is continually declining due to the changes brought about by modern agricultural and socio-economic practices. Studies on landrace genetic diversity are a prerequisite for efficient conservation and management, registration purposes and effective use of landraces in breeding programmes. The considerable advances in molecular genotyping and databasing technologies in recent years are beginning to make the variation and resources of landraces more accessible for exploitation. High-throughput genotyping enables genebank accessions with uncertain provenance to be elucidated and thereby enable validation of associated phenotypic data, making them much more useful. In the quest to bridge the phenotype-genotype gap (Miflin, 2000; Parry and Shewry, 2003) and exploit landrace variation, recent advances in genotyping and whole-plant field phenotyping methodology are discussed.

2 History of Cereal Landraces

The Fertile Crescent and Turkey (in Asia Minor) is known as one of the important centres of diversity of many field crops, particularly wheat and barley, and therefore has considerable genetic diversity. Plant domestication from this region over thousands of years has also resulted in the development of enormous diversity. Progressive adaptation to a wide range of environments, responding to various selection pressures including biotic, abiotic and human intervention, has resulted in characteristic intra-specific diversity and differentiation (Teshome et al., 2001) represented by many landraces with specific histories and eco-geographical origins. To better understand the importance of these resources it is necessary to highlight their history.

Archeobotanical data shows that the first domesticated wheat species were einkorn (*Triticum monococcum* ssp. *monococcum*) and emmer (*Triticum turgidum* ssp. *dicoccon*). These evolved from their wild relatives (*T. boeticum* and *T. dicoccoides* respectively) about 10 000 years ago (Heun et al., 1997). Both species were the staple food of the human population until the end of the Bronze Age when naked *Triticum* species became dominant in agricultural lands. In a recent expedition of the Hellenic Genebank, at the edges of the centre of diversity in the northern eastern part of Greece in Thrace natural populations of *Triticum monococcum* subsp. *boeticum* and *Aegilops speltoides* progenitors of the genomes A and B were found in co-existence and in situ conservation has been planned (Kotali, 2008).

Durum wheat (*T. turgidum durum*) has been of great historical significance, because it provided a range of sub-species that were cultivated widely across the globe for thousands of years (Feuillet et al., 2007). Durum wheat spread out from the Fertile Crescent and through southern Europe, reaching north Africa around 7000 BC (Feldman, 2001). It came into cultivation originally in the Damascus basin in southern Syria about 9800 BC (Zohary and Hopf, 2000). A second route of migration occurred through north Africa during the Middle Ages (Moragues et al., 2006a). The beginning of modern agriculture transformed durum landraces in obsolete 'cultivars' which were gradually replaced by today's elite cultivars where only a fraction of the crop diversity is exploited (Feuillet et al., 2007). Nowadays, wheat landraces are kept and maintained in germplasm bank collections around the world, but are grown in practice only in the more marginal agricultural environments of their origins.

Bread wheat (*T. aestivum* L. subsp. *aestivum* L.) first emerged in cultivated wheat fields approximately 5–6000 years ago (Zohary and Hopf, 2000), resulting from a hybridization between *T. turgidum* L and *T. tauschii* L. The D genome of bread wheat originated from *Aegilops tauschii* and carried alleles adapted to the more continental climate and thus enabled bread wheat to be cultivated over more extensive geographic environments than emmer wheat (Feuillet et al., 2007). The D genome also encodes proteins that restore the softness of the grain endosperm (Chantret et al., 2005) thereby improving bread-making properties. Use for leavened bread production has contributed to its migration to Europe and subsequent widespread cultivation around the world with the development of several different landraces with a diversity of local adaptations driven by different climates and agricultural practices. Hexaploid bread wheat accounts for approximately 90% of world wheat production today (Feuillet et al., 2007).

Barley (*Hordeum vulgare* L.) also became domesticated in the Fertile Crescent, about 10 000 years ago, migrated through Europe, and local agricultural practices and natural selection have led to locally adapted landraces (Jaradat et al., 2004). Valuable information about domestication of cultivated barley, ecogeographical diversity and relevant issues are found in the recent book on barley diversity by Bothmer et al. (2003). In archaeological records, oats, along with rye, were found as a weed contaminant of wheat and barley samples. As a cultivated crop oats was present on a significant scale towards the end of prehistory, mainly in northern European regions (Moore-Colyer, 1995). Domestication of oats is complex, with independent domestication occurring at each ploidy level (Harlan, 1977). Britain was the main area of cultivation of the hexaploid oats. Until the early 1900's cultivated germplasm was landraces adapted to local growing conditions. Nowadays, a large number of modern cultivars trace back to a restricted number of landraces, such as 'Kherson' from which 14 cultivars developed by re-selection and another 80 had 'Kherson' in their pedigrees (Wesenberg et al., 1992).

Maize (*Zea mays* L. ssp. *mays*) was domesticated from its wild progenitor teosinte (*Zea mays* ssp. *parviglumis*) through a single domestication event, in southern Mexico, between 6000 and 9000 years ago (Matsuoka et al., 2002). After this initial event, introgressions from other teosinte types may have contributed to the maize gene pool and thereby help explain the remarkable phenotypic and genetic diversity (Matsuoka et al., 2002). The same authors suggested also that from early diversification in the Mexican highlands, two paths or lineages of dispersal occurred. One path traces through western and northern Mexico into the south-western USA and then into the eastern USA and Canada. A second path leads out of the highlands to the western and southern lowlands of Mexico into Guatemala, the Caribbean Islands, the lowlands of South America and finally the Andean mountains. There is evidence that these two maize germplasm pools were introduced to Europe at different times and locations. The first introduction came from the Caribbean germplasm, and remained confined to southern Spain probably due to poor adaptation to the mboxEuropean conditions as late maturing populations (Brandolini, 1969). From the 17th century onwards, the north American flint populations, relatively insensitive to day length and with low temperature requirements for flowering, were introduced in northern Europe. Since the introduction of maize to Europe five centuries ago, cultivated populations have evolved under the different selective pressures imposed by the environment and farmers. Adaptation to many environmental niches of European countries for many years explains the large variability and number of landraces which can be observed today (Gauthier et al., 2002).

Clearly landraces have very rich and complex ancestry representing variation in response to many diverse stresses. These are vast resources for the development of future crops deriving many sustainable traits from their heritage. How these resources can be made accessible and exploited will be addressed below.

3 Diversity and Germplasm Collections

3.1 *Bread Wheat Landraces Diversity*

Bread wheat landraces are characterized by their diversity and heterogeneity. However, this genetic diversity needs to be described and measured if it is to be used effectively in breeding and management of plant genetic resources. Traditionally used markers for the description of landrace genetic diversity are morphological and agronomic traits known as descriptors and established by the International Plant Genetic Resources Institute (IPGRI) (IPGRI, 1985). Among them plant height, flowering and anthesis time, spikelet and leaf emergence (Motzo and Guinta, 2007), grain size (Ferrio et al., 2007), grain yield and weight, spikes per unit area (Moragues et al., 2006a,b) and harvest yield index (De Vita et al., 2007) are considered to be the most important ones, and have been successfully used in the phenotyping of bread wheat landraces. Besides IPGRI descriptors other morpho-physiological traits have also been used (Autrique et al., 1996; Nachit et al., 1988; Dencic et al., 2000). Even though morphological descriptors are highly heritable characters and expressed in all environments (Frankel et al., 1995), they are limited in number, and agronomic traits are affected by environmental conditions.

In recent years several physiological, biochemical, molecular and technological traits have been commonly used to characterise bread wheat landraces and to asses their importance as a resource and for food security. Among the biochemical and physiological markers, carbon isotope (13C) discrimination (Ferrio et al., 2007), biomass accumulation (De Vita et al., 2007; Moragues et al., 2006b), storage protein patterns (Gregová et al., 1999, 2004, 2006), High Molecular Weight (HMW) glutenin subunits polymorphism (Caballero et al., 2001) and mineral content (Oury et al., 2006) have been evaluated. Technological parameters, i.e. grain hardness, starch and protein content, viscosity (Igrejas et al., 2002) have been applied also, providing different approaches to the evaluation of landraces diversity and quality. Although biochemical and molecular markers allow fast screening of landrace diversity to detect useful variation for breeding programmes (Gregová et al., 2006) or management of the genetic resources in germplasm collections (Dreisigacker et al., 2005), they are complementary to but cannot replace morphological characterisation (Moragues et al., 2006a; dos Santos et al., 2009; Zeven, 1998). Major difficulties arise if one attempts to interpret molecular or biochemical data generated from germplasm accessions in the absence of their morphological characterisation (Gregová et al., 2006; Zeven, 1998). Overall a significant decrease of genetic diversity has been observed related to the replacement of bread wheat landraces by elite cultivars which appears to be associated with loss of some quality traits such us protein content and glutenins quality (Gregová et al., 2006; Caballero et al., 2001). At the same there exist enormous gaps in our knowledge that needs to be fulfilled concerning landraces structure, within and among landraces diversity and useful traits.

3.2 Durum Wheat Landraces Diversity

Durum wheat is a primary cereal crop in several regions of the Mediterranean Basin, including the southern peninsular of Italy (Motzo and Guinta, 2007), southern Anatolia of Turkey (Akar and Özgen, 2007) and southern Spain (Ruiz and Martín, 2000). Durum wheat has a great economic importance due to the long tradition of pasta making in Italy (De Vita et al., 2007) and bulgur making in Mediterranean countries (Akar and Özgen, 2007). Archaeological findings suggested that bulgur and cracked wheat could be distinguished as two basic ingredients of Mediterranean cooking and bulgur was known at least since the 3rd millennium BC (Valamoti, 2002). A screen of Madeiran and Canary Islands wheat accessions showed that durum accounts only for 19 and 15% of the germplasm collections respectively (Andrade et al., 2007).

In these and other regions, durum wheat landraces are cultivated by farmers to a very limited extent (Moragues et al., 2006a; dos Santos et al., 2009; Ruiz and Martín, 2000; Zhang et al., 2006; Cherdouh et al., 2005; Teklu et al., 2005; Kebebew et al., 2001; Ben Amer et al., 2001).

As in the case of bread wheat, durum landraces have been replaced in modern farming systems by elite cultivars. The studies performed to evaluate the diversity of durum landraces are predominantly based on germplasm collections that preserve accessions of populations abandoned by farmers. Such studies show the existence of considerable crop heterogeneity and genetic variability (Medini et al., 2005; Masum Akond and Watanabe, 2005; Teklu et al., 2005; Queen et al., 2004; Pagnotta et al., 2004; Alamerew et al., 2004; Kebebew et al., 2001; Ben Amer et al., 2001). Among the DNA molecular markers, Randomly Amplified Polymorhic DNA (RAPD) (Mantzavinou et al., 2005), microsatellites or Simple Sequence Repeats (SSRs) (Zhang et al., 2006; Hao et al., 2006), Amplified

Fragment Length Polymorphisms (AFLPs) (Martos et al., 2005) and Restriction Fragment Length Polymorphisms (RFLPs) (Autrique et al., 1996) have been used to genotype durum wheat landraces. However, it is not always easy to determine which material authors have analysed. For example Ruiz and Martín (2000) showed that from 619 durum wheat entries of the Spanish Plant Genetic Resources Center (CRF-INIA (Spanish: Centro de Recursos Fitogenéticos - Instituto Nacional de Investigación Agraria)) collection, 428 were *turgidum* and 126 were durum accessions and claimed that they were representative of durum landrace diversity in southern Spain. However, no detailed information about the origin and sampling methodologies were given.

As a result of the increased economic importance of durum wheat, several breeding programmes aim to develop new cultivars and release old durum landraces (De Vita et al., 2007; D'Amato, 1989). In southern Italy local durum landraces of *Mediterraneum typicum* were cultivated until the beginning of the 1950 when a process of their replacement by modern cultivars began (Motzo and Guinta, 2007; Ruiz and Martín, 2000). As with bread wheat landraces, there has been a significant decrease of genetic diversity in this process which appears to be associated with some loss of quality traits (Oak et al., 2004; De Vita et al., 2007). Overall, it can be concluded that our knowledge on durum landraces need to be improved.

3.3 Barley Landraces Diversity

Barley landraces show stability under adverse climatic conditions. This is attributed to the heterogeneity present and that provides them with a buffering capacity. The 'within' as well as 'among' landraces diversity has been the subject of many studies, for example with respect to their agronomic traits, and morphological characters (Assefa and Labuschagne 2004; Abdellaoui et al. 2007). Biochemical markers used for assessing diversity (Kolodinska Brantestam et al., 2003) or complementing morphological data include isozymes (Jaradat and Shahid, 2006) and hordeins (Demissie and Bjørnstad, 1997). Molecular markers used include restriction fragment length polymorphisms (RFLPs) (Bjørnstad et al., 1997; Demissie et al., 1998; Backes et al., 2003), random amplified polymorphic DNA (RAPDs) (Abdellaoui et al., 2007; Manjunatha et al., 2007; Papa et al., 1998) microsatellites or simple sequence repeats (SSRs) (Yahiaoui et al., 2008; Feng et al., 2006; Hamza et al., 2004; Jilal et al., 2008), amplified fragment length polymorphisms (AFLPs) (van Treuren et al., 2006; Assefa et al., 2007) and Inter-Sequence Simple Repeats (ISSRs) (Kolodinska Brantestam et al., 2004).

A controversial issue of genetic diversity studies in landraces is the loss of genetic diversity overtime, as different trends in genetic diversity changes have been observed for different countries. A study carried out on barley germplasm derived from the Nordic and Baltic countries using ISSRs showed that while there were no significant changes of genetic diversity observed during the last century in the northern parts of this geographical region a significant decrease was observed in the southern parts (Kolodinska Brantestam et al., 2004). Similar results were obtained when biochemical markers were employed (Kolodinska Brantestam et al., 2003).

3.4 Oat Landraces Diversity

Oat landraces or oat mixtures were widely grown in Europe until early 1900, while in the USA most cultivars developed up until the 1970s traced back to only seven landraces introduced from Europe (Coffman, 1977). Oat landraces had fairly uniform morphological characters but were heterogeneous for alleles that conditioned reactions to diseases. The 14 cultivars developed as a single-plant selection from the uniform morphologically landrace 'Kherson' referred to above (Coffman, 1977) differed mainly in reactions to diseases and, to a lesser extend, morphological characters. Furthermore, from a single plant selection in a potato field in Cumberland, United Kingdom, the short straw 'Potato Oat' was obtained from which a number of sub-cultivar selections were isolated. We might therefore expect a rather narrow genetic basis to be present but the number of cultivars developed from 'Kherson' and 'Potato Oats' indicates otherwise.

In a number of experiments with many genotypes, morphological characters were shown to possess great phenotypic plasticity in their environmental response (Diederichsen, 2008; Katsiotis et al., 2009). Diederichsen (2008) tested 10 105 entries, including landraces, over four years in a single location and found eight environmentally stable characters, while during the RESGEN CT99-106 project (Katsiotis et al., 2009), 1011 entries were tested in four diverse environments over three seasons but only two stable characters were found, namely panicle shape and kernel covering. In both cases the morphological diversity within the oat accessions did not differ between landraces and modern cultivars.

Using molecular markers non-landrace entries showed the same polymorphism as landraces, with the majority of the AFPLP marker variation (89.9%) residing within accessions of each country, revealing the success of oat breeding programmes in maintaining genetic diversity within elite cultivars (Fu et al., 2005). Finally, in a study among Canadian oat cultivars released between 1886 and 2001 using 30 SSRs, a range of increasing and decreasing patterns of allelic changes was observed at different loci and significant allelic decrease was detected in cultivars released after 1970 coming from specific breeding programmes (Fu et al., 2005).

3.5 Maize Landraces Diversity

Maize domestication resulted from a single event involving its wild progenitor teosinte (*Z. mays* subspecies *parviglumis*), introgression from other teosinte types and the segregation into two European germplasm pools (see above) between which much hybridisation occurred. The idea of hybridisation rather than a slow northward dispersion accompanied by selection for earliness is supported in the case of Spain and Portugal where many maize landraces are still cultivated. The Iberian maize germplasm display no close relationship with any American types, sharing alleles with both Caribbean and North American flints (Rebourg et al., 2003; Vaz Patto et al., 2004). Other studies have shown that maize landraces can be distinguished by morphological and agronomic traits (Pinheiro de Carvalho et al., 2008; Brandolini and Brandolini, 2001; Goodman and Paterniani, 1969), biochemical traits such as zeins (de Freitas et al., 2005) or molecular markers (Reif et al., 2005; Rebourg et al., 2001; Gauthier et al., 2002). More recently SNP markers have been increasingly applied to study useful landraces (Tenaillon et al., 2001). These studies also show that a significant landraces diversity well adapted to agro-ecological conditions still exist in several countries (Pinheiro de Carvalho et al., 2008; Vaz Patto et al., 2007; Brandolini and Brandolini, 2001; Ruiz De Galarreta and Alvarez, 2001).

In summary there are many germplasm collections of landraces of the major cereals worldwide exhibiting much variation in valuable morphological, agronomic and biochemical traits. The germplasm has been characterised to variable degrees and in many different ways including molecular markers which can assist selection.

4 Genebanks and Conservation of Cereal Landraces

Throughout the centuries farmers have been the major guardians of genetic diversity. The importance of these crop resources for agriculture and food security was stressed in the International Agricultural Congress at Rome in 1927 (Zeven, 1998). The extinction of traditional farming systems, the aging and exodus of rural population, globalisation, and environmental degradation, have led to extinction of many cereal landraces and much of this diversity has been eroded. As a consequence, during the last century most of this unique cereal biodiversity has disappeared and the information regarding traditional cultivars is presently very scarce. According to FAO (1998), it is estimated that 75% of the genetic diversity of crop plants was lost in the last century. The erosion of these resources results in a severe threat to the world's long-term food security. Although often neglected, the urgent need to conserve and utilize landraces genetic resources as a safeguard against an unpredictable future is evident (Hammer et al., 1999).

The first organised attempts to conserve landrace resources by growing them on farm, (in situ conservation) were made in Austria, during the 1930s (Zeven, 1996). Nowadays the germplasm collections are major guardians of landraces diversity. Increasingly the new tasks of these genebanks are related to the conservation of plant resources and the need to keep accessions representing the landraces diversity and genetic structure (van Treuren et al., 2006). Cereal landraces represents a group of populations, sharing common morphological and agronomic traits, geographical origin and history, and uses (Camacho Villa et al., 2005). These populations are often composed of several genotypes, which together make up the cereal landraces characteristics (Jaradat and Shahid, 2006). For these reasons the genebanks need to collect or conserve rare alleles and avoid genetic drift when accessions are sampled both during field collection and sample regeneration (Mantzavinou et al., 2005). Recently, enormous effort has been made to capture biodiversity being lost by collecting as many germplasm accessions as possible from different geographic regions, especially from the rich centres of diversity. However, it seems that in many cases the sampling strategies were inadequate and the data collected incomplete and scarce, and insufficient attention was paid to ensuring the maintenance of the collected material throughout the lifetime of the genebank reducing the utility of the resource (Sackville Hamilton and Chorlton, 1997). Based on this evidence concern was expressed about the erosion of genetic diversity of the landraces held in the genebanks. It became evident that the management and research on biodiversity requires renewed approaches (Hammer and Gladis, 1996; Hammer and Spahillar, 1998). This problem is increased by the scarcity of knowledge about landrace structure or by the varying understanding of landraces definitions. During the last few years genebank management procedures, such as collecting strategies and techniques, conservation methods, monitoring viability, and regeneration strategies aiming at maintenance of integrity and characterisation of the accessions have been improved by IPGRI (2003). However a major effort is still needed to standardize methodologies used by different genebanks (Engel and Visser, 2003).

A second major problem is the undetermined level of duplicates within and between collections. In order to improve conservation efficiency there is an urgent need to rationalise collections by identifying and minimising unnecessary duplication (Dobrovolskaya et al., 2005) and to develop germplasm core collections, a concept proposed by Frankel and Brown (1984). The first step in the identification of probable duplicates is based on the available passport data (Hintum and Knüpffer, 1995), followed by characterisation data such as agro-morphological, molecular and protein traits. There is an absence of research dealing with this problem and little information is accessible on global information systems. However, there are good examples of research where germplasm collections have been evaluated identifying specific agronomic or quality traits for breeding purposes (Pecetti et al., 2001; Raciti et al., 2003) or assessing accession duplications. For example, Ruiz and Aguiriano (2004) confirmed the existence of 90% of the duplications among 106 cases in durum wheat collections with 266 accessions, using their gliadin patterns. Agro-morphological traits and biochemical or molecular markers are important tools in accession descriptions which will help to fill in information gaps which would otherwise diminish the ability to exploit such material by farmers and plant breeders. In principle, the better managed and more comprehensive a collection, the more valuable it is. The European Cooperative Programme for Plant Genetic Resources (ECP-GR, http://www.ecpgr.cgiar.org/) is a good example of a successful collaboration which should help ensure the continued protection and conservation of cereal landraces through standardised procedures, compatible data documentation systems, and compatibility with European frameworks for better management, study and exchange of resources. Valuable collections of cereal landraces are held at several European and world genebanks (see World Information and Early Warning System (WIEWS) on Plant Genetic Resources for Food and Agriculture (PGRFA), http://apps3.fao.org/wiews/wiews.jsp).

The Convention on Biological Diversity (CBD, 1993) recognizes the contribution of farmers to the conservation and development of genetic diversity. Sharing of benefits and the concomitant increased recognition of the value of the resources are the most effective ways to promote conservation and to ensure the continued availability of plant genetic resources. There is a need to make an economic evaluation of genebank conservation including the issue of benefit sharing but this has not received much attention among those formulating legal measures for the implementation of the CBD. Conservation involves both preservation and evolution. Therefore, ex situ preservation alone cannot provide the lasting benefits that accrue from the conservation of habitats and ecosystems rich in biodiversity (Swaminathan, 2002). This interaction among genebanks and those that are motivated to preserve the traditional seeds will be fundamental to overcoming the problems facing genebanks with seed regeneration, such as genetic drift, contamination and loss.

4.1 On-Farm Conservation

Two prevalent methods to conserve plant diversity are in situ and ex situ conservation. In ex situ conservation the genetic resources are conserved outside of their natural habitat (or cultivation territory) in identified genebanks. Unfortunately, in the ex situ conservation methods the variability that has been collected remains static because the natural evolution process is not allowed to continue (Dhillon et al., 2004). In contrast, in situ approaches to conservation are at the level of ecosystems and natural habitats, and include the maintenance and recovery of viable population of species in their natural surroundings, or in the case of domesticated species, in the surroundings where they have developed their distinctive properties. This approach involves two methods, (1) the genetic reserve and (2) on-farm conservation (Hawkes et al., 2002). The former is defined as location, management and monitoring of genetic diversity of natural wild populations within defined areas for active long-term conservation, for example the natural conservation sites in the near-east where the wild *Triticum* species are conserved in their place of origin. On-farm conservation is the sustainable management of genetic diversity of locally developed traditional crop cultivars along with associated wild and weedy species or forms within traditional agricultural systems. Such in situ methods, including on-farm conservation, have an advantage over ex situ methods since they provide a natural laboratory for evolution to continue and help the continued gradual build-up of traits imparting adaptation to specific ecogeographical regions and those matching the requirements of local tribes, communities and populations. New and more adapted types evolve and thus diversity is augmented. The need for on-farm conservation of original landraces is one of the most important recent questions in plant genetic resource management (Dhillon et al., 2004).

The ‘European Plant Conservation Strategy’ (Council of Europe, Planta Europea, 2001), the ‘European Community Biodiversity Strategy’ (European Commission, 2000), the ‘Convention on Biological Diversity’ (Convention on Biological Diversity, 1992) and the ‘International Treaty on Plant Genetic Resources for Food and Agriculture’ (http://www.planttreaty.org) all stress the need to improve the efficiency of conservation techniques, particularly those related to in situ conservation of endangered crops and crop wild relatives. However, little progress has been made on the methodologies for on farm conservation of plant genetic diversity (Maxted, 2003), especially in case of cereal landraces. Although there is a general agreement between conservationists on the fact that landraces should to be conserved on farm, i.e. in the place of their natural origin (Kovács, 2006b), in practice this is often very difficult.

In situ conservation programmes were initiated in most European countries (Maxted, 2003), using participatory approaches (see below), but with limited success. The main problems were that the original places are no longer agricultural land, that traditional farming systems and knowledge has disappeared in the given region, or that the in situ conservation is not economic for the farmers and therefore they do not participate in such projects (Kovács, 2006b).

Some very successful in situ conservation strategies have been established in some neighbouring countries. In case of cereals, Turkey, Israel and other countries of the Fertile Crescent have active genetic conservation programmes (Jaradat et al., 2004). One of the first projects was established in Ammiad to conserve wild wheat and wheat and barley diversity in its place of origin (Anikster and Noy-Meir, 1991). However, in Europe there are few such good examples. For example, the AEGRO (AGRI GEN RES 057) project which attempts to promote an integrative approach for the conservation of crop wild relatives and landraces in situ and on farm, such us in the case of Avena landraces. The Scottish landrace protection scheme was set up in 2006 to compile an Inventory of identified landraces and traditional cultivars which are still being grown and used in agriculture in Scotland providing a safety net for the continued use of landraces by storing seed produced by each grower each year (N. Green and G. Saddler, SASA, Edinburgh, personal communication). The farmers are an aging population and the future of Scottish landraces depends on the continued regeneration of the landraces, so if seed harvest fails, the landrace would otherwise be lost.

Clearly much landrace germplasm is being maintained across the world both in long-term storage in major collections and on farm where it continues to evolve, both of which have their merits and problems. There is much concern about loss of variation, identification, description and accessibility of accessions despite international strategies for addressing these issues.

5 Genotyping and Phenotyping

Although the diversity within landraces has been demonstrated to be a powerful means to improve barley yields in marginal environments in recent times (Ceccarelli, 1996), to fully realise and utilise the potential of such resources they need to be accurately and appropriately genotyped and phenotyped and the data made readily available in forms which can be easily interpreted by the plant breeding community.

The genetic structure of landrace collections, when linked to geographical and environmental data, may reveal genomic signatures of selection which are valuable information for breeding for specific environments, farming methods and users. For example, a collection of landraces was made in 1981 in Syria and Jordan from farmers who had been using their own seed for generations (Syrian Jordanian landrace collection (SJLC), Weltzien, 1988). These landraces, sampled from a gradient of agroecological conditions, showed a wide range of responses to drought stress as established by extensive field trials. Preliminary morphological evaluation revealed considerable variation between and within collection sites for many agronomically important characters (Ceccarelli, 1987) and disease reactions (van Leur, 1989). Russell et al. (2003) assayed genetic variability at 21 nuclear and 10 chloroplast microsatellite loci for a stratified subset of 125 barley landrace accessions from the SJLC collection, sampled from five different ecogeographical regions. Chloroplast polymorphism was detected, with most variation being attributable to specific differences between the five regions. A total of 244 nuclear alleles were detected, only 38 of which were common to the five regions sampled, most variation being within sites. There were strong associations between the chloroplast and nuclear SSRs and linkage disequilibrium, the non-random association of alleles, at both linked and unlinked SSR loci, clearly showing that these landraces have ‘adapted gene complexes’ which might be advantageous for breeding programmes and the genetic diversity and population structure was clearly driven by a drought gradient.

In another study of barley, Bjørnstad et al. (1997), compared cultivated accessions from Europe, north America and Japan with Ethiopian landraces, and found that the Ethiopian germplasm was significantly less diverse than the cultivated germplasm, but that it was also genetically more distinct. Similar studies to those in barley have been carried out in wheat and other cereals. Al Khanjari et al. (2007) surveyed Omani wheats using SSR markers and Stodart et al. (2007) used Diversity Array Technology (DArT®) markers to examine 705 accessions from the Australian Winter Cereals Collection and found much diversity, the latter study identifying Nepal as a unique gene pool of particular value.

An exciting development in the potential exploitation of landrace germplasm is the considerable interest in using association-based approaches to identify candidate genes or regions underpinning complex traits (Gaut and Long, 2003; Flint-Garcia et al., 2003; Gupta et al., 2005). Large-scale investigations of sequence variation within genes and across genomes have only just begun for plant species. Such studies are required to determine the distribution and extent of linkage disequilibrium, since this will determine the resolution power of association-based mapping strategies. From studies in other plant species it is clear that the natural decay of linkage disequilibrium with distance occurs at a considerably slower rate in inbreeding systems because effective recombination is severely reduced and genetic polymorphisms remain correlated over longer physical distances (Nordborg et al., 2002; Morrell et al., 2005).

Recently sequence diversity and patterns of linkage disequilibrium were investigated across a 212 kb region in cultivated, landrace and wild barley to determine the impact of inbreeding and evolution history (domestication and selection) (Caldwell et al., 2006). High levels of association were found to stretch across the whole region in the cultivated sample, with linkage disequilibrium values extended across the entire 212-kb region. In contrast, linkage disequilibrium and its significance decreased as a function of increasing distance in both landraces and wild barley (Fig. 1). These

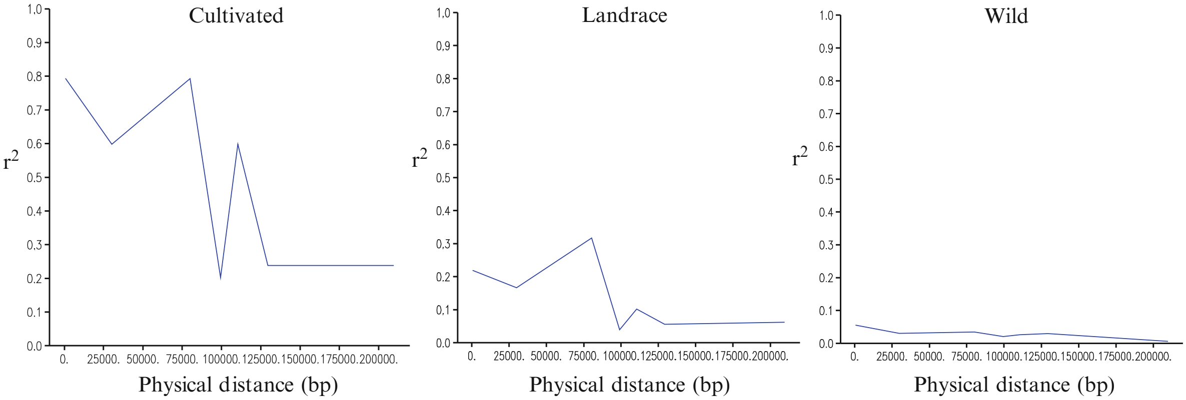

Fig. 1 Plots of linkage disequilibrium as measured with the commonly used statistic (r^2) (y axis) for each group of pairwise comparisons against the corresponding median distance for cultivated, landrace and wild barley accessions (Caldwell et al., 2006)

contrasting patterns exist despite similar levels of inbreeding and most likely reflect different population histories associated with the occurrence of bottlenecks and selection within the domesticated germplasm. Therefore, large linkage disequilibrium regions in cultivated, low-resolution whole-genome scans could be deployed to identify candidate gene regions; this would then be complemented by fine-scale, high-resolution linkage disequilibrium mapping utilising landraces and wild barley to identify candidate genes.

5.1 Genotyping Technology

The developments in association genetics have been facilitated through advances in DNA molecular marker technology reviewed above and by Buckler and Thornsberry (2002). An example of the latest technology is the high throughput Illumina ‘Golden Gate Assay’ SNP approaches which gives a high density of markers across many genotypes, enabling association genetic approaches to become highly effective. Such surveys provide information on genomic diversity, domestication and evolution, identify geographic regions, which contain high levels of diversity, and discriminate between groups of similar accessions. With the large amount of sequence information in barley, over 400 000 Expressed Sequence tags (ESTs), a platform was set up for high resolution genotyping known as Illumina Oligo Pool Assay (OPA) with 1536 Single Nucleotide Polymorphisms (SNPs) in each assay. Genes which are transcriptionally responsive to abiotic stress were chosen in particular for examining landrace populations.

A subset of the 176 landrace accessions from the SJLC referred to above were subjected to high throughput genotyping on the OPA platform and 72% of the 1536 SNPs were polymorphic and well distributed across the 7 barley chromosomes. Using the available phenotypic data also, whole genome association scans were used to identify and validate genes and markers linked to performance under drought stress (J. Russell et al., personal communication). Again five distinct groups clustering around key ancestors and regions of origin of the germplasm were identified, the accessions from North East Syria and South Jordan being particularly contrasting. Differences in the patterns of diversity between regions of origin were observed along the chromosome, highlighting selection signatures of adaptation to the environment and/or agronomic practices prevalent in these regions.

5.2 Genotype-Phenotype Association

Another example of strategies to combat drought stress using landrace germplasm is found in the work of Comadran et al. (2007) who identified barley genomic regions influencing the response of yield and its components to water deficits in a collection of 192

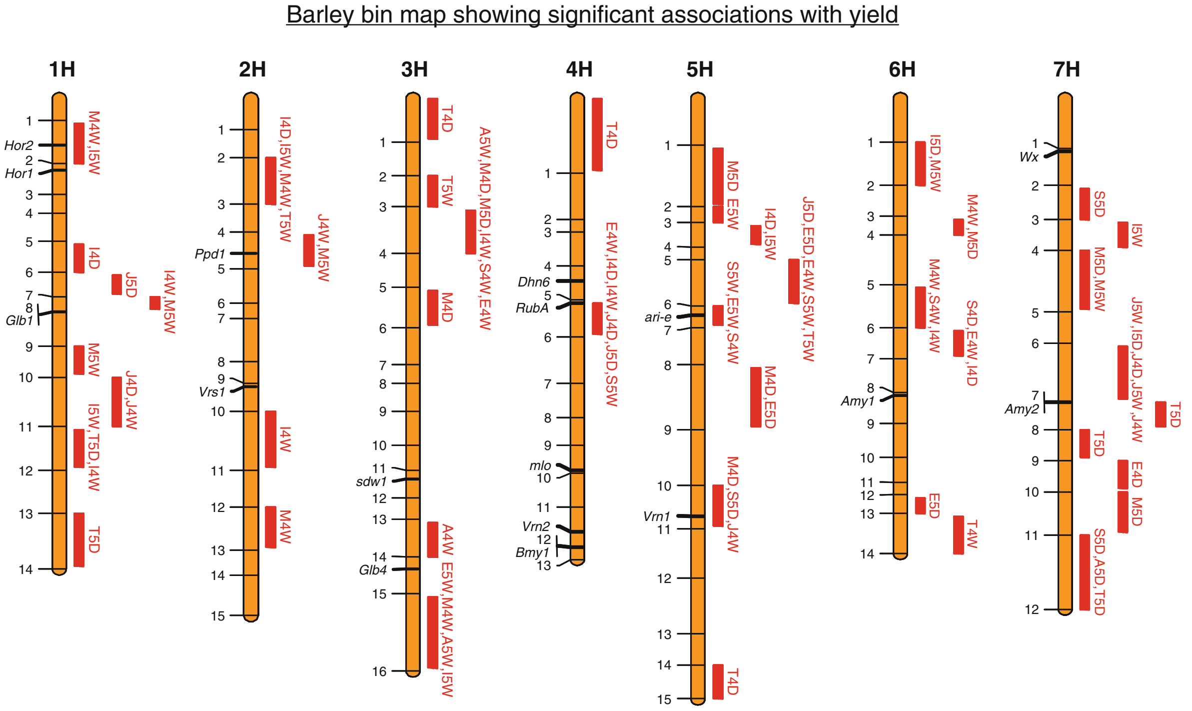

Fig. 2 Barley chromosome 'bin' map showing the location of phenotypes associated with yield under drought stressed environment conditions

genotypes that represented landraces, old, and contemporary cultivars sampling key regions around the Mediterranean basin and the rest of Europe. They used a stratified set of 50 genomic and EST derived molecular markers, 52 of which were SSRs, and 1131 DArT® markers which together revealed an underlying population sub-structure that corresponded closely to the geographic regions in which the genotypes were grown. The population was phenotyped for yield at two contrasting sites in each of seven Mediterranean sites for two years leading to marker-trait associations to understand the genetic and physiological dynamics underlying barley domestication and intensive breeding carried out in the last century and its relation to adaptation to drought. The yields observed for individual genotypes ranged from 10 t/ha to complete failure of individual genotypes to produce any seed due to the stress. As this was a highly structured sample, after accounting for this in the analysis, multi-environment QTLs were detected most frequently on chromosomes 3H, 4H, 5H and 7H (Fig. 2). One of the encouraging findings of this study was the detection of significant genetic variation for yield in the eight severely stressed environments where the mean yield was less than 2 t/ha, with one of the most consistent genomic regions being that on chromosome 7H, where four out of the five significant associations came from the Jordanian sites with mean yield ranging from 0.3 to 1.2 t/ha. The detection of QTLs in the low yielding environments offers the prospect of developing Marker Assisted Selection protocols for yield improvement in such situations.

5.3 Accurate Whole-Plant Field Phenotyping for Exploiting Variation Within Landraces

In plant breeding literature, the term "phenotypic selection" is often used interchangeably with "visual selection", even though the two are not synonymous. With the current rapid advancements in high-throughput molecular genotyping technologies described above, it is becoming increasingly clear that the limiting factor in applying those powerful technologies to molecular breeding programmes is no more the capacity of genotyping, but the potential for accurate or precision phenotyping (Campos et al., 2004; Fasoula, 2004). Phenotyping becomes a particular challenge when moving from qualitative to quantitative traits, like yield and stability, which have the greatest interest for breeders (Thomas, 2003). A way to bridge the genotype-phenotype gap (Miflin, 2000; Parry and Shewry, 2003) is provided when the unit of evaluation and selection in plant breeding becomes the individual plant (individual genome) and the confounding effects of competition and soil heterogeneity on selection efficiency are addressed with appropriate experimental designs (Fasoulas and Fasoula, 1995, 2000).

Fasoula (2004) demonstrated a methodology for accurate whole-plant field phenotyping using the analysis of crop yield potential, i.e. yield, stability, and responsiveness to inputs (Fasoula and Fasoula, 2002, 2003) which resulted in extracting superior lines from breeder's seed of two local barley (cv. Athenaida) and durum wheat (cv. Kyperounda) cultivars of landrace origin. An excerpt of this analysis is presented in Table 1.

Developments in genotyping technologies are making the variation available in landraces ever more accessible. However, high quality, extensive and detailed, relevant and appropriate phenotyping needs to be associated with the genotyping to enable it to be exploited successfully. We also need to understand the complexity of the genetics of these desirable traits in order to develop new germplasm.

6 Nutrient Uptake and Utilisation

Landraces have developed mostly in environments with low nutrients availability, and may therefore represent a source of variation for selection of varieties adapted to low fertiliser input cropping systems. While the literature on N and P uptake and utilisation of landraces is relatively rich, little has been documented for other nutritional elements. Landraces differ from elite cultivars in their heterogeneous genetic structure as well as for several typical morpho-physiological traits. However, the focus below will be on the morphological and physiological aspects associated with uptake and utilisation of nitrogen and phosphorus by cereal landraces.

The main factor determining nutrient uptake is the root system. This has been shown to be more developed in wheat landraces than in high yielding elite germplasm, especially semi-dwarf varieties (Siddique et al., 1990; Waines and Ehdaie, 2007). Good soil exploration by roots has been shown to be essential for absorption of phosphorus (Gahoonia and Nielsen, 2004a, 2004b) and nitrogen (Cox et al., 1985; Edwards et al., 1990; Feil et al., 1990; Laperche et al., 2006; Wieseler and Horst, 1994), though in the latter case the results are more divergent (Heuberger and Horst, 1995; Kuhlmann et al., 1989; Van Beem, 1997). Thus, appropriately selected landraces with well-developed root systems could be a source of variation for the improvement of nutrient uptake, but its use would require suitable methods for the assessment of the root system, which are still lacking.

Table 1 Ranking of selected lines within the local Cyprus durum wheat cultivar Kyperounda, of landrace origin, based on the analysis of crop yield potential for accurate whole-plant field phenotyping, using the R-19 honeycomb design, capable to evaluate 19 entries. The original cultivar Kyperounda (control) was assigned the design code 19 and ranked second last in terms of mean yield per plant

	Yield g/plant		Stability of performance		Responsiveness to inputs		Expected response to selection	
Line code	$\bar{x}$	%	$\bar{x}/s$	%	$(\bar{x}_{sel} - \bar{x})/s$	%	$\bar{x}(\bar{x}_{sel} - \bar{x})/s^2$	%
7	142.52^{a}	100	2.69	97	1.60	80	4.30	78
14	142.12^{ab}	100	2.28	82	1.76	89	4.01	73
5	140.87^{ab}	99	2.58	93	1.72	87	4.44	81
17	138.63^{ab}	97	2.78	100	1.78	90	4.95	90
Other intermediate lines								
6	123.26^{bcd}	86	2.44	88	1.77	89	4.32	79
13	123.09^{bcd}	86	2.29	82	1.72	87	3.94	72
3	116.89^{cde}	82	2.15	77	1.70	86	3.66	67
Kyperounda (19-control)	111.00^{cde}	78	2.03	73	1.67	84	3.39	62
8	101.10^{e}	71	2.44	88	1.78	89	4.34	79

Arbuscular mycorrhizas may considerably increase the active absorbing surface with minor cost for the plant, compared to the formation of roots and root hairs, enhancing P uptake and to a certain extent, uptake of other nutritive elements (Bolan, 1991). The degree of colonialisation has been shown to depend on the host genotype in wheat (Hetrick et al., 1993; Kapulnik and Kushnir, 1991; Manske, 1989, 1990; Manske et al., 1995) and barley (Baon et al., 1993). However, high colonisation rates are not always associated with correspondingly high symbiosis benefits for the plant as this also depends on the host genotype (Hetrick et al., 1993; Manske, 1989, 1990; Manske et al., 1995). There is evidence that certain landraces benefit more from symbiosis than high yielding elite cultivars (Kapulnik and Kushnir, 1991; Manske, 1990). Thus, improvement of the efficiency of symbiosis based on selected landraces might be possible.

Nitrogen-fixing bacteria in the rhizosphere are also important root symbiotic relationships for nutrient uptake, especially in Azospirillum species. These associations are also influenced by the host genotype and are particularly developed in several wheat landraces and wild ancestors originating from the southern Mediterranean basin (Kapulnik et al., 1983, 1985, 1987). Although the bacteria contribute to N nutrition of the host plants, the major benefit for the plant was from stimulation of root growth and thus drought tolerance (Kapulnik et al., 1983, 1985, 1987).

Landraces and old varieties are often later maturing than modern cultivars, especially those bred for dryer environments (Canevara et al., 1994). This may be of importance for the uptake of N in N-limited environments. If little or no N fertiliser is applied, N supply depends on mineralisation of soil organic matter, organic fertilisers and crop residuals. The time course of mineralisation is not always compatible with that of the crop's requirements (Panga and Lethaya, 2000) and varies greatly from year-to-year due to factors such as the weather and preceding crops. The potential uptake by cereal plants is mostly higher than the actual mineralisation in the soil, especially in the later growth stages (Baresel et al., 2008). Late-maturing genotypes may consequently absorb more N overall, and may therefore be better adapted to these conditions, if water availability is not limiting.

Under N-limited conditions, wheat landraces and varieties with a taller growth habit and lower harvest index have been shown to absorb and translocate more nitrogen into the grain than modern cultivars (Baresel et al., 2005). Figure 3 shows that old landraces and very old cultivars with similar morphological habit may absorb much more nitrogen under low-yielding conditions than modern cultivars and breeding lines. One reason might be greater pre-anthesis uptake and buffering capacity in genotypes with high vegetative biomass (Baresel et al., 2008), but this aspect has been little investigated. Nitrogen absorbed before grain filling is remobilised and then translocated to the grain after anthesis. Translocation efficiency might therefore also contribute considerably to efficient N utilisation. However, only small differences between genotypes could be detected in the absence of leaf diseases (Bertholdsson and Stoy, 1995; Johnson et al., 1967; Papakosta and Garianas, 1991; Pommer, 1990) and therefore genetic variation of translocation efficiency does not appear to offer much opportunity to improve N efficiency.

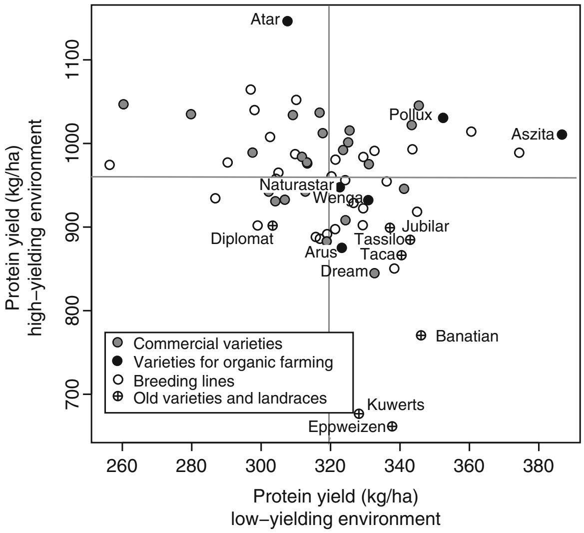

Fig. 3 Protein yields of modern varieties and breeding lines, varieties bred for organic farming and landraces or very old varieties in an environment with high and low average protein yield, respectively. The latter group have relatively high protein yields in the low-yielding environments, but low performance in the high-yielding environments. Results of a linear regression analysis on 70 genotypes in 9 environments; see Baresel et al. (2005) for details

There are many possible combinations of environmental factors which determine nutrient availability and landraces generally reach their genetic equilibrium in environments with reduced nutrient availability and variable conditions from year to year. They therefore show many different adaptive types

(Attene and Veronesi, 1991) and if maintained through on-farm conservation, will offer a valuable resource for finding ways of improving nutrient use efficiency without loosing genetic plasticity.

Nutrient use efficiency is a very important criterion for sustainability. Landrace material offers a potential source for crop improvement although these traits are highly interactive with their environment, particularly developmental stage, soil conditions and other organisms affecting roots and their environment.

7 Nutrition and Quality

Early landraces and wild species provide a broad representation of natural variation not only in agronomically important traits, but also in nutraceuticals, which have decreased during the breeding of modern cultivars. The nutritional status of the most important staple foods, such as the cereals is ultimately dependent on their metabolic composition (Galili et al., 2002). Nevertheless, while traits associated with yield and resistance have been the focus of most research, quality traits that are dependent on chemical composition are less well studied. There are some notable exceptions, such as protein content and structure of wheat cultivars and landraces (Láng, 2006; Rakszegi et al., 2006). Mineral content in modern wheat cultivars has significantly decreased including copper, iron, magnesiou, manganese, phosphorous, selenium, and zinc (Murphy and Jones, 2006). Looking for breeding sources for higher levels of iron and zinc has revealed that the highest levels can be found in landraces and low yielding genotypes (Monasterio and Graham, 2000). An ancestral wild wheat gene was found that accelerates senescence and increases nutrient remobilisation from leaves to developing grains. The gene was found in all wild emmer accessions and most domesticated emmer but not in durum lines or hexaploid wheat (Uauy et al., 2006). Cereal seed mineral content have been analysed in a wide range of cereal genebank accessions (Bálint et al., 2003) to find optimal sources to increase mineral content of modern wheat cultivars. Emmer wheat is a promising source of genetic variation for protein, zinc and iron content.

In bread wheat, the concentration of carotenoids is low, but they are more abundant in, for example, durum wheat, emmer and einkorn cultivars and landraces, having higher concentration in landraces then in cultivars (Panfili et al., 2004). Tocols, in contrast, are abundant both in bread wheat and durum wheat (Panfili et al., 2004). As carotenoid and tocol contents are independent traits (Hidalgo et al., 2006) that were not subject to conscious human selection and are selectively neutral, the wide range of natural variation still exists in the cereal genetic resources and landraces. In recent experiments bread wheat landraces, emmer and einkorn were found to be the best sources of tocols, while durum landraces and emmer were the best sources of carotenoids (Hidalgo et al., 2006).

Total phenolics in wheat varied both with cultivar and farming site (Gélinas and McKinnon, 2006). The phenolic compounds flavonoids, saponins, lignans and sterols are found in oat grain, but in minor quantities. Their concentrations are very low compared to avenantramides and tocols but have antioxidant and other bioactivitie properties (Peterson, 2004). Tocopherols and tocotrienols have higher concentrations in cultivars with high total lipids (Bryngelsson et al., 2002) and landraces of black oats have higher concentrations of antioxidants than elite cultivars (Mannerstedt-Fogelfors, 2001).

Maize contains appreciable amounts of carotenoids (Wurtzel, 2004) and has a greater total phenolic content and total antioxidant activity than wheat, oats or rice (Adom and Liu, 2002). In the maize kernel, tocopherol and oil content may be physiologically associated (Kurilich and Juvik 1999) while the natural variation detected in maize landraces kernel pigmentation may be associated with increased availability of certain antioxidant compounds. White maize polyphenolics have shown to have antioxidant and anti-carcinogenic effects (Del Pozo-Insfran et al., 2006). Blue, purple and red-pigmented maize kernels are also rich in anthocyanins with well-established antioxidant and bioactive properties (Del Pozo-Insfran et al., 2006). Purple maize, that has been cultivated for centuries in the Andean Region, is a good example of this (Pedrechi and Cisneros-Zevallos, 2006).

In general, cereal landraces and old varieties are among the best sources of phytonutrients accompanied with optimal micronutrient concentrations. Grains, fruits and vegetables contain a broad variety of phytonutrients, which show a significant effect on reducing the incidence of aging-related and chronic diseases. Among the numerous antioxidant compounds present in these foods, grain fat-soluble antioxidants and their unique bioactive compounds play an important role in disease prevention. The additive and synergistic effects of such phytochemicals in fruits, vegetables and whole grains are thought to be primarily responsible for their health benefits.

Landraces are clearly a potential source of traits for improved nutrition of cereal crops, particularly antioxidants, phenolics in general, carotenoids and tocol in particular. They also have the potential to improve mineral content, particularly iron and zinc, if these traits can be successfully transferred to improved varieties.

8 Biotic and Abiotic Stress Resistance and Tolerance

The replacement of landraces by homogeneous cultivars entails a significant loss of genetic variation for resistance to biotic and abiotic stresses. Elite cultivars may not possess the combined resistances already present in the landrace that they are intended to replace. Also, landraces might be a good reservoir of resistance mechanisms other than the hypersensitive mechanism typically exploited in modern cultivars due to its simple inheritance and complete expression. Most of the studies cited and others are either screening for qualitative resistance or more detailed mapping of the major genes responsible. There are few studies of landraces for partial and polygenic resistance due to the increased resources needed to obtain quantitative data. However, such studies are needed to determine whether multiple sources of partial resistance from such sources offer genes more likely to be durable than those in the elite gene pools. Below we will review the biotic stresses for just wheat and barley where landraces have provided valuable sources of resistance, then disease tolerance and some abiotic stresses for cereals in general.

8.1 Wheat Diseases

8.1.1 Septoria Leaf Blotch

The fungus *Mycosphaerella graminicola* (Fuckel) Schr. (sexual stage of *Septoria tritici* Rob. in Desm) or Septoria Tritici Blotch (STB) is currently a major disease of worldwide distribution. Most currently grown wheat cultivars are more or less susceptible to *M. graminicola*. The Italian landrace Rieti, an ancestor of many modern European wheat cultivars has been identified as very resistant to all studied isolates (Arraiano and Brown, 2006). The presence of *Stb6* gene in both European and Chinese landraces suggests that this gene has been present in cultivated wheat since the earliest times of agriculture (Chartrain et al., 2005). Resistance has also been found in Czech and Slovak landraces (Vechet and Vojácková, 2005).

8.1.2 Powdery Mildew

Powdery mildew of wheat is a foliar disease caused by the obligate biotrophic fungus *Blumeria graminis* (DC.) Speer f. sp. *tritici* (syn. *Erysiphe graminis* DC. f. sp. *tritici* G. Marchal) that can cause loss in both grain yield and quality. Screening of old wheat cultivars, landraces and related species for resistance to powdery mildew started in the 1930's (Hsam and Zeller, 2002) and *Pm* genes have since been identified in many different, widely distributed wheat cultivars and landraces. Non-major gene resistance has been sought such as the durable adult plant resistance found in the landrace accession k-15560 (Peusha et al., 2002).

8.1.3 Fusarium Head Blight

There is little resistance available to the major disease Fusarium Head Blight caused by *Fusarium graminearum* Sch. although some has recently been identified in landraces (Zhang et al., 2000).

8.1.4 Bunts and Smuts

Common bunts (*Tilletia foetida* (Wall.) Liro and *T. caries* (DC.) Tul.) are important diseases that are easily controlled by seed dressing, but might become more important in organic and low input agriculture. More than 15 resistance genes (*Bt1-15*) have been identified in wheat and the landrace PI178383, originally collected in Turkey, carries resistance genes *Bt-8, Bt-9* and *Bt-10* plus an unidentified factor (Goates, 1996). Resistance has also been reported in landraces of bread wheat (Hubert and Buertsmayr, 2006) and of durum wheat (Mamluk and Nachit, 1994). Karrnal bunt (*Tilletia indica* Mitra) is the most recently described smut of wheat and resistance has been identified in Indian landraces (Anon, 1943).

8.1.5 Rust Diseases

Stem rust (*Puccinia graminis* Pers.) resistance transferred to bread wheat from Yaroslav emmer (*Sr2* complex) in combination with other genes seems to have provided the foundation for durable resistance to stem rust in CIMMYT germplasm (Roelfs, 1988) in the last 50 years. Sources of durable resistance to stem rust in durum wheat have been reported, like the durum wheat Glossy Huguenot, effective in Australia over the past 100 years (Hare, 1997). Interestingly, its resistance is also quantitative and based on a reduced number of pustules in adult plants and a delayed onset of disease. As indicated by Roelfs (1988), of the 41 known genes for stem rust resistance, 20 originated in species other than *T. aestivum* and *T. turgidum*; of the 35 known genes for leaf rust resistance, 12 originated in species other than *T. aestivum* and *T. turgidum*. Among the genes originating from *T. aestivum* for resistance to either rust, a number of these are from landraces (McIntosh et al., 1998). The recently reported spread of the stem rust race Ug99 and the dependence of so many elite cultivars of wheat on the *Sr31* resistance gene has spurred renewed interest in surveying landrace collections for novel resistance sources (Bonman et al., 2007).

Durable resistance to leaf rust (*Puccinia recondita* f.sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson and *P. triticina* Erikss.) of wheat is thought to be more difficult to obtain than to stem rust but resistance against leaf rust has been identified that appears more durable than the norm. Resistance in the bread wheat cultivars Americano 44D, and Frontana, derived from resistant landraces, appears to be particularly durable. It appears that *Lr12* and *Lr13,* both genes for hypersensitive resistance that is expressed only in the adult plant stage, in combination with *Lr34*, are the basis of most of this resistance (Roelfs, 1988; Rubiales and Niks, 2000). Americano 44D, a Uruguayan landrace of unknown origin (called Universal 2 in Argentina), was used by Klein in breeding early Argentinean lines and is now considered another important source of durable resistance to leaf rust (Van Ginkel and Rajaram, 1992).

Durable resistance to yellow rust (*Puccinia striiformis* Westend.) has been described in wheat landraces from China, Italy and the Netherlands (Van Dijk et al., 1988; Zhang, 1995). Partial resistance combined with temperature-sensitive resistance have been suggested as the major components of the durable resistance found in old winter wheats in the Netherlands (Van Dijk et al., 1988). Sources of partial resistance to leaf rust have been recently reported in landraces from various origins (Fekadu and Parlevliet, 1997; Martínez et al., 2001a, b; Shtaya et al., 2006a, b).

Histological studies on interactions between plants and rusts can help both to discern the various resistance mechanisms and to combine them in a genotype in the hope to increase durability. The commonly used hypersensitivity resistance, typically conferred by single genes with race-specific effectiveness, is due to a post-haustorial defence mechanism. This type of resistance is very common in non-host interactions (Niks and Rubiales, 2002). It also is the mechanism responsible for the partial resistance of some wheat landraces to wheat leaf rust (Martínez et al., 2001c).

8.1.6 Aphids

Resistance to the Russian wheat aphid (*Diuraphis noxia* Kurd.) has been found in landraces of wheat from Iran and the former Soviet Union (Du Toit, 1987).

8.2 Barley Diseases

8.2.1 Powdery Mildew

In most barley growing regions powdery mildew (*Blumeria graminis,* (syn.: *Erysiphe graminis*) (D. C. Speer) f. sp. *hordei*) is very common. Intensive studies were carried out on barley landraces from Ethiopia (Negassa, 1985a, b), Jordan and Syria (van Leur, 1989) and other countries of the Near East (Weltzien, 1988), Europe (Honecker, 1938), India (Freisleben, 1940), Japan (Hiura, 1960) and world-wide (Moseman, 1955; Nover and Mansfeld, 1955, 1956; Hoffmann and Nover, 1959; Rigina, 1966; Wiberg, 1974a, b; Moseman and Smith, 1976;

Czembor, 2002). The main history of incorporation of powdery mildew resistance genes in cultivated barley and the exploration of their genetic diversity in Europe is described by Wolfe and Schwarzbach (1978).

The situation regarding diversity of genes for partial resistance to powdery mildew in cereal landraces is less clear. However, many assessments of partial resistance to pathogens in barley have been made with powdery mildew (Wright and Heale, 1984; Asher and Thomas, 1983, 1984, 1987; Anderson and Torp, 1986; Carver, 1986; Heun, 1986; Geiger and Heun, 1989; Newton, 1990; Kmecl et al., 1995).

8.2.2 Rust Diseases

Three rust species commonly occur on barley: leaf rust (*Puccinia hordei* Otth), stripe rust (*P. striiformis* West. f. sp. *hordei*) and stem rust (*P. graminis* Pers.: Pers. f. sp. *tritici* Eriks. et Henn.).

The origins of resistance sources were similar to those of mildew-resistant landraces from the Mediterranean region where both the host and the pathogen are indigenous and have co-evolved (Anikster and Wahl, 1979). Israel in particular is part of the centre of origin and genetic variation of wild native *Hordeum* species *H. vulgare* ssp. *spontaneum* Koch, *H. bulbosum* L. and *H. murinum* (Wahl et al., 1988; Kandawa-Schulz, 1996). Some interesting material has also been found in Azerbaijan and Turkmenia (Bakhteev collection) and Iran (Kuckuck collection) (Nover and Lehmann, 1974; Walther and Lehmann, 1980). Many studies described the activities in evaluation and in breeding for major genes and partial resistance (Clifford, 1985; Reinhold and Sharp, 1986; Yahyaoui et al., 1988; Khokhlova et al., 1989; Jin et al., 1995; Lukyanova and Terentyeva, 1997; Alemayehu and Parlevliet, 1997). Resistance against a new pathotype of *Puccinia hordei* with virulence for the resistance gene *Rph7* has been identified in barley landraces (Shtaya et al., 2006c).

Stripe rust is known in most of the barley growing regions. Screening for new sources of resistance has been carried out by many groups (Nover and Lehmann, 1966, 1970, 1975; Upadhyay and Prakash, 1977; Stubbs, 1985; Van Leur et al., 1989; Okunowski, 1990; Luthra et al., 1992; Hill et al., 1995). As with wheat rusts, much durable resistance is pre-haustorial and is known to be the mechanism responsible for the partial resistance of some barley landraces to barley leaf rust (Shtaya et al., 2006a, b). Sources of partial resistance to leaf rust (*P. hordei*) have been recently reported in barley and wheat landraces from Spain and from Fertile Crescent (Martínez et al., 2001a, b; Shtaya et al., 2006a, b).

8.2.3 Scald

Evaluations of barley for resistance to scald (*Rhynchosporium secalis* (Oudem.) J. J. Davis *f. sp. hordei*) have been carried out in many countries (Fukuyama et al., 1998; Yitbarek et al., 1998). Recent examples of such reports are: novel alleles at the *Rrs1* and other loci have been found for *Rhynchosporium secalis* resistance (Grønnerød et al., 2002; Bjørnstad et al., 2004).

8.2.4 Net Blotch

Studies of landraces for resistance to net blotch (*Pyrenophora teres* (Died.) Drechsl. f. *teres*) have been carried out by many scientists (Schaller and Wiebe, 1952; Buchannon and McDonald, 1965; Gaike, 1970; Smirnova and Trofimovskaya, 1985; Proeseler et al., 1989; Lukyanova, 1990; Faiad et al., 1996). Sato and Takeda (1994) studied the variation of host resistance of 2233 accessions of the barley world collection and found sources of resistance in accessions from Ethiopia, North Africa and Korea. New sources with resistance to up to eight races of *P. teres* were found among Peruvian landrace accessions (Afanasenko et al., 2000).

8.2.5 Barley Stripe

Many studies were conducted to identify new sources of resistance to barley stripe (*Pyrenophora graminea* Ito & Kuribayashi) including landraces (Baigulova and Pitonya, 1979; Nettevich and Vlasenko, 1985; Skou and Haahr, 1985; Van Leur et al., 1989; Su et al., 1989; Lukyanova, 1990; Bisht and Mithal, 1991; Ceccarelli et al., 1976; Kirdoglo, 1990; Skou et al., 1992, 1994).

8.2.6 Common Root Rot and Spot Blotch

Several germplasm collections have been evaluated and resistance to common root rot and spot blotch

(*Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur) has been identified from several resources (Banttari et al., 1975; Velibekova, 1981; Rochev and Levitin, 1986; Lehmann et al., 1988; Lukyanova, 1990; Gilchrist et al., 1995; Semeane, 1995; Faiad et al., 1996).

8.2.7 The Smuts

Three species of cereal smut attack barley: *Ustilago nuda* (Jens.) Rostr. (*U. segetum* var. *nuda*), *U. nigra* Tapke (*U. segetum* var. *avenae*) and *U. hordei* (Pers.) Lagerh. (*U. segetum*). Many studies on resistance to these pathogens were conducted (Shchelko, 1969, Nover et al., 1976; Damania and Porceddu, 1981; Onishkova, 1987; Dunaevskij et al., 1989; Surin, 1989; Lukyanova, 1990; Dubey and Mishra, 1992) identifying sources of resistance from landraces from Ethiopia, Yemen, Tibet, Canada and USA.

8.2.8 Fusarium Complexes

Many scientists described differences in fusarium resistance between cultivars (Grigor'ev et al., 1988; van Leur, 1989; Gu, 1989; Corazza et al., 1990; Khatskevitch and Benken, 1990; Lukyanova, 1990; Takeda, 1992; Filippova et al., 1993; Nelson and Burgess, 1994; Perkowski et al., 1995, 1997). Based on these reports it can be concluded that valuable sources of resistance were identified in the East Asian region landrace accessions in particular (Takeda and Heta, 1989).

8.2.9 Viruses

Yasuda and Rikiishi (1997) evaluated a total of 4342 barley accessions from the world collection on a field in Japan infected with strain I (Kashiwazaki et al., 1989) of BaYMV for resistance. The percentage of asymptomatic cultivars was highest among Ethiopian landraces followed by those from Japan. Cultivars showing severe disease symptoms were frequently found among Chinese, Nepalese, southeast Asian, north African, north American and European accessions. Field resistance to barley yellow dwarf was detected in several Ethiopian barleys (Schaller et al., 1964).

8.2.10 Aphids

The spring two-rowed barley RWA 1758 has been developed via selection from CIho 4165, a landrace originally collected in Afghanistan (Bregitzer et al., 2008).

8.3 Disease Tolerance

A character much neglected in elite breeding programmes is disease tolerance, not least because of the varied definitions of the term and the difficulty of measuring it (Bingham and Newton, 2009). However, interspecific variation has been found in cereals, for example powdery mildew-infected leaves of a wild oat showed a smaller reduction in net photosynthetic rate than a cultivated oat genotype under comparable infection severities (Sabri et al., 1997). The wild oat leaves also showed a slower rate of disease-associated senescence. In a comparison of wild and cultivated barley genotypes similar effects were reported (Akhkha et al., 2003) but the effect of this variation on tolerance at the scale of the crop canopy has not been determined. Several landrace accessions appear amongst the accessions screened for tolerance in barley (Newton and Thomas, 1994; Newton et al., 1998, 2000), though not disproportionately with more modern cultivars.

8.4 Abiotic Stresses

Generally the genus *Hordeum* shows a high degree of adaptation to different stressful environments.

8.4.1 Drought Tolerance

Drought is the most common abiotic constraint for stable barley production in rain-fed areas. Under Mediterranean conditions, water stress is particularly common at the end of barley life cycle (Passiuora, 1996). In comparison to other cereals, barley is well adapted to arid environments and the immediate progenitor of cultivated barley *H. vulgare* ssp. *spontaneum* can grow in desert condition (Nevo, 1992; Zohary and Hopf, 1998). Such ecotypes were identified in desert locations in Jordan (Jaradat et al., 1996). The study of drought stress on yield in Mediterranean environments noted above (Comadran et al., 2007) identified genomic regions in landraces that may be very valuable for combating such stress.

8.4.2 Frost Tolerance

Selection of highly frost tolerant lines from Turkish barley landraces has resulted in conversion of spring based production into winter based and enlargement of winter sown barley production in the Turkish highlands since 1940. Today 60% of barley production has been provided by winter sown barley, yield has doubled and these landraces have been routinely and widely used as parents in many breeding programmes in Turkey (Akar et al., 2009).

8.4.3 Salinity Tolerance

In the investigation of Abo-Elenin et al. (1981) 1163 entries were tested in the field and 777 in lysimeters. In this study ‘Abyssinia’ was the most tolerant. Mano et al. (1996) screened 6712 accessions for salt tolerance at germination. Accession variation showed a normal distribution and the most tolerant ones could germinate in sea water. However, six-rowed cultivars were more tolerant than two-rowed, hull-less than hulled, normal than semi-dwarf ‘*uzu*’, and winter than spring. In another study Mano et al. (1995) evaluated 5182 barley cultivars for salt tolerance at seedling stage. Generally the geographical differentiation among tested accessions was not clear.

Tolerance to salinity is more frequent in bread than is durum wheat, as bread wheat has a salt-exclusion mechanism but durum does not. This was found in landraces originating from saline areas of the Middle East and is conferred by *Nax* genes which therefore could be incorporated into both durum and bread wheat (Munns, 2005).

8.4.4 Acid and Alkaline Soils and Tolerance to Metal Toxicity

Cereal adaptation to acid and alkaline soils is limited by two major problems, the aluminium and manganese toxicity in acid environments and boron toxicity in alkaline ones. The soil acidity is a serious agricultural problem, affecting as much as 40% of the world’s arable land and up to 70% of the world’s potentially arable land (Kochian et al., 2005; Hede et al., 2001). Aluminium toxicity is a main growth and yield-limiting factor on soils with pHs below 5.0 (Davies, 1994), and can directly reduce yield by up to 60% (Tang et al., 2003). Amelioration of soil surface layer is not a reasonable solution in low input and organic agriculture, and because plant roots develop in lower acid layers to reach critical water and nutrient supplies. Selection and development of genotypes with enhanced tolerance to acid soils and toxic levels of aluminium is considered to be a more effective solution to this problem.

Cereal crops show very different responses to aluminium toxicity and soil acidity. The highest aluminium tolerance is detected among rye (Little, 1988), followed by oat (Slaski, 1992), wheat (Aniol and Madej, 1996), barley (Foy et al., 1965), and corn (Horst et al., 1997). Some experimental evidence shows a dramatic variation in aluminium tolerance among cultivars, which can be related to their genetic variability (Carver and Ownby, 1995). Landraces are important sources of this variability to improve the aluminium and acid soils tolerance in breeding programmes, but most research on aluminium tolerance has been carried out on elite cultivars or isogenic lines (Kochian et al., 2005) and there are very few studies to identify landrace tolerance for these traits (Pinheiro de Carvalho et al., 2003; Gudu et al., 2001; De Sousa, 1998; Cosic et al., 1994). Forty-eight accessions representing 16 Madeiran wheat landraces were screened for their aluminium tolerance using erichrome staining and root elongation (Pinheiro de Carvalho et al., 2003, 2004) and the accumulation of callose in the root types (dos Santos et al., 2005). The variability of landrace responses to the presence of aluminium and the existence of high performing accessions with better performance has been shown by comparison with elite cultivars such as Maringa. Durum wheat landraces show less variability and are moderately sensitive or tolerant to the presence of aluminium (Pinheiro de Carvalho et al., 2003; Cosic et al., 1994). De Sousa (1998) published a classification of aluminium tolerance of 76 wheat cultivars, including several landraces introduced in earlier twentieth century. The major sources of aluminium tolerance in wheat are considered to be originated from Brazil (Zhou et al., 2007). However, Stodart et al.

(2007) and Zhou et al. (2007), through the screening of wheat accessions from different countries showed the existence of potential new sources of aluminium resistance among the landraces germplasm originated from Bulgaria, Croatia, India, Italy, Nepal, Spain, Tunisia, and Turkey. The sources of aluminium tolerance in barley are limited to old cultivars and landraces, and represent multiples alleles of a single locus (Nawrot et al., 2001). The evaluation of corn germplasm, in two different studies screening of 76 accessions of unknown number of Kenyan maize landraces (Gudu et al., 2001) and 40 accessions of five Madeiran maize landraces (Pinheiro de Carvalho et al., 2004) also showed their high variability in aluminium tolerance, with several accessions presenting better performance then commercial standards.

The soil alkalinity also affects agricultural crops, growing in arable soils with pHs between 8 and 10. In barley, boron toxicity is directly responsible for yield penalties of up to 17% (Cartwright et al., 1984). The screening of 444 accessions of winter barley and 19 accessions of durum wheat, including landraces from Europe, west Asia and north Africa showed that boron tolerance is associated with geographic origin (Yau, 2002; Yau et al., 1995).

Landraces have long been assumed to be valuable sources of resistance to pathogens and the literature demonstrates that there is much to be gained from such sources. Transfer of resistance genes from landraces to modern cultivars is likely to be less problematic than from wild accessions.There is clearly also potential, largely unrealised, for disease tolerance and resistance or tolerance of pest and various abiotic stresses too including to toxic environments.

9 Breeding: Conversion of Landraces into Modern Cultivars

In this section the advantages of breeding cereal landraces, the methodology of improvement and modifications of breeding assumptions will be proposed.

9.1 Yield-Based Selection

Breeding from landrace accessions is a strategy being used to improve yield and yield stability in less favourable agricultural system with lower input levels. Heritabilities are higher in more favourable than poor environments (Blum, 1988). The stagnation of yields in these areas (Annicchiarico and Pecetti, 1993) is mainly related to the narrow genetic base of the more recently bred, high-yielding cereals (Pecetti et al., 2002). Two different approaches may be followed to raise yields in the long-term: one is based on increasing yield potential of broadly adapted cultivars, while the other relies on the better exploitation of the adaptive features of genotypes by fitting cultivars to specific target environments (Acevedo and Fereres, 1993). Improvement of grain yield potential in small-grain cereals has traditionally relied on direct selection for this trait (Annichiarico and Pecetti, 1998). Traditional breeding is based on a combination of bulk-pedigree method of selection, applied selection in the presence of stress, and use of adapted germplasm (Ceccarelli and Grando, 1997).

The opportunity to complement traditional breeding with use of indices of indirect selection for yield including sets of morpho-physiological traits, also known as an analytical breeding, has been put forward, especially for less favourable regions (Richards, 1982). There are two strategies in analytical breeding for identifying morpho-physiological traits usable as tools for selection (Fischer, 1981; Jackson et al., 1996). The first, called the ‘black box’ strategy, consists of assessing a germplasm pool for correlated response to yield gain deriving from selection for sets of putatively useful traits. The second, defined as the ‘ideotype strategy’, is based on the assessment of traits chosen a priori, through comparison in isolines or prediction of performance in crop growth models of different trait levels (Annichiarico and Pecetti, 1998).

9.2 Adaptability

In landraces an understanding of the relationship between amount of genetic diversity expression of morphological and agronomic characters and adaptation to stress environments may elucidate whether the success of landraces in less favourable areas is due to a population buffering mechanism or to a particular architecture of morpho-physiological traits, or both. This may in turn clarify whether ‘pure line breeding’ is the correct approach for less favourable areas (Ceccarelli et al., 1987). Pure line breeding can be successful only if genotypes with a very high degree of phenotypic plasticity are identified.

Evans (1980) pointed out that selection for adaptation may result in yield increases but may not represent selection for greater yield potential. However, it has already been shown for durum wheat (Pecetti et al., 1992) and barley that some of the material selected under unfavourable conditions is able to retain its superiority in a more favourable environment (Ceccarelli et al., 1991). For barley the proportion was about 20% of the selected genotypes, and for durum wheat about 30%. In both cases such a proportion was higher than the proportion of lines selected under favourable conditions which were also able to perform well in a less favourable environments, and this is in agreement with previous observations (Pecetti et al., 1994).

Breeding for specific adaptation is particularly important in the case of crops predominantly grown in unfavourable conditions, because unfavourable environments tend to be more different from each other than favourable environments (Ceccarelli and Grando, 1997). The specific adaptation strategy may be explored on the basis of yield response of the germplasm pool that is representative of the available genetic base tested across a representative sample of sites within the target region (Annichiarico, 2002).

9.3 Conversion into Density-Neutral Modern Cultivars

Inter-plant competition, i.e., the unequal sharing of growth resources due to genetic (pre-existing) or acquired differences among plants, can be quantified by the drastic increase in the coefficient of variation (CV) of individual plant yields in the crop stand (Fasoula and Fasoula, 1997). The difference between the genetically heterogeneous landraces and the genetically highly homogeneous modern cultivars means that a landrace stand involves genetic competition among plants, whereas a modern cultivar stand is devoid of genetic competition. A systematic study of the relationship between yield and competitive ability within a bread wheat cultivar (Fasoula, 1990) found a high and significant negative correlation ($r = 0.94$). The study demonstrated that highly competitive plants, i.e., those yielding less at the ultra-low planting density (1 plant/m^2; absence of competition), out-yielded the low competitors in mixed stands, i.e., plants yielding more at the ultra-low density. Conversely, in pure stands, the performance of highly competitive genotype plants lagged behind that of the poor competitors.

Genetic heterogeneity, such as that found in landraces, involves genetic competition among plants, in addition to acquired competition, which is also encountered in stands of single genotypic cultivars. In principle, genetic competition can be eliminated when landraces are converted into desirable homozygous lines, but the acquired competition is more difficult to control. The intensity of acquired competition increases in marginal or low input environments, which possess inherent heterogeneity in the distribution of resources. The above offers an insight into the reasons that led to the eventual replacement of landraces by pure single genotype modern cultivars in favourable environments and their persistence in marginal environments. In favourable environments, competition in the crop stands is reduced because of elimination of both the genetic (single genotype) and the acquired (ample resources) components. This results in increased crop yields, reflected in the reduced CV of individual plant yields (Fasoula and Fasoula, 1997; Tollenaar and Wu, 1999).

Yields of modern single genotype pure cultivars can be either density-dependent as in the case of maize hybrids that yield optimally under high plant densities only (Duvick, 1992), or density-neutral, i.e., remain optimal under a wide range of plant densities. With appropriate breeding methodology, it is eventually possible to convert the density-dependent into more density-neutral cultivars (Fasoula and Fasoula, 2000; Tokatlidis et al., 2001). To effectively exploit landrace diversity for breeding purposes, it is important to appreciate the significance of creating more density-neutral genotypes, particularly for marginal environments, which usually suffer from drought stress. In drought-prone environments, the use of more density-neutral cultivars allows use of lower seeding rates, limiting the damage due to drought. A subtle point is the understanding that density itself is not a stress; it simply enhances the existing differences that lead to competition in the stand. Therefore, particularly in more uniform environments, it is possible to have a high yielding dense stand with reduced inter-plant competition, as measured by the reduced CV of individual plant yields.

There is a lot of evidence that mixtures can be valuable, especially when combining various disease resistances of the components. A word of caution is, however, presented as to the interpretation behind the phenomenon. In favourable environments, the evidence of yield stability due to heterogeneity is often counter-balanced by experiments and theory, indicating that increasingly homogeneous cultivars have higher yield potential across sites and years. A case in point is the well-known, superior stability of performance of the genetically homogeneous modern single-cross maize hybrids over the older genetically heterogeneous double-cross hybrids (Duvick, 1992), because the adverse effects of genetic competition are restricted by genetic uniformity. This hints to the non-universality of the superiority of populational (Allard and Bradshaw, 1964) buffering. However, the requirement for superior individual buffering is that genes conferring tolerance to the biotic and abiotic stresses are being gradually incorporated into a few or a single individual(s). Further, the cause of the superior stability of certain mixtures has been indicated (Fasoula and Fasoula, 1997) to mainly rest in the reduced stability of the individual components (quantified by the higher CV of single plant yields in pure stands), appearing as reduced under-compensation in the mixtures, mimicking the effects of true over-compensation (pseudo-overcompensation).

9.4 Seed Degradation – Cultivar Degeneration

An additional detrimental effect of the negative correlation between yield and competitive ability relates to the observed landrace or cultivar "seed degradation". Zeven (1999) provides an interesting array of evidence about what he calls the often inexplicable seed replacement by traditional farmers. A traditional practice to combat seed degradation has been the periodic seed replacement of farmers' own seed with seed from elsewhere. Zeven (2000) reports a widely existing belief that the home-grown cultivar degenerates after several generations of re-sowing. He further states (Zeven, 2000) that most farmers do not actually perform traditional maintenance breeding; as they and their ancestors probably have experienced that traditional maintenance breeding does not result in a better crop. Apparently, "farmers must have thought that seed replacement was a better method to maintain the yielding capacity of their crops".

A proposed explanation for the practice of "inexplicable" seed replacement and the avoidance of traditional maintenance breeding by farmers relates to the consequences of the existing, but mostly unsuspected, negative correlation between yield and competitive ability. The problem of cultivar or landrace degeneration (Fasoula, 1990) can be addressed by applying the concept of non-stop selection (Fasoula and Fasoula, 2000) for superior lines at ultra-low plant densities. The outcomes of non-stop selection exceed that of conventional maintenance breeding (Fasoula and Boerma, 2007). Experimental data of honeycomb selection within breeder's seed of the old barley cultivar Athenais and the old durum wheat cultivar Kyperounda, both of local landrace origin, demonstrated the existence of useful adaptive variation persisting within homozygote lines, as well as the potentialities of non-stop selection (Fasoula, 2004).

Adaptive variation is genome-monitored, de novo and heritable across generations. It is directional and constantly released by the sensory mechanisms of the genome in response to environmental stimuli (McClintock, 1984; Rasmusson and Philips, 1997). This epigenetic variation stems from the interaction between genotype and environment (Goldberg et al., 2007) and allows profitable exploitation of limited resources and continual incorporation of gene variants for resistance to changing biotic and abiotic stresses. Continuous exploitation of adaptive variation is synonymous with the continuous genetic upgrading of landraces and cultivars.

Because of the reported negative correlation between yield and competitive ability, integration of yield and stability genes into fewer, improved genotypes is more efficient when the unit of evaluation and selection becomes the individual plant grown at ultra-low planting densities. When this negative correlation is considered at the level of the individual plant, it means that a plant possessing genes for high yield potential will also possess genes for low competitive ability. At the level of the variety crop stand, the negative correlation means that the greater the inter-plant competition in the stand, quantified by the yield CV of individual plants, the greater the crop yield reduction. At the level of selection and landrace/variety maintenance, it means that high competitors are selected at the expense of higher yielding genotypes. As a result, the variety eventually degenerates (Fasoula, 1990). This phenomenon offers a novel explanation for the previously reported practice of landrace seed replacement amongst traditional farmers and their avoidance of traditional maintenance breeding.

In summary, single gene traits are generally easily transferred from landrace germplasm to modern cultivars, but most of the desirable traits characteristic of landraces are complex and difficult to express in different genetic backgrounds. Maintaining these characteristics in heterogeneous landraces is also problematic. Breeding, selection and deployment methods appropriate to these objectives should be used rather than those used for high input intensive agriculture plant breeding.

10 Participatory Breeding

Participative approaches to agricultural research and development are now extensively used throughout the world to help define and address the practical research needs of farmers. They have proved useful in solving practical problems in complex and diverse farming systems characteristic of organic farming and low input systems. During the last few years participatory research divided into several different topics and in the field of plant breeding there are already many very different strategies like Participatory Varietal Selection, true or complete Participatory Plant Breeding (Witcombe et al., 1996) and an intermediate approach, Efficient Participatory Breeding (Morris and Bellon, 2004). The essential advantages of participatory plant breeding over conventional plant breeding involve: better targeting of local environmental conditions, better definition of selection criteria important to the end-users, faster and greater adoption of improved cultivars by the farmer, and increase or maintenance of genetic variability. Participatory plant breeding also gives voice to farmers and elevates local knowledge to the status of science (Ceccarelli and Grando, 1997).

Very effective participatory plant breeding projects in cereals are active all over the world. In Europe in particular, a few participatory plant breeding projects are running with success either in cross-pollinated and self-pollinate cereals. In Portugal, the VASO (Vale do Sousa-Sousa Valley) project, running since 1984, is a maize Participarory Plant Breeding project developed to cover the needs of small maize farmers, with scarce land resources, in poly-cropping systems for human uses, particularly bread production (Moreira, 2006). Local germplasm is used, adapted to the local conditions over centuries of cultivation, quality being the first priority over quantity. This Participarory Plant Breeding project concerns mainly flint-type open-pollinated landraces with quality for the production of the traditional maize bread called ‘broa’. This quality depends on traits not present in the available commercial hybrid cultivars (Brites et al., 2008). ‘Broa’ production still plays an important economic and social role in Central and Northern Portuguese rural communities. Selection takes place at the farmers’ field by the farmer in close collaboration with the breeder.

The genetic diversity evolution through this participatory breeding project was evaluated using molecular markers and it was concluded that though interesting phenotypic improvements were achieved, the level of genetic variability was not significantly influenced and diversity was maintained (Vaz Patto et al., 2007). Several maize open-pollinated landraces were selected within this project with the joint collaboration of the breeder and the farmers. In Sweden, the Allkorn project, running since 1995, is a cereal Participarory Plant Breeding project for organic farming (Larsson, 2006). The goal of the project is to identify and find interesting cereal cultivars for organic farming in Sweden. Cultivars should be of high quality for human nutrition and be well adapted to local soils and climate. They should have good weed-suppressive traits as well as tolerance to diseases and pests. The farmer can select the best cultivar, from his point of view, from several quality cultivars that he has chosen from the project and tested in his own fields. The main aim of this breeding is not to conserve the cultivars but to develop them for the future use in organic farming all over the country. Cultivars of nearly all species of cereals which have historically been used in Sweden are tested in this project, namely *Triticum monococcum*, *T. dicoccum*, *T. spelta*, winter and spring wheat *T. aestivum*, winter and spring rye *Secale cereale*, hulless barley, spring barley, oats, and black oats. These cultivars have a broad diversity and cover primitive cultivars, older landrace cultivars and early Swedish breeding cultivars from 1900 to 1950, since in this early breeding period one of the parents of the cultivars was often a local landrace. The cultivars are selected each year for better adaptation to organic conditions. The idea is to form province groups of farmers that could help each other with seed supply, seed cleaning, shelling of spelt wheat, milling and selling. Regional production groups are formed for the local market with help of local mills and local bakers and the consumer can then find heritage cultivars from each region, country or province. The goal of this type of participatory research is empowering the farmers: supporting the formation of groups capable of assessing their own needs and addressing them either directly or through demands on research organisations.

In Hungary several Participatory Varietal Selection projects are running both in on farm cereal landrace conservation and organic farming research. The on-farm conservation Participatory Varietal Selection strategy is mainly connected with the regeneration of traditional Hungarian landraces and old cultivars which were maintained only in ex situ collections over the last 20 years. Several organic farmers are involved in such projects and their main interest is related to the traditional use of special local food and heritage farming in relation to agro-tourism. In such Participatory Varietal Selection programmes, the Hungarian National Genebanks provide accessions to several farmers participating in the in situ conservation project, and the locations for long-term maintenance are chosen by all participants in order to try to find the optimal place (environmental condition) and landrace to maintain, considering the social background of the region too (Holly, 2000). In recent years new projects have been initiated in the field of Participatory Varietal Selection in Hungary. One of the biggest ones, entitled "Selection of suitable cultivars for organic farming" was carried out in a real Participatory Varietal Selection system in which most of the important stakeholders - the breeders, seed producers, farmers and end users - were involved in cultivar selection for several field crops, including wheat, barley, sunflower, pea, etc. (Kovács, 2006a). In this project breeding institutions provided both landraces and old and modern cultivars and farmers and end user made the cultivar evaluation independently of the providers' opinion in each locality taking into consideration the end users priorities (Kovács et al., 2006). A similar research programme is running for underutilised cereal species, such as einkorn, emmer and macha wheat in the country regions where such species were traditionally used. In this case the re-introduction of the forgotten species into modern agriculture it is important to exploit the genetic diversity which still exists in such cereals (Kovács, 2008).

A participatory breeding programme in tetraploid wheat was initiated in 2001 at INRA-Montpellier, France, based on a demand for organic pasta by the industry (Desclaux, 2005). The quality of the wheat cultivars being produced under organic conditions did not meet the requirements of the processing industry. To identify the main causes, a multidisciplinary public research team, bringing together plant breeders, soil scientists, ecologists, agronomists and sociologists was assembled to work in close collaboration with the farmers and end-users. The two main French territories involved were Camargue and Pays Cathare (Desclaux et al., 2002). The lack of cultivars adapted to low nitrogen conditions became apparent rapidly in such organic crop systems. The main aim of the on-farm and participatory breeding is to take on-board farmers preferences and to better target local environmental conditions by increasing and managing the genetic variability.

Old durum wheat cultivars, segregating or advanced pure lines and populations resulting from crosses between durum wheat and emmer or wild species were provided to the farmers by the breeders. Selection and evaluation are done in close collaboration and results are discussed between all the participants. Information is feed back and can lead to re-examination of the objectives of the breeding scheme (Desclaux, 2005). This French project is neither a farmer-led nor a formal-led project but it is led by both professionals and researchers and requires farmer's critical participation right from the first steps of the breeding scheme, i.e. it is collegiate and decentralised (Desclaux, 2005).

In the UK, a project for developing appropriate participatory methodologies for cereal seed production and cultivar selection under organic conditions has been established (Clarke et al., 2006). This project aimed to overcome the deficiencies of the UK official Recommended List of Cereals for organic farmers. In winter wheat comparative trials using cultivars and mixtures of cultivars were established at 19 UK farms the 2003/04 and 2004/05 seasons (Clarke et al., 2006; Jones et al., 2006). A collegiate participatory method was used to balance statistical rigour with farmer's objectives of managing a whole farm system. Participating farmers sowed the seed in large marked plots using their standard methodology within a field containing wheat. Researchers gained information from each of the farmers about their farming system, field and trial. These experiments showed the large variability of organic systems in the UK and the difficulty of selecting a single cultivar suitable for them all. This project also helped to develop a small core of trained farmers and researches which can be exploited in further participatory projects.

The diversity available within landraces is useful for breeding purposes in at least four different ways (Ceccarelli et al., 1987), including the release of the highest yielding lines as pure line cultivars, the utilisation of superior lines as parents, the evaluation of multilines built with a variable number of pure lines, and the identification of lines showing extreme expression of specific attributes.

Participatory plant breeding and variety selection has proven more successful than the approach used in high input breeding programmes for landrace improvement in stress-prone environments where sustainable approaches are a high priority. Despite being more complex to carry out, it not only delivers improved germplasm, but also aids uptake and communication between farmers, researchers and advisors for the benefit of all.

11 Legal Issues

Landraces as an important genetic resource have been included in international treaties and national decrees that protect and enhance their use in their local environment. The objectives of the convention on biological diversity are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources. The objectives of the International treaty on plant genetic resources for food and agriculture (http://www.planttreaty.org) are the conservation and sustainable use of plant genetic resources and the fair and equitable sharing of benefits derived from their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security. The treaty promotes or supports, as appropriate, farmers and local communities' efforts to manage and conserve on-farm their genetic resources for Food and Agriculture.

In Europe the marketing of seeds of landraces and conservation of cultivars is ruled by the strict seed trade and cultivar protection laws. Cultivars have to meet the Distinctiveness, Uniformity and Stability standards to be registered legally. Agricultural crops have to meet the Value of Cultivation and Use criteria and to be listed on the "European common catalogue of cultivars of agricultural plant species" to be tradable within Europe. Combined with the extension of the intellectual property rights in the UPOV (French: *Union internationale pour la protection des obtentions végétales*) 91 act, the maintenance of agro-biodiversity on-farm and the conservation of local cultivars are generally threatened. According to the Biodiversity Action Plan for agriculture presented by the European Commission in 2001 "the conservation and improvement of in situ/on farm plant genetic resources also depends on the effective possibility of sustainable uses and on legislation which makes it possible to market diversified genetic materials".

The directive of conservation cultivars is still limiting the diversity of seed. The most recent draft of a directive of conservation cultivars has the same goals as the UPOV standards: Uniformity, stability and distinctness, but less documentation is needed to fulfil the requirements. Landraces are never uniform, neither are they stable. Therefore, landraces will not be allowed according to the directive of conservation cultivars. The directive of conservation varieties therefore applies for old out-dated approved cultivars, and will not contribute to the biodiversity of arable land (SANCO, 2006). An international commission has written a manifest on the future of seed in order to strengthen the movement toward sustainable agriculture, food sovereignty, biodiversity and agricultural diversity, to help defend the rights of farmers to save, share, use and improve seeds and enhance our collective capacity to adapt to the hazards and uncertainties of environmental and economic change (Shiva, 2007). The most recent European Union Commission Directive 2008/62/EC published on 20th June 2008 does provide for certain derogations for acceptance of agricultural landracs and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed of those landraces and varieties.

Clearly legislation was designed primarily to protect trade and return royalty income to modern plant breeders with expensive programmes to fund. As the desirability of using landraces becomes more apparent to achieve greater sustainability, legislation changes are being made to facilitate this trade too. However, more changes are needed to promote the exploitation of diversity in landraces and encourage their use.

12 Conclusions

The position of cereal landraces as valuable genetic material in contemporary agriculture is gaining renewed importance. A lot of recent research effort has gone into collecting, organising, studying and analysing them with a primary goal being to incorporate their positive qualities in new cultivars or populations for a more sustainable agricultural production, particularly in response to recent climate changes. Positive attributes include landraces being a source of novel resistance genes or combination of genes with a good deployment strategy. Particularly important is the fact that resistance found in cereal landraces has been durable in many instances, in contrast to the gene-for-gene type of resistance that is usually encountered in modern cultivars. However, the durability may be associated with and dependent on deployment in heterogeneous populations.

A major part of this valuable landrace diversity is conserved in the world's gene banks network and should be exploited systematically for traits such as quality and specific adaptation to stress environments. The available genetic variation in adaptive responses to soil and climatic conditions conserved in landraces is little understood, known and even less used. More uniform and user-friendly documentation about collection and characterisation of landraces, either morphologically or with molecular tools, is needed to access this variation more effectively. Gene banks should aim at adopting a common concept of landraces and plan special inventories for them. The level of diversity should be monitored during their conservation so that the original level of variation is maintained. More studies are needed in order to investigate if their long-term maintenance by farmers resulted in increasing genetic variation.

New high-throughput genotyping platforms and phenotyping data in common databases will enable powerful association genetics approaches to be utilised for improvement and utilisation of landrace resources. Knowledge of linkage disequilibria in landraces compared with elite germplasm will help focus breeding for stress-adapted cultivars or populations. With the current rapid advancements in high-throughput molecular genotyping technologies, it is becoming increasingly clear that the limiting factor in applying those powerful technologies to molecular breeding programmes is no more the capacity of genotyping, but the potential for accurate or precision phenotyping. Particularly important and challenging is phenotyping for the so-called quantitative traits.

The renewed focus on cereal landraces for breeding purposes is also a response to some negative consequences of modern agriculture and conventional breeding, such as the liberal use of high inputs, the loss of genetic diversity, and the stagnation of yields in less favourable areas. To deliver this, participatory plant breeding and variety selection practices have emerged as a powerful way to merge breeders' knowledge and farmers' selection criteria, emphasizing decentralised selection in the target environments with the active participation of local farmers. Location-specific adaptation of these diverse landraces will be important for further selection at the farmer level. New strategies are emerging to produce "modern landraces" based on multiple cross populations of einkorn, emmer and bread wheat in combination with on farm site-specific selection to obtain highly adaptable populations for local and regional production.

The reported practice of the "inexplicable seed replacement" by traditional farmers is connected to the gradual seed degradation during landrace maintenance, brought about by the established negative correlation between breeding and competitive ability. Further enhancement of productivity and stability is achieved through practicing "non-stop selection" within landraces across the marginal production environments, to exploit the constantly released by the genome useful adaptive variation. The procedure and results of non-stop selection exceed those of conventional maintenance breeding, combating seed degradation and resulting to a constant cultivar upgrade. An additional essential breeding consideration is the creation of density-neutral landrace germplasm, since its use, particularly in marginal environments, permits lower seeding rates, limiting the damage due to drought.

The issues of conservation and sustainable use of landraces have been so far included in international convention treaties and directives which need to be implementated on European and national levels. In this review we have highlighted the value of landraces as resources for the future sustainability of cereal crop production, the methods to enhance their genetic makeup and avoid seed degradation, and emphasised the level of coordination and resourcing needed to realise their great potential.

Acknowledgements We thank the European Union for funding the COST Action 860 "Sustainable variety development for low-input and organic agriculture" (2004–2008) through which this review was initiated.

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Mineral Sources of Potassium for Plant Nutrition

David A.C. Manning

Contents

Abstract Recently published assessments of nutrient budgets on a national basis have shown that K deficits for developing countries are so substantial that a doubling of world production of potash fertilisers would be required to balance inputs and offtake, simply to meet demands in Africa alone. The price of potassium fertiliser raw materials has increased by a factor of 4 during 2007–2009, approaching \$1000 per tonne in some markets. Thus an annual investment of the order of US\$5600 million is required to replenish soil K stocks in Africa. In this context it is appropriate to review current knowledge of alternative sources of K, which is the seventh most abundant element in the Earth's continental crust, present in feldspars and (much less commonly) feldspathoid minerals including nepheline and leucite. Theoretical considerations based on the experimental determination of mineral dissolution rates indicate that nepheline dissolves 100 times more quickly than potassium feldspar, and this suggests that nepheline-bearing rocks are more effective as sources of K for plant growth than granitic rocks, even though these have higher K contents. Crop trials with silicate rocks and minerals as sources of K show increased K availability and uptake for nepheline-bearing rocks compared with granitic rocks. Under conditions where soils are rapidly leached (especially tropical soils such as oxisols that contain quartz, aluminium oxy-hydroxides and kaolinite), with low capacity to retain soluble nutrients, the use of potassium feldspar or crushed granite does give a yield response, although no greater than for conventional fertilisers. In other experiments with crushed ultramafic, basaltic and andesitic rocks improvements in crop yield are claimed, although this cannot be unambiguously related to the mineralogical or chemical

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_11,

from Manning, Agron. Sustain. Dev. 30 (2010) 281–294. DOI: 10.1051/agro:2009023

composition of the rock used. In conclusion, the present high cost of conventional potassium fertilisers justifies further investigation of potassium silicate minerals and their host rocks (which in some cases include basic rocks, such as basalt) as alternative sources of K, especially for systems with highly weathered soils that lack a significant cation exchange capacity. Such soils commonly occur in developing countries, and so this approach provides an opportunity to develop indigenous silicate rock sources of K as an alternative to sometimes prohibitively expensive commercial fertilisers.

1 Introduction

Of the three main plant nutrients, N, P and K, potassium (K) and phosphorus (P) are exclusively sourced from geological materials (Manning, 1995; van Straaten, 2007). Both nutrients are mined and processed to give fertiliser products that vary in the amount of chemical treatment involved in their preparation. Both can be used as mined (sylvinite (mixed KCl + NaCl) and phosphate rock). However, the majority of commercial phosphate fertilisers are manufactured from phosphoric acid, including composite N-P fertilisers, such as diammonium phosphate (DAP), which incorporate N as urea, ammonium or nitrate. The manufacture of nitrogen fertilisers depends largely on chemical processes that derive N from atmospheric sources or from fossil fuel materials, with subsequent energy-intensive processing to give a final product. Per unit of nutrient, N fertiliser production typically uses an order of magnitude more energy than required to produce P or K fertilisers (Lægrid et al., 1999).

World trade in fertilisers is substantial: in 2007–8, consumption is reported to be 98 million tonnes N, 40 million tonnes P (as P_2O_5), and 28 million tonnes K (as K_2O: FAO, 2008). However, trade does not necessarily reflect agricultural need, but ability to purchase. This can be demonstrated using nutrient budgets on a national and international scale, one based on trade (e.g. FAO, 2008), and the other based on scaling up farm offtakes and inputs (e.g. Sheldrick et al., 2002).

In terms of the global trade in fertiliser products, the Food and Agriculture Organisation of the United Nations (FAO, 2008) indicates that global supply of fertilisers is sufficient to meet demand. For potassium, the FAO states that global demand is likely to increase annually at 2.4%, and that supply will balance demand. However, in an analysis of the regional situation, the FAO notes that only 10 of 57 African countries consume significant quantities of fertiliser of any kind. Consumption in Africa of K fertilisers is currently approximately 485 000 tonnes (2008–9), expected to increase by 2% annually, all of which has to be imported (FAO, 2008). Other regions that rely on imported K include Oceania and Asia, with the bulk of commercial supply derived from Europe and North America.

In an assessment of nutrient budgets, Sheldrick et al. (2002) describe a nutrient audit model, in which offtakes are expressed in terms of nutrients removed from the land, and inputs include fertilisers, crop residues and manures. These are then balanced and assessed on a national, regional or global scale to determine whether or not nutrient supply is balanced by inputs. Importantly, Sheldrick et al.'s (2002) work has shown that on a global basis it is not phosphorus supply that is of most concern, but supply of potassium, with an annual global deficit of 20 kg K ha^{-1}. In an assessment of nutrient audits for Africa, Sheldrick and Lingard (2004) have shown that overall, and in most African countries, nutrient depletion has increased since 1961, with annual deficits in 1998 of 3.5 million tonnes of N (17.4 kg N ha^{-1} $year^{-1}$), 0.7 million tonnes of P (3.3 kg P ha^{-1} $year^{-1}$) and 4.1 million tonnes of K (20.0 kg K ha^{-1} $year^{-1}$). The highest nutrient depletion rates are for potassium.

Using data from Sheldrick and Lingard (2004) for African countries, Fig. 1 shows the extent of

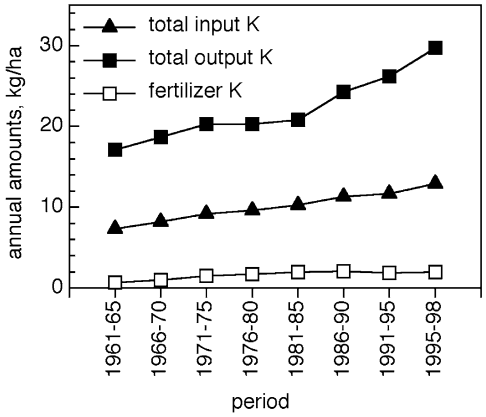


Fig. 1 Potassium inputs and outputs combined for all African countries for the latter part of the 20th Century (Sheldrick and Lingard, 2004)

the imbalance between the supply and offtake of K. Between 1961 and 1998, the total output of K through offtakes has almost doubled, to 30 kg K ha^{-1} year^{-1}. In contrast, fertiliser K input has remained very low, at 2.1 kg K ha^{-1} year^{-1} or less, whilst other inputs (from crop residues and manures) have increased. Potassium fertilisers make up 10% or less of the total K input, and this proportion has declined between 1961 and 1998.

The nutrient deficits identified by Sheldrick and Lingard (2004) and the problems of fertiliser supply identified by the FAO (FAO, 2008) for Africa have major implications for the supply of potash to agriculture, more widely within the developing world. This is because potash ores have a rather limited distribution globally (Rittenhouse, 1979; Moores, 2009b), with the bulk of the world's potash mined in Canada, Europe and the Middle East. Thus there is currently very little scope for many developing countries to be self-sufficient in potash using conventional fertilisers.

Furthermore, consideration of trade in the context of geographical factors, infrastructure and transport costs confirms, for example, that many (but by no means all) African countries do not compete on a level playing field with more developed countries. Limão and Venables (2001) carried out a detailed analysis using gravity modelling to assess the impact of a number of variables on the costs of trade. For sub-Saharan African countries, transport costs between countries are 136% higher than for trade between other African countries, and infrastructure costs account for half of this figure. Poor infrastructure plays a highly significant part in inhibiting trade within Africa, including transit from coastal ports to landlocked countries (Longo and Sekkat, 2004; Limão and Venables, 2001).

Focusing on potash fertilisers, and considering the situation in Africa, Fig. 2 shows the amount of muriate of potash that would be needed to compensate for potassium offtake for the period shown in Fig. 1. Figure 2 shows that in 1961 inputs from fertilisers equivalent to 3 million tonnes of KCl were required to balance potassium offtake, rising to 7 million tonnes annually for the period 1995–1998. This is 70% of the annual consumption of muriate of potash by the United States during the same period (10 million tonnes per annum; Searls, 2000), and about 20% of world consumption at that time. Assuming that demand for K in Africa has not increased since 1998, the value of the annual fertiliser need for Africa identified in this way is of the order of US$5600 million using prices for the end of 2008, likely to be an underestimate given that demand is expected to have increased since 2000.

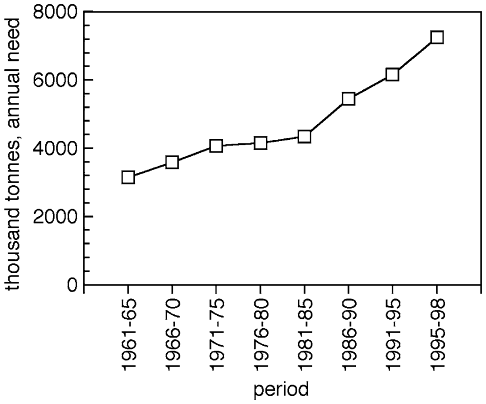


Fig. 2 Estimated total requirement of K for African countries, based on difference between outputs and existing inputs

The situation described above concerns an entire diverse continent, and varies from one African country to another. But similar deficits occur elsewhere: for 1998, China has a K deficit of 60 kg K ha^{-1} year^{-1}, or a requirement of 8.3 million tonnes per year. To maintain this deficit and to feed its growing population, Sheldrick et al. (2003) consider that China needs to see growth in K fertiliser use at an annual rate of 8%. According to the FAO, East Asia's K deficit is in excess of 9 million tonnes per year, dominated by China (FAO, 2008). In contrast to the developing world, the USA and Europe have no potash deficit; in general, developed countries add K well in excess of offtake.

Using figures from Sheldrick et al. (2002) for the end of the 20th century, globally, there is a deficit of 20 kg K ha^{-1} year^{-1}, corresponding to an annual global fertiliser need of 30 million tonnes of K. This corresponds to a requirement for an additional 60 million tonnes of KCl (35 million tonnes of K_2O equivalent), which is slightly greater in quantity than the most recent published estimates of current world production (approx. 30 million tonnes of K_2O equivalent; Ober, 2007).

Thus to satisfy demand from world crop production for K, the amount of additional potash required is equivalent to slightly more than current global mined production. Production and consumption of potash fertilisers must more than double to sustain soil K stocks. At present, the potassium deficit that arises from offtake of crops is being supplied by the soils. Using K as an indicator of other mineral-derived nutrients, it

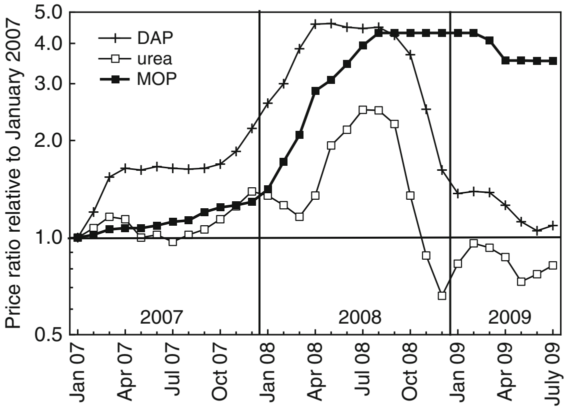


Fig. 3 Changes in prices to July 2009 of muriate of potash (MOP), urea and diammonium phosphate (DAP) fertilizers, relative to January 2007 prices (calculated using data from ICIS; http://www.icis.com)

appears that global agricultural production is responsible for nutrient mining on a huge and evidently unsustainable scale.

The above review of Sheldrick's work is based on nutrient balances for the late 20th Century. The first decade of the 21st Century has seen the growth of China as an industrial power, with concomitant demand for natural resources, including fertilisers. This has led to substantial demand for K, and world prices have risen accordingly.

Figure 3 compares changes in price (not corrected for inflation) for three fertilisers since the start of 2007: urea, diammonium phosphate (DAP) and muriate of potash (MOP). For N and P fertilisers, it clearly shows an increase and subsequent decrease that coincides broadly with trends in oil prices that occurred in the latter part of 2008, reflecting the close links between these and the fossil fuel industry. Prices for these fertilisers return to values similar to those of January 2007 (DAP; approximately US$ 280/tonne compared with US$ 250/tonne in January 2007) or are lower (urea; approximately US$ 280/tonne compared with US$ 350/tonne in January 2007). In contrast, the price of potash (muriate of potash, price Freight On Board (FOB) Saskatchewan) for the period rose by a factor of 4 in this period, from US$180/tonne to US$650/tonne, with a peak price of US$ 800/tonne for much of late 2008, and spot market prices of US$1000/tonne reported on the internet. Most importantly, the price of K has not decreased in parallel with the observed decrease in price of N and P fertilisers. This observation is discussed in more detail by Moores (2009a, b), and fundamentally distinguishes potash fertilisers. The high price of potash reflects factors in addition to those associated with manufacture, including inability of supply to keep pace with demand.

Given the apparent obstacles to trade and the high cost of potash, it is important to consider how the African potash deficit identified by Sheldrick and Lingard (2004) can be addressed. One possibility is to identify new sources of K. There are known deposits of potash salts (e.g. Republic of Congo, De Ruiter, 1979; Thailand, El Tabakh et al., 1999) that presently are not mined. The Republic of Congo deposits (located 16 km east of the port of Pointe-Noire) are (according to internet sources) at an advanced stage of mine planning and may represent a significant source in due course. However, in general the distribution of conventional mined potash salts has changed little since Rittenhouse (1979) considered global supply and demand, and is unlikely to change given the very substantial cost of developing a producing mine (US$2000 million; Moores, 2009b). In any case, given the difficulties of intra-African trade (Limão and Venables, 2001), the possible development of the Pointe-Noire potash deposits is unlikely to improve the availability of K in those countries that most need it, especially as the products of the mine are likely to be sold on the world market at a similar price to those from other sources.

In these circumstances it is appropriate to consider the availability of K within soil systems from first principles, and to consider unconventional sources of K that are not necessarily attractive on a commercial basis to the global fertiliser industry but that might be appropriate in circumstances where farmers are presently excluded from global fertiliser markets.

In soil systems, retention of potassium is wholly dependent on the cation exchange capacity of the soil. This, in turn, is largely controlled by the presence within soils of predominantly clay minerals that have an interlayer site capable of accommodating K, such as the illite group or vermiculites (Sposito, 1989). In two of the major soil orders, oxisols and ultisols, cation exchange capacity is very low, reflecting the leaching from these of cations as a consequence of the extreme weathering process that they have undergone. Such soils are characterized by the presence of quartz, kaolinite and Fe-Al oxy-hydroxides, representing a residual mineral assemblage from which nutrients such as K have been leached. These soils occur widely in tropical

Table 1 Chemical formulae and potassium contents (expressed as element and oxide) for potash ore minerals and for common potassium silicate rock forming minerals

Mineral	Formula	Weight % K	Weight % K_2O
Potash ore minerals			
Sylvite	KCl	52.35	63.09
Carnallite	$MgCl_2.KCl.6H_2O$	14.05	16.94
Kainite	$KMgSO_4Cl.3H_2O$	15.69	18.91
Langbeinite	$2MgSO_4.K_2SO_4$	18.84	22.71
Silicate minerals			
Potassium feldspar	$KAlSi_3O_8$	14.03	16.91
Leucite	$KAlSi_2O_6$	17.89	21.56
Nepheline	$(Na,K)AlSiO_4$	13.00	15.67
Kalsilite	$KAlSiO_4$	24.68	29.75
Muscovite	$KAl_3Si_3O_{10}(OH)_2$	9.03	10.88
Biotite	$K_2Fe_6Si_6Al_2O_{20}(OH)_4$	7.62	9.18
Phlogopite	$K_2Mg_6Si_6Al_2O_{20}(OH)_4$	9.38	11.30

areas of Africa, South America and Australia. Their cultivation requires addition of nutrients including K, which are removed by through drainage as well as by plant uptake.

For an agricultural system in a poor country in which oxisols dominate, the supply of K is a critical limiting factor. In this context it is appropriate to consider unconventional geological sources of K that, whilst less effective than the soluble salts that make up commercial fertiliser products, are widely available globally and might be expected to weather rapidly within a soil, releasing sufficient K to provide agronomic benefit. Such sources include silicate rocks, whose use as a source of plant nutrients has been discussed by a number of authors (van Straaten, 2006, 2007; Leonardos et al., 1987; Harley and Gilkes, 2000; Gillman et al., 2002).

The purpose of this review is first to present the theoretical background that justifies the use of crushed silicate rocks as a source of plant nutrients, focusing on K, and secondly to provide an overview of the results of existing published trials using crushed rocks as an alternative to conventional fertilisers. It then considers in principle whether or not crushed silicate rocks should be considered as one source of K that can address deficits on a basis that is appropriate for sustainable crop production, and highlights the need for strategic and consistent future trials.

2 Mineral Sources of K

In the context of meeting the world's need for potash fertilisers, there is no doubt that conventional soluble salts will continue to dominate in developed countries. Table 1 lists the principle potash fertiliser ore minerals, together with the dominant rock-forming potassium silicate minerals. In natural soils, potassium is derived ultimately from the potassium silicate minerals, which can be subdivided into those that are formed at high temperatures by igneous and metamorphic processes (feldspars and feldspathoids; primary micas), and those that are produced on weathering of the high-temperature minerals (clay minerals, especially illite). In deeply weathered, highly oxidized soils that have low organic matter, it is essentially the clay minerals that are responsible for the exchangeable K that is measured in soil analysis; the high temperature silicate minerals provide a reservoir of fixed K.

It is normally assumed that the weathering of the feldspars and feldspathoids is very slow, and that their addition to soil will not be beneficial for crop growth, especially when compared with conventional potash fertilisers, which, being salts, are readily soluble as well as having high K contents. A number of studies have investigated silicate rocks as sources of K (and other nutrients), in response to the following drivers:

(1) nutrient supply for organic farmers, principally in developed countries, who seek an alternative to salts (partly in response to environmental protection demands). This is in part related to the 'Remineralise the Earth' philosophy of plant nutrition using rock dust (http://remineralize.org);
(2) nutrient supply for farmers in developing countries who lack funds to purchase conventional fertilisers.

Both areas of application have shown that in some circumstances potassium silicate rocks can act as effective sources of K, with long term crop yields that meet the needs of the producer. A number of commercial enterprises use potassium silicate rocks in long-standing businesses with satisfied customers, particularly in the organic farming sector (e.g. http://www.glensideorganics.co.uk). However, the scientific evidence for the possible agronomic value of potassium silicate rocks is dispersed in a number of publications, and these do not generally refer to specific commercial products.

3 The Potassium Aluminium Silicate Minerals: Stability and Dissolution Rates

Potassium occurs as an essential component within the feldpars and felspathoid mineral groups. In both groups, the mineral structure is a three dimensional network of silica tetrahedra, in which substitution of Al for Si leads to a charge deficiency that is balanced by the presence of Na^+, K^+ or Ca^{++}. The potassic aluminosilicate minerals and their formulae are summarised in Table 1, which also gives their theoretical K_2O contents, ranging from 18–30 wt% (equivalent to 15–25 wt%; 150–250 g kg^{-1} K). Of the aluminosilicate minerals, potassium feldspar is most commonly found, occurring in a wide range of rock types (granite, sandstone, gneisses, etc). Orthoclase (Or) and microcline (Mc) are common varieties of potassium feldspar, differing in their crystal structure (Deer et al., 1992). Leucite (Lc), nepheline (Ne) and kalsilite (Ks) are much less widely distributed, predominantly occurring in nepheline syenites and related volcanic rocks, and some alkaline basalts. Nepheline is relatively readily available as a commodity, as it is mined as a raw material for ceramic and glass manufacture. End-member nepheline is a sodium aluminium silicate; natural nepheline typically contains potassium to the extent of 20% of the cation site. Leucite and kalsilite are not readily available as commodities, and often occur mixed with secondary products of their weathering and alteration.

The thermodynamic stability of the potassium aluminium silicates can be assessed using the approach of Curtis (1976). A balanced chemical reaction can be written in which aluminium is conserved and the weathering products include mineral phases that are known to be stable under Earth-surface conditions. This approach provides a thermodynamic basis for empirical stability series such as that produced by Goldich (1938; Curtis, 1976). Table 2 shows the reactions for the aluminosilicates, including the plagioclase feldspars anorthite (An) and albite (Ab) for comparison. The calculated free energy changes for the reactions are given in Table 2, taking into account variation in the written formula by expressing the free energy change per gram atom rather than per mole (this approach allows comparison of like with like in terms of the numbers of bonds being broken as the framework silicate weathers; Curtis, 1976). The least stable mineral is kalsilite, followed by nepheline, then leucite and finally potassium feldspar (microcline). On the basis of these thermodynamic data, kalsilite and nepheline stabilities are similar to those of the calcic plagioclase feldspars.

In addition to variation in their thermodynamic stability, the potassium aluminosilicates vary in the kinetics of their dissolution reactions. Data for these are available for the feldspars (summarised by Blum and Stillings, 1995) and for nepheline (Tole et al., 1986); there are no comparable dissolution rate data for leucite or kalsilite. Dissolution of the aluminosilicates takes place as a consequence of hydrolysis reactions at the mineral surface (Brantley and Stillings, 1996); these reactions are irreversible. They liberate cations to the solution and disrupt the framework structure of the mineral. Thus they differ fundamentally from the reversible cation exchange reactions that characterise the interlayer site of the clays and micas and the exchange site of the zeolites.

Table 2 Weathering reactions for framework aluminosilicate minerals (Curtis, 1976)

	Reaction	ΔG_r, kJ/mol	ΔG_r, kJ/ gram atom
Ks	$2KAlSiO_4 + 2H^+ + H_2O = 2K^+ + Al_2Si_2O_5(OH)_4$	–115.25	–6.07
An	$CaAl_2Si_2O_8 + 2H^+ + H_2O = Ca^{2+} + Al_2Si_2O_5(OH)_4$	–98.49	–5.47
Ne	$2NaAlSiO_4 + 2H^+ + H_2O = 2Na^+ + Al_2Si_2O_5(OH)_4$	–91.02	–4.79
Lc	$2KAlSi_2O_6 + 2H^+ + H_2O = 2K^+ + Al_2Si_2O_5(OH)_4 + 2SiO_2$	–88.00	–3.52
Ab	$2NaAlSi_3O_8 + 2H^+ + H_2O = 2Na^+ + Al_2Si_2O_5(OH)_4 + 4SiO_2$	–87.74	–2.83
Mc	$2KAlSi_3O_8 + 2H^+ + H_2O = 2K^+ + Al_2Si_2O_5(OH)_4 + 4SiO_2$	–67.70	–2.18

Table 3 Dissolution rates for potassium silicate minerals and other feldspars (date from Blum and Stillings, 1995)

Mineral	Dissolution rate Log k+	Relative dissolution rate
Potassium feldspar	–9.93 (pH 1-5.7)	1.9
	–10.2 (basic)	1
Leucite	Not known	–
Nepheline	–8.2 (pH5)	100
	–8.6 (pH7)	40
Kalsilite	Not known	–
Anorthite	–5.87	21000
Albite	–9.69	3.2

The available dissolution rate data for the feldspars and for nepheline are given in Table 3. It is important to compare corresponding experimental conditions, as a wide range of temperatures, pH and other factors has been considered in kinetic studies. Blum and Stillings' review (1995) assembles directly comparable information in one source. It is also important to note that these experimentally-determined dissolution rates are not directly applicable to the soil environment (e.g. White, 1995). Of particular importance is the experimental difficulty in determining the reacting surface area of the mineral. The feldspars (and presumably the feldspathoids) characteristically are riddled with holes and structural defects at a very fine (molecular) scale (Lee and Parsons, 1995), and these can have a major effect on dissolution kinetics.

Table 3 also shows relative dissolution rate data for the potassium aluminosilicates, albite and anorthite. Dissolution rates vary according to pH; for comparable pH values, nepheline's dissolution rate exceeds that of potassium feldspar by as much as 100 times. Because of its lower thermodynamic stability compared with nepheline, it might be predicted that kalsilite has a higher dissolution rate, although its similar framework structure means that its dissolution rate is likely to be similar to that of nepheline. Leucite is expected to have a dissolution rate that is intermediate between nepheline and potassium feldspar.

4 Potassium in Silicate Rocks

The potassium silicate minerals typically occur mixed with other silicate minerals, and this dilutes the K content. In general terms, K is the seventh most abundant element in the Earth's continental crust, the composition of which is dominated by rocks of granitic composition and the products of their weathering and erosion. Potassium feldspar is an essential component of granitic rocks, which also contain the micas biotite and muscovite. Weathering of granitic rocks initially produces secondary muscovite and chemically-similar illitic clays, and if conditions are right ultimately produces the potassium-free clay mineral kaolinite.

Although potassium feldspar, muscovite and biotite are very common, the other potassium silicate minerals are rare. Nepheline occurs in very specific igneous rock types that occur sporadically, mainly as intrusive rocks but also within lavas. Kalsilite and leucite both occur mainly in volcanic rocks (Deer et al., 1992), in potassium-rich lavas from specific regions. These include rift valleys, such as the East African Rift (Woolley, 2001).

Examples of typical rock compositions are given in Table 4. This table also shows estimated compositions for the upper continental crust, based on the observed composition of till derived from glacial erosion of (a) igneous rocks and (b) sedimentary (Carboniferous) sequences. Table 4 also gives the composition of particulates carried by the Ganges, which contribute nutrients to soils when deposited in annual floods. Table 4 shows that the potassium content of some readily available, and some rare, rock materials are quite similar, ranging from 2.7–5.6 wt% K_2O.

Table 4 also recalculates the composition of the rock or sediment in terms of the equivalent hypothetical, normative, content of orthoclase (K-feldspar), nepheline and leucite. The normative composition shows that glacial sediments derived from granitic terrains are chemically very similar (in terms of K content) to their source, justifying consideration of the use of tills of this type for soil remineralisation. However, glacial till can be highly variable in composition (Barlow, 1996), its composition reflecting mixing between different mineral components within the substrate. Thus the samples reported by Barlow (1996) show mixing between quartz (derived from sandstone) and a clay-rich fraction derived from mudstones. Tills derived from sedimentary rocks may have lower K contents, and lower normative orthoclase, as a consequence of weathering prior to till formation.

Table 4 Typical compositions of igneous rocks (Le Maitre, 1976) and estimates for continental crustal composition based on till and suspended sediment composition (Goldschmidt, 1933; Rudnick and Gao, 2003; Martin and Meybeck, 1979)

	Granite	Trachyte	Diorite	Nepheline syenite	Basalt	Nephelinite	Upper continental crust (glacial sediments)	Glacial till (clay-rich)	Ganges river particulates
Comments:	Common	Rare	Moderately common	Rare	Common	Very rare	Igneous/ Metamorphic crust	Sedimentary rock source	
Oxide									
SiO_2	71.30	61.21	57.48	54.99	49.20	40.60	62.22	79.31	66.9
TiO_2	0.31	0.70	0.95	0.60	1.84	2.66	0.83	0.39	0.96
Al_2O_3	14.32	16.96	16.67	20.96	15.74	14.33	16.63	7.54	16.0
Fe_2O_3	1.21	2.99	2.50	2.25	3.79	5.48	–		–
FeO	1.64	2.29	4.92	2.05	7.13	6.17	6.99*	3.21*	5.2*
MnO	0.05	0.15	0.12	0.15	0.20	0.26	0.12	0.05	0.14
MgO	0.71	0.93	3.71	0.77	6.73	6.39	3.47	1.11	2.3
CaO	1.84	2.34	6.58	2.31	9.47	11.89	3.23	1.16	4.1
Na_2O	3.68	5.47	3.54	8.23	2.91	4.79	2.15	0.57	1.5
K_2O	4.07	4.98	1.76	5.58	1.10	3.46	4.13	1.74	2.7
P_2O_5	0.12	0.21	0.29	0.13	0.35	1.07	0.23	0.09	–
Normative potassic minerals									
Or	24.50	29.41	10.42	32.98	6.53	3.16	24.41	10.28	15.96
Lc	–	–	–	–	–	13.57	–	–	–
Ne	–	–	–	21.77	–	21.95	–	–	–

*Total iron reported as FeO

5 Evidence of Silicate Minerals as Sources of K

A number of studies have addressed the ability of different potassium silicate minerals to yield nutrients under laboratory and pot trial conditions.

The ability of feldspar to dissolve in soil conditions, yielding K, has been determined by Riggs et al. (1993). Potassium feldspar was added to soil and subjected to leaching and incubation experiments. These showed that feldspar leaching was effective in open system leaching experiments, more so that in closed-system incubations. Similarly, Blum et al. (1989) compare a number of different rock types as sources of mineral plant nutrients. Assuming that micas and feldspars are concentrated in the silt and sand fractions of a soil, Mengel et al. (1998) compared the extractable K contents and the performance of ryegrass (*Lolium perenne*) for whole soil and for the same soil from which the clay fraction had been removed. Finding no difference between the two soils, Mengel et al. (1998) conclude that K is as easily available from the sand and silt fraction as from the whole soil. In earlier trials with soils that differ in their sand, silt and clay content (again with no detailed mineralogical or geochemical analysis), Mengel and Rahmatullah (1994) observed potassium deficiency in wheat (*Triticum aestivum* cv. Faisalabad 83), maize (*Zea mays* L. cv Gohar) and barley (*Hordeum vulgare*), but adequate K nutrition of elephant grass (*Pennisetum purpureum*).

The interaction between silicate minerals and microbial systems has been investigated by a number of authors. In the context of fungal community development on natural weathered rock surfaces, Gleeson et al. (2005) show that individual minerals are associated with distinct fungal community structures that relate to the chemical composition of the mineral. Similarly, Rosling et al. (2004) showed that eight different ectomycorrhizal fungal cultures responded differently to culturing in the presence of minerals (quartz, apatite, potassium feldspar and marble).

In laboratory experiments in which the fungus *Aspergillus fumigatus* was applied to a potassium-rich shale (containing principally K-feldspar), K release was enhanced by two orders of magnitude (Lian et al., 2008). In addition to the role played by organic acids exuded by the fungus, Lian et al. (2008) observed increased K yields when the organism was in contact with the mineral substrate, suggesting that surface interactions, some of which are mechanical, enhance K release.

Wallander and Wickman (1999) compared ectomycorrhizal and non-mycorrhizal cultivation of *Pinus sylvestris* seedlings in pot trials where the source of K was potassium feldspar (microcline) or biotite. In both cases, release of K from the minerals (as measured by biomass K contents) was enhanced by the presence of mycorrhizal fungi.

6 Use of Potassium-Bearing Silicate Rocks as a Fertiliser

Proposals to use potassium silicate rocks and minerals as sources of plant nutrients are not new; Lloyd (1918) comments that it is economically not viable to extract K from silicate rocks to produce fertilisers, despite the availability of a number of different methods. Instead of regarding potassium silicate-bearing rocks as an ‘ore’ from which to extract K artificially, it is more appropriate to consider their direct use, allowing plants and their root processes to extract the K that they need.

Selection of a silicate rock potash fertiliser can be based on the absolute potash content, which is then tested for availability (e.g. Barral Silva et al., 2005). An alternative approach is to select on the basis of dissolution rate. The value of this approach is illustrated in Fig. 4, which shows the relative rates of loss of K to solution from two silicate minerals, potassium feldspar and nepheline, at neutral pH and pH 5 over a period of 1 year, for a surface area of 1 m^2 g^{-1}. Figure 4 clearly shows that the release of K from nepheline, with 3.8% K, is an order of magnitude greater than release from potassium feldspar (15% K), and that this observation applies to pH values that are appropriate for manures and soil solutions influenced by active root growth. Thus rocks that contain nepheline (and by implication kalsilite and leucite) are likely to be more effective as sources of K than those that contain K-feldspar alone.

7 Crop Trials with Potassium Silicate Minerals

Table 5 summarises a number of trials that have used silicate rock materials as a source of plant nutrients, focusing on K. These trials include pot (greenhouse) and field experiments, using a range of differing crops, for differing time scales and in different climates.

7.1 Trials with Granite and Related Rocks

A consistent set of trials has been carried out in Western Australia, in which the same granite was used in field trials on wheat, and pot trials on wheat, clover and ryegrass (Coreonos et al., 1996; Hinsinger et al., 1996; Bolland and Baker, 2000). Although a consistent source of granite powder was used, the K content varied from one study to another, from 2.2–3.8% K_2O. Application rates also varied, up to the equivalent of

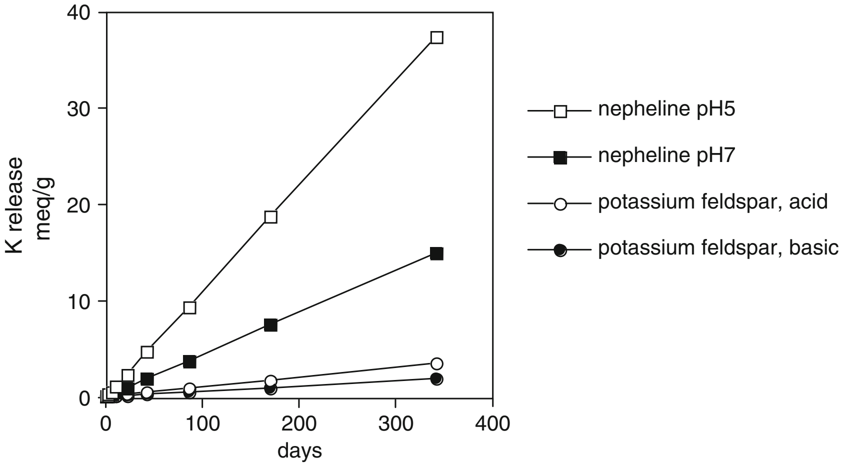


Fig. 4 Release of K (expressed as milliequivalents/gram) from nepheline and potassium feldspar calculated from mineral dissolution rates

Table 5 Summary of trials with silicate rocks as nutrient sources (K). Ksp: potassium feldspar; Ne: nepheline; Bi: biotite

Crop	Species/cultivar (where stated)	Mineral/rock	Trial type	Duration (months)	Agronomic benefits	Reference
Alfalfa	*Medicago sativa* L. cv Asta and cv Haifei	Gneiss	Pot	1.75	Insignificant	Wang et al., 2000
Clover	*Trifolium subterraneum*	Granite	Field/pot	12/1-1.5	Insignificant	Bolland and Baker, 2000
Clover	*Trifolium subterraneum* cv Trikkala	Granite	Pot	5	Increased yield and K uptake	Coreonos et al., 1996
Grass	Timothy *Phleum protense* L., Meadow fescue *Festuca protense* L.	Ksp and Ne+Bi-rich mine tailings	Field	36	K uptake similar for Ne-bearing tailings and KCl; Ksp-hosted K inadequately available.	Bakken et al., 2000
Grass	*Brachiaria dyctioneura*	Feldspar (Ksp)	Field	14	Insignificant	Sanz Scovino and Rowell, 1988
Grass	Italian ryegrass *Lolium multiflorum* L.	Gneiss	Pot	1.75	Significant K mobilization	Wang et al., 2000
Grass	Perennial ryegrass *Lolium perenne* L.	Gneiss	Pot	1.75	Significant K mobilization	Wang et al., 2000
Grass	Rye grass *Lolium rigidum* cv Standard	Granite	Pot	5	Increased yield and K uptake	Coreonos et al., 1996
Italian ryegrass	*Lolium multiflorum italicum* var . Turilo	Ksp and Ne+Bi-rich mine tailings	Pot	6	Ksp-hosted K inadequately available. Increased yield for Bi and Ne-bearing rocks.	Bakken et al., 1997
Legume	*Pueraria phaseoloides*	Feldspar (Ksp)	Field	14	Insignificant	Sanz Scovino and Rowell, 1988
Maize	*Zea mays* l. cv ND60	Gneiss	Pot	1.75	Significant K mobilization	Wang et al., 2000
Okra	*Abelmoschus esculentus* cv Eskandrani and cv Balady	Feldspar (Ksp)	Field	24	Increased pod yield compared with conventional fertiliser	Abdel-Mouty and El-Greadly, 2008
Onions	*Allium cepa*, L.	Feldspar (Ksp)	Field	24	15% less yield than equivalent chemical fertiliser	Ali and Taalab, 2008
Pak choi	*Brassica campestris* L. *ssp. Chinensis* L.	Gneiss	Pot	1.75	Significant K mobilization	Wang et al., 2000
Rice	*Oryza* sp.	Phlogopite	Pot		Increased grain yield	Weerasuriya et al., 1993
Spruce	*Picea* sp.	Phonolite	Field	60	Increased uptake	von Wilpert and Lukes, 2003
Tomatoes	*Solanum lycopersicum*: variety not stated	Feldspar (Ksp)	Field	12	Increased yield	Badr, (2006)
Wheat	*Triticum aestivum*	Granite	Field/pot	12/1–1.5	Insignificant	Bolland and Baker, 2000
Wheat	*Triticum aestivum* cv Gutha	Granite	Pot	2.5	Increased yield	Hinsinger et al., 1996
Wheat	*Triticum aestivum* cv Gutha	Diorite	Pot	2.5	Insignificant	Hinsinger et al., 1996

30 T rock per hectare (up to 1 T ha^{-1} K). Contrasting results were obtained. For pot experiments with wheat, Hinsinger et al. (1996) used granite (2.29% K_2O) and found significantly increased (by 10%–20%) biomass yields for applications of 7.5 T rock ha^{-1} (equivalent to 0.24 t K ha^{-1}). Experiments with ground diorite (0.3% K_2O) gave no increase in yield. Other work with wheat was inconclusive: Bolland and Baker report wheat yields for field and pot trials with applications of 2, 5 and 20 T rock per hectare (again 2.29% K_2O), with no increase in yield compared with the control. In contrast, pot trials using clover and ryegrass with the equivalent of 20 T rock per hectare (3.85% K_2O; Coreonos et al., 1996) showed that application of granite powder did enhance both yield and shoot K content significantly compared with control. In all

cases similar sandy soils with very low exchangeable K were used. From this work, it was concluded that the benefits of using crushed rock materials as a source of K are not sufficient in view of the cost differential compared with conventional soluble K sources (Harley and Gilkes, 2000). As the price of K has risen by a factor of at least 4 since 2000 (US$ 120/tonne), this conclusion may no longer be valid.

Pot trials with gneiss, which is a highly metamorphosed rock mineralogically similar to granite, are reported by Wang et al. (2000). The mineralogical and chemical compositions of the gneiss used in these experiments are not given, apart from the K content (29.6 g K kg^{-1} or 3.57% K_2O), and the observation that feldspar, muscovite and biotite are present. The purpose of the experiment was to compare the ability of different plants to extract K from the minerals within the gneiss, using maize (*Zea mays* L. cv. ND60), pak choi (*Brassica campestris* L. ssp. *chinensis*), Italian ryegrass (*Lolium multiflorum* L.), perennial ryegrass (*Lolium perenne* L.) and two alfalfa cultivars (*Medicago sativa* L. cv. Asta and cv. Haifei). Dry yields were found to be similar for plants grown with gneiss compared with those supplied with a corresponding nutrient solution, but K uptake was lower for plants grown with gneiss. Comparing different grain sizes for the crushed gneiss, K uptake was greatest for finer size fractions of gneiss, and in all cases greatest for maize, then ryegrass and pak choi. Wang et al. (2000) conclude that plant growth facilitates the release of K from gneiss, which contains the potassium minerals feldspar, muscovite and biotite, with varying efficiency that relates to root biomass and root activity.

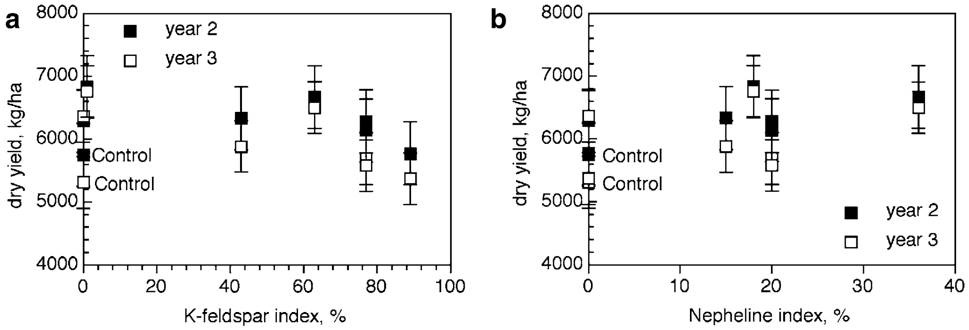
7.2 Trials with Nepheline-Bearing Rocks and Mineral Residues

To compare the possible effects of different potassium-bearing silicate minerals, studies reported by Bakken et al. (1997 and 2000) present data for experiments involving (a) Italian ryegrass (*Lolium multiflorum italicum* var. Turilo) in pot trials (Bakken et al., 1997) and (b) the growth of a mixture of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* L.) in field trials in central and northern Norway (Bakken et al., 2000). The sources of potash were mine tailings (nepheline-bearing carbonatites (an igneous carbonate rock); nepheline syenites), a quarried epidote schist, and commercial products (adularia shale, biotite concentrate). All K sources, including KCl for comparison, were applied at the rate of 50 kg K ha^{-1} in the first and second years only. Over a three year period, dry yield and K offtake were determined.

In their analysis of the results from these trials, Bakken et al. (2000) note that dry yields with KCl application were greatly in excess of those for the rock applications for years 1 and 2, but insignificantly different for year 3. Treatments with feldspar-rich rocks did not differ significantly from control, but those with biotite and nepheline-bearing rocks did show increased dry yields. However, in pot trials, Bakken et al. (1997) note that K uptake is similar for applications of nepheline-bearing carbonatite fines and KCl, and that results for experiments with K feldspar gave similar uptake to controls.

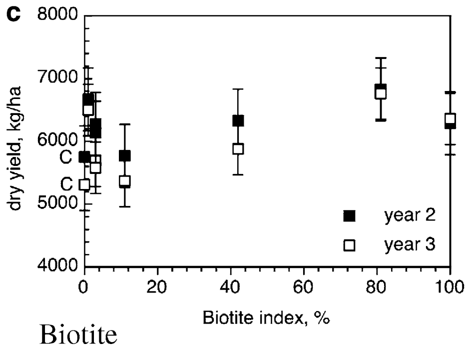
Figure 5 illustrates the different performance of the minerals used in Bakken et al.'s (2000) trials. In Fig. 5, the relative contributions of potassium feldspar, nepheline and biotite to the potassium content of the rock are expressed as an index (K-feldspar index = %K-feldspar/(%K-feldspar + %nepheline + %biotite), etc.) that allows the different rock types to be compared. These figures show that in general there is an increase in yield, compared with control, as the proportions of both nepheline and biotite increase. The zero nepheline index samples include the control and results for trials with 100% biotite. Because these figures concern relative proportions of the three minerals, the apparent decline in yield with increasing K-feldspar index (Fig. 5a) reflects the decreasing proportion of either nepheline or biotite.

A related trial with potentially nepheline-bearing rock is reported by von Wilpert and Lukes (2003), who used phonolite from the German Kaiserstuhl carbonatite complex in experiments with spruce (*Picea* sp.) on a glacial loam. Although the detailed mineralogy and origin of the phonolite is not given, in the context of published descriptions of phonolites from this location it can be assumed that if correctly named it contains nepheline (a characteristic of this rock type). von Wilpert and Lukes (2003) report that the phonolite used in their trials contains 4.23% K (5.1% K_2O), and that the rock may have contained zeolite minerals (giving it enhanced cation exchange capacity). The trials compared dolomite, phonolite and potassium sulphate, and showed that after 5 years the application



Potassium feldspar

Nepheline



Biotite

Fig. 5 Comparison of the performance of rock types used by Bakken et al. (2000) on the basis of their relative proportions of potassium feldspar, nepheline and biotite

of the two rock powders had influenced soil properties measurably, with an increase in the exchangeable ion pool. Nutritional effects were determined from the K content of the spruce needles, which after 4 years increased with the application of phonolite (but still remained below the deficiency threshold). Application of potassium sulphate eliminated the potassium deficiency, but led to deficiency in Ca and Mg, and had no major effect on soil properties. The value of phonolite as a long term source of nutrition was questioned in terms of an observed release of sodium, but the rock used in this trial was unusually rich in sodium for a phonolite (4.37% Na; 5.9% Na_2O); other phonolites, or related trachytes (which can be extremely K rich, up to 12–13% K_2O; e.g. Sutherland, 1967) may not have this problem.

In a separate study involving pot trials with rice (*Oryza* sp.), Weerasuriya et al. (1993) used phlogopite mica (a magnesium equivalent to biotite, in which Fe is substuted entirely by Mg; Deer et al., 1992) and potassium feldspar, both treated with concentrated nitric acid to enhance the availability of K. Application rates were equivalent to 200 kg ha^{-1} for phlogopite and 500 kg ha^{-1} for feldspar, corresponding to approximately 10 kg K ha^{-1} as total K. Significant increases in rice yield (panicle number and seed weight) were observed for the use of phlogopite compared with both control and muriate of potash.

7.3 Trials with Feldspars

Other work with feldspars alone has been carried out in Egypt, in field trials with okra (Abdel-Mouty and El-Greadly, 2008), onions (Ali and Taalab, 2008) and tomatoes (Badr, 2006). In trials with okra (*Abelmoschus esculentus* cv Eskandrani and cv Balady), potassium feldspar was used as one of a number of treatments designed to assess crop response (Abdel-Mouty and El-Greadly, 2008). Significant increases in pod yield were observed in treatments with potassium feldspar compared with a control, and these increases were enhanced with foliar application of gibberellic acid.

Similarly, Ali and Taalab (2008) report increasing individual bulb weight for onions (*Allium cepa* L.) with increasing feldspar application, and increasing overall yield, from 18 $\mathrm{T\,ha^{-1}}$with application equivalent to 95 $\mathrm{kg\,K\,ha^{-1}}$, to 30 $\mathrm{T\,ha^{-1}}$ with 285 $\mathrm{kg\,K\,ha^{-1}}$. Crop yields were however lower than those recorded for equivalent chemical fertiliser applications, by about 15%.

In experiments with mixtures of compost and feldspars, Badr (2006) reports an increase in the amount of available K with increasing proportions of added feldspar. There was also a clear increase in tomato yield with increasing proportions of feldspar in the composts used, from yields of 27 t/ha (control) to 45 $\mathrm{T\ ha^{-1}}$ with an application equivalent to 360 $\mathrm{kg\,K\,ha^{-1}}$. Potassium content of fruits also increased. Again, fruit yields were 10–15% less than observed for application of chemical fertiliser (in this case potassium sulphate).

The Egyptian experiments with potassium feldspars suggest that feldspar is almost as effective as a soluble potash fertiliser. Sanz Scovino and Rowell (1988) report trials with a mix of the grass *Brachiaria dyctioneura* and the legume *Pueraria phaseoloides* with the potassium feldspar sanidine, compared with muriate of potash. An increase in yield was observed, with maximum applications equivalent to 80 $\mathrm{kg\,K\ ha^{-1}}$, but this was statistically insignificant. Sanz Scovino and Rowell (1988) comment that application of potassium feldspar may be beneficial as an alternative to KCl in Colombia, where economic and agricultural conditions, including the occurrence of oxisol soils, give rise to disadvantages with the use of KCl.

7.4 Trials with Non-Granitic Bulk Rocks

Bulk rocks other than granites and rocks rich in alkali feldspars have been used as a source of soil nutrients in a number of studies, dominated by the use of phosphate rock which is well-established commercially (van Straaten, 2007). In general, trials with silicate rocks, especially volcanic rocks, have not focused on their possible value as a source of K, but as a source of a range of major and trace mineral nutrients.

Leonardos et al. (1987) approach the problem of nutrient supply in laterite systems, where soils (oxisols) are predominantly extremely depleted in nutrients, and have little or no capacity (by virtue of their mineralogy being dominated by quartz, kaolinite and aluminium oxy-hydroxides) to retain nutrients supplied by soluble fertilisers. Leonardos et al. (1987) strongly criticise conventional fertiliser practice that is driven by the industrial world: "Unfortunately, the standard concept and technology of soil fertilizer… is behind that of the superphosphate concept developed by J.B. Lawes in England, 150 years ago. … Had this technology been originally developed for the deep leached laterite soils of the tropics instead for (*sic*) the glacial and rock-debris-rich soils of the northern hemisphere our present fertilizers might have been quite different."

Preliminary data for trials with beans (*Phaseolus vulgaris*), napier grass (elephant grass; *Pennisetum purpureum*), and *Eucalyptus pellita* were carried out near Brasilia, using a range of different rock types (Leonardos et al., 1987). For beans, the greatest yields were observed in experiments with basalt applied at the equivalent of 6 $\mathrm{T\ ha^{-1}}$ and mica schist (as a source of K) applied at the equivalent of 60 $\mathrm{kg\,K\,ha^{-1}}$, with N and P applied as ammonium nitrate and triple superphosphate. For napier grass, yields increased with the application of limestone, basalt and the metamorphic rocks mica schist (mica-rich) and migmatite (feldspar-rich), up to 30% greater than for a control. Eucalyptus' response was measured by comparing plant height and trunk diameter; both were greatest with application of basalt.

More recently, other experiments have been carried out using volcanic rock (ultramafic rocks from the Mata da Corda; Theodoro and Leonardos, 2006; 30–36% SiO_2). This rock forms bedrock in an area of forest known for its fertility compared with neighbouring soils. Application at the rate of 2.5–3 $\mathrm{T\,ha^{-1}}$ was recommended to farmers who conducted the trials, which were designed to raise awareness in local people of possible benefits of indigenous rock sources. Soil fertility improved, with measurable increases in Ca, Mg, P and K, and reduction in exchangeable Al. Results were presented based on records of application and yield supplied by participating farmers, including their observations of plant growth. Maize (*Zea mays*), sugar cane (*Saccharum officinarum*), rice (*Oryzea sativa*), manioc (*Manihot esculenta*) and corn (*Zea mays*) were used in the trials.

Theodoro and Leonardos (2006) found in every case that application of the rock fertiliser compared to conventional practice with chemical fertilisers gave increased crop yields, most notably for sugar cane. However, in some cases manure was used in combination with the rock dust, and so it is difficult to attribute the observed benefits to rock dust alone. Theodoro and Leonardos (2006) report that the reaction of farmers engaged in the project was very positive to rock dust, and that the project gave many valuable social benefits in addition to the perceived agronomic benefits. The use of rock dust in tropical soils is developed further (without evidence from trials) by Fyfe et al. (2006), on the basis of a range of societal benefits.

Other work has been carried out to determine the behaviour under soil conditions of basaltic rocks (45% SiO_2; Hinsinger et al., 2001; Gillman et al., 2002) and andesitic rocks (typically 58% SiO_2; Meheruna and Akagi, 2006).

In laboratory studies designed to understand weathering phenomena, Hinsinger et al. (2001) assessed the influence of plant growth on the weathering of basalt, and report the results of experiments to assess the extent to which crop plants enhance the release of cations from the rock. In these experiments, lupin (*Lupinus albus* cv Lublanc), oilseed rape (*Brassica napus* cv Drakkar), banana (*Musa paradisiaca* cv Cavendish CV901) and maize (*Zea mays* cv Mona) were compared. The plants were grown in contact with a mesh bag that contained the powdered basalt (0.1–0.2 mm, irrigated with a nutrient solution. The effluent solution was analysed for elements liberated from the basalt that were lacking in the irrigation solution (especially Fe, Ca, Mg, Na, Si). Plant uptake of these elements was monitored with time, so the rate of basalt weathering could be compared between species. Rates of release to the leaching solution were greatest for Mg and Na with lupins, and for Fe with maize. Mass balance calculations showed that maize had greatest uptake of Na, Fe and Si, and banana had greatest uptake of Mg, Fe and Ca. The calculated weathering rates for the basalt are compared in Fig. 6. Bearing in mind that the reaction rate is expressed as a logarithm, Figure 6 shows that weathering rate varies from one plant species to another, and differs for different elements. With the exception of Ca, all weathering rates increase in the presence of plant growth, by an order of magnitude for Fe, and by a factor of up to 5 for Mg and Na, for banana, lupin and maize.

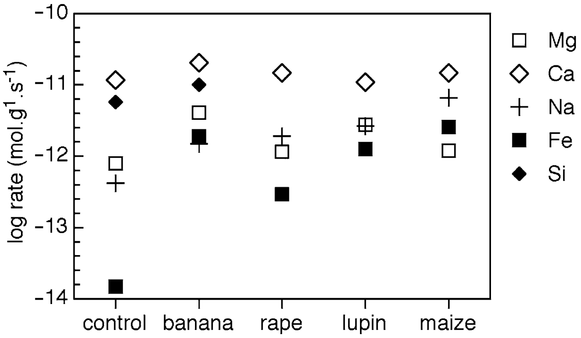


Fig. 6 Comparison of dissolution rates for basalt in the presence of different crops (data from Hinsinger et al., 2001)

Other laboratory experiments are reported by Gillman et al. (2002), who compared the effect of addition of basalt dust on the soil properties for a range of Queensland soil types in experiments involving incubation for 3 months at field moisture capacity. Soil properties (pH, CEC, exchangeable cations, P sorption capacity, extractable P and extractable Si) were determined before and after leaching with the equivalent of 2750 mm rainfall. Exchangeable H and Al were reduced, with an increase in exchangeable Ca and Mg. Basalt released both Si and P to the soil, and gave increased extractable P.

Meheruna and Akagi (2006) carried out experiments similar to those of Hinsinger et al. (2001), using andesite instead of basalt, and growing rice (*Oryza sativa*), maize (*Zea mays*) and soybean (*Glycien max* L.) under hydroponic conditions. Weathering was determined from the release of Si, Ca, Mg, Mn, Fe and Al, which was enhanced in all cases, most notably for Ca and Mg with maize. Weathering rate data were not given, and so cannot be compared directly with results presented by Hinsinger et al. (2001).

Although crushed rock materials have been promoted as nutrient sources for some time, this has been largely through the alternative or organic farming sectors (e.g. Lisle, 1994; Walters, 1975). The use of rock dusts underpins the “Remineralize the Earth” movement (http://www.remineralize.org), but as yet there are no published, statistically sound, trials that clearly demonstrate the agronomic value of rock dusts in isolation from other applications to the land, such as manures or composts. The use of rock powders in farming is, however, sufficiently acceptable and widespread to sustain commercial activity, especially with the growth in popularity of organic food.

8 Conclusion

Investigations of the possible use of silicate rocks as sources of K have yielded varying results. In general, there is a lack of consistency in terms of the design of individual trials, limiting comparison and extrapolation. Soil type and properties are rarely reported. Given its inherent inconsistency, the work reported here is limited in the extent to which it can be interpreted. However, it is undeniable that the price of potash has risen considerably since much of the experimental work was carried out, justifying further research.

Laboratory studies of the dissolution of feldspars and nepheline, with or without the presence of mycorrhizal soil microbial communities, have shown that in principle these minerals are capable of releasing K on a timescale that is appropriate to meet the needs of growing plants. When trials have been carried out, results have been variable, and have not always been substantiated by statistically rigorous experimental design or analysis. Nevertheless, the most positive results have been obtained for soils with a very low cation exchange capacity, typical of those of oxisols encountered in tropical/sub-tropical regions and that have poor nutrient retention characteristics. Thus the application of crushed rock materials, that might be derived from local sources, has to be considered seriously as an alternative to conventional chemical fertilisers where those might be poorly accessible on the grounds of cost or availability.

The price of conventional sources of K is much greater now than at the time of the trials reported here. Thus those reports that dismissed the use of crushed rock materials, especially granite, on economic grounds need to be reconsidered. In circumstances commonly encountered in developing countries, where the price of potash fertilisers on the world market is prohibitively high (especially following recent price rises), it is beneficial to explore the use of locally-sourced igneous rocks as a source of K.

There is clearly scope for careful selection of the silicate rock potassium source. On the basis of dissolution rate data, priority should be given to rocks that contain nepheline. These include nepheline syenites, phonolites and trachytes, rock types that are characteristic of rift valley tectonic settings, and so are available in many countries that might have difficulty funding adequate conventional fertiliser purchases. In geological descriptions, there is some overlap between trachyte and basalt, and areas mapped as basalt may include trachytic rock types. Thus the behaviour of basaltic rocks in crop production systems is relevant in consideration of sources of K, although such rocks generally have relatively low K contents.

If nepheline-bearing rocks are not available, it is appropriate to consider the use of potassium feldspars, one of the commonest minerals in the Earth's crust. Potassium feldspar often occurs in coarse grained pegmatites, from which it can easily be separated by eye, as well as in granites. Published trials in African and Columbian production systems demonstrate the benefit of application of feldspar for a range of crop types, as a material almost equivalent in performance to soluble potassium fertilisers.

For northern hemisphere countries in which agricultural soils are developed on tills or mechanically weathered rock substrates, or are rich in clay minerals, the ability to retain mineral nutrients is much greater than in tropical regions. In these circumstances, the use of crushed rocks as a source of K is less likely to be beneficial in general terms, although the long-term replenishment of K from crushed rock sources may be desirable in specific farming practices, such as organic farming.

One of the major problems associated with the use of crushed rocks as a source of K (or other nutrients) is the lack of robust experimental trials that are consistent in terms of both their agronomic and their mineralogical design. It is essential that the two disciplines of biology and mineralogy have equal weight in experiments of this type, as expertise in both is needed. Such trials require substantial investment in terms of time, and so need to be considered strategically. The current high cost of conventional potassium fertilisers coupled with increased demand for crop production both justify strategic investment of this type.

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Glandless Seed and Glanded Plant Research in Cotton

Yingfan Cai, Yongfang Xie, and Jinggao Liu

Contents

Abstract Recently the world has been entangled by insufficient food such as the lack of rice which threatens the safety of world food and affect sustainable development of the world economy, resulting in rising of food price. To address this issue, cotton appears as a possible source of both fiber and food. The research in recent years indeed showed bright prospects for this expectation. However, gossypol stored in the glands of cotton is toxic to nonruminant animals and humans, which wastes large amounts of cottonseed protein that could potentially provide the annual protein requirements for half a billion people. *Gossypium* species are characterized by their lysigenous glands containing terpenoid aldehydes, important secondary phytoalexins consisting mainly of gossypol, which constitute one of the important plant's defense system against pests and diseases. The best approach to address this issue is to create glandless seed and glanded plant cotton. A breakthrough in this field would realise the fulfilment of making cotton both a fiber and a food crop, which would be a feat of great magnitude for sustainable development of agriculture. Research on the relationship between glands and their secondary inclusions at the molecular level would be one approach for genetic engineering to control the glands and gossypol content. In this article, we review recent progress on glands and gossypol content for diverse gland types in *Gossypium* species, inheritance of glands and gossypol content, traditional breeding of glandless seeds and glanded plant cotton, the terpenoid aldehyde biosynthesis pathway, molecular cloning of the related genes, the strategy for genetic engineering, and future prospects.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_12,

from Cai et al., Agron. Sustain. Dev. 30 (2010) 181–190. DOI: 10.1051/agro:2008024

Keywords Cotton • Pigment gland • Gossypol • Inheritance and breeding • Gene cloning and genetic engineering

1 Introduction

Cotton, genus *Gossypium* L. containing 49 species, is a leading fiber and potential food crop, and cottonseed has compared favorably with other traditional food sources as a source of protein in several human nutritional studies (Lusas and Jividen, 1987). Cottonseed is among the most abundant protein meal and it represents 6.9% of world protein meal production only next to soybeans, rapeseed (Ash and Dohlaman, 2006). Global cottonseed production can potentially provide the annual protein requirements for half a billion people, for every kilogram of fiber produced by the plant, 1.65 kg of seed are also produced (Sunilkumar et al., 2006; Bertrand et al., 2005; Gerasimidis et al., 2007). But all cotton species have characteristic lysigenous glands containing terpenoid aldehydes, predominantly the sesquiterpenoid gossypol which is toxic to nonruminant animals and humans (Fryxell, 1968; Bell, 1969; Stipanovic et al., 1977). The pigment glands, as the storage organs of terpenoid aldehydes, including gossypol, are on the surfaces of cotton organs or tissues (Punit et al., 1991).

Gossypol was first characterized in a classic series of studies by Adams et al. in 1938 (Heinstein et al., 1962). Actually gossypol in cotton is a double-edged sword. It provides constitutive and inducible resistance against a variety of pests and diseases and acts as an important phytoalexin (Wang et al., 2004; Townsend et al., 2005). In vitro, gossypol also acts as an anticancer (Liu et al., 2002; Ye et al., 2007; Oliver et al., 2005), anti-HIV (Lin et al., 1993; Bourinbaiar Lee-Huang 1994; Keller et al., 2003), antibacterial and nonhormonal male contraceptive (Coutinho, 2002; Tegos et al., 2002; Lopez et al., 2005; Badawy et al., 2007). However, on account of its toxicity, the nutritional value of cottonseed is reduced much, in addition gossypol discolors cottonseed oil (Cherry, 1983) which results in a tremendous waste of seed protein and oil. To reduce gossypol in cottonseed, studies have developed glandless cotton, glandless both in seeds and plants, with no or very low gossypol content (McMichael, 1959; Vaissayre and Hau, 1985). The protein and oil of the glandless seed are free of gossypol and can therefore be used directly as food (Lusas and Jividen, 1987), but plant resistance to pests is reduced and the yield of cotton fibers is reduced due to the absence of gossypol and other terpenoid aldehydes, the glandless cotton has been applied in very limited area (Cherry, 1983; Vaissayre and Hau, 1985).

Cotton has been anticipated to become a crop that produces both fiber and food for decades, and thus developing a cotton variety that has gossypol within glanded roots, leaves, and stems to maintain the resistance trait but has glandless seeds for safe food utilization is now necessary (Cai et al., 2004). In other words, the ideal cotton cultivar would delay gossypol biosynthesis until germination, a phenotype seen only in some Australian *Gossypium* species (Brubaker et al., 1996). Many studies have examined the relationship between cotton glands and gossypol production, and recent efforts have shown much progress in this field, especially in molecular biology and genetic engineering. Such breakthroughs will revolutionize cotton breeding, cotton planting, industrial processing of cotton seed, and even use as animal feed and human food, and eventually will be beneficial to sustainable development of agriculture and human being and guarantee the safety of the world food.

2 The Relationship Between Gossypol and Glands: Diversity of Glands Occurence in *Gossypium* Species

Gossypol is synthesized in cotton roots, and transported and stored within pigment glands of cotton above ground (Smith, 1962). The pigment glands are spherical on leaves, bracts, and ovary surfaces; elliptical on stems and stigma surfaces; and spherical to oval on calyx surfaces. Genotypes differ markedly in gland distribution (Punit et al., 1991). The content of gossypol in cotton is dependent on the genetic types of pigment glands. The gland number of cotyledonary leaves is significantly and positively associated with free gossypol and positively associated with the number of gossypol-producing glands and free gossypol content in seeds (Punit et al., 1995).

High performance liquid chromatography, HPLC, analysis of gossypol in different gland genotypes of cotton revealed major differences among glands and species of *Gossypium*, including the glandless and glanded *G. hirsutum* L. and *G. barbadense* L. Especially *G. hirsutum* variety Xiangmian 18 is characterized by glandless seeds with very low gossypol content and low number of glands in foliage (Cai et al., 2004). "Glandless-seeded trait" in Australian *G. sturtianum* Willis reportedly lacks terpenoid aldehydes, and thus may represent an important genetic resource in the development of cottonseed oils and protein free of these toxins (Brubaker et al., 1996). The special trait of glands in this Australian species involves delayed pigment gland morphogenesis (Zhu et al., 1999). The studies on gossypol of the cotyledons during seed germination and the relationship between gossypol and gland formation in five wild Australia *Gossypium* species such as *G. sturtianum*, *G. nandewarense* Dereda, *G. australe* Mueller, *G. nelsonii* Fryxell, and *G. bickii* showed little gossypol in dormant seeds and cotyledons 24 h before seed germination. However, 48 h after germination, gossypol began to accumulate in cotyledons, although the content was very low. After 1–2 days of growth, the gossypol content in cotyledons increased markedly and was highest 9 days after germination. The dynamics of gossypol accumulation during seed germination was basically the same in all five wild species, but the time at which gossypol first appeared in the cotyledons differed significantly among them. Presumably some genetic regulation exists between glands and gossypol content (Zhu et al., 1999). Pigment glands in *Gossypium* species have diverse characteristics; glanded cotton normally contains gossypol in both seeds and other plant parts, and the content of gossypol is highly correlated with the number of pigment lands in glanded cotton (Singh and Weaver, 1972). However, in *G. somalense* Hutchinson (Genome E2) and *G. herbaceum* L. (Genome E1), pigment glands are found in the seeds but the gossypol content is unmeasurable.

In general, the following five types of pigment glands are found in *Gossypium* species: glandless cotton, with no or very low gossypol and no glands in seeds, roots, stems, and leaves; glanded cotton, with gossypol within glands in seeds, roots, stems, and leaves; cotton with a delayed gland morphogenesis trait (Zhu et al., 1999), specifically the Australian *Gossypium* species *G. bickii* (Genome G1), *G. australe* (Genome C3), and *G. sturtianum* (Genome C1-n), which contain no or very low gossypol and have no glands in seeds, but have gossypol and glands in roots, stems, and leaves; cotton with glands in seeds, but very low gossypol content, e.g., *G. stocksii*; and cotton that has no or few glands and low gossypol in seeds, but has some glands and gossypol in plant parts. Hence, the diversity of glands in *Gossypium* and the relationship between glands and gossypol content warrant further exploration.

3 Inheritance of Glands and Gossypol

Results on the genetics of glands in cotton have differed because different gland materials have been used. The glandless trait is thought to be controlled by two or three recessive or dominant genes in different gland types from different cotton species. According to McMichael (1960), the glandless trait is controlled by two recessive genes, gl2 and gl3, found in lines of Hopi cotton (McMichael, 1960). In other varieties, however, such as Hai 1 and others, it is controlled by dominant genes such as GL2e (Carvalho and de Vieira, 2000). Yet other scientists have reported that the glandless trait is a quantitative trait controlled by minor polygenes (Lee et al., 1968). GL2e in *G. barbadense* is dominant and produces glandless plants and seeds. The free and total gossypol contents in F1 seeds from this variety were similar to those of the glandless parent, showing complete dominance of this gene (Carvalho and de Vieira, 2000).

Twenty-one gossypol glanded cultivars from Turkey were crossed with two Turkish gossypol-free cultivars, Suz-86 and 129. Of 5588 plants raised in the F2 generation, 340 were entirely glandless. According to the two independent pairs of genes (glanded: GL2 GL2 GL3 GL3; glandless: gl2 gl2 gl3 gl3), the expected 1/16 ratio of glandless plants in the F2 generation was in accordance with a chi-square test (Arshad Mahmood et al., 2004).

A mutant of Xiang X9628 (Xiangmian 18) in upland cotton (*G. hirsutum*) has normal glanded leaves and a low gossypol content in seeds (Zhang et al., 2001). Genetic analysis indicated that the glanded leaves and low gossypol content of the mutant are controlled by two pairs of recessive duplicate genes. Allelic tests showed that one of these is allelic to the gl2 gene and the other is a multi-allele of the gl3 loci. This was therefore considered to be a new gland-forming gene in *Gossypium* and was named gln3. Data from crosses using line 247-1, which is rich in glands and has a high gossypol content in the flower buds, and plants homozygous for various combinations of gland-determining alleles at the gl1, gl2 and gl3 loci indicate that the high gossypol content of 247-1 could be explained by the presence of a high-potency allele at the gl3 locus, plus another factor or factors independent of the other gland-determining alleles (Wilson and Smith, 1976).

Studies on the inheritance of the high-glanding (HG) trait in cotton (*G. hirsutum*) using crosses made among HG, normal glanding (NG) and glandless genotypes, and with isolines of the HG breeding line XG15, which has the genotypes 2 (Gl2gl3) and 2 (gl2Gl3), showed that isoline XG15 gl2Gl3 expressed the HG phenotype, suggesting that HG is conferred by a special Gl3 allele derived from XG15. This allele, derived originally from Socorro Island cotton, was tentatively designated Gl3s (Calhoun, 1997). Additive effects accounted for more than 90% of the total genetic variance in seed gossypol level in all trials.

Epistatic effects, though small, were frequently significant. In *G. barbadense*, Gl2 and Gl3 were found to be associated with the production of similar amounts of gossypol, whereas previous trials with cultivated varieties of *G. hirsutum* showed that more than twice in Gl2 was expressed compared to Gl3. The higher average productivity of seed gossypol in cultivated *G. barbadense* compared to *G. hirsutum* is attributable to higher activity at the Gl3 locus in the former species (Lee, 1973).

Additive and dominance effects and other digenic interactions are associated with gossypol content, with the additive effects and additive × additive epistasis being more prominent. The variety of interactions indicate that the major gland determining the loci gl2 and gl3 are not the only determinants of gossypol content, but that the trait shows polygenic inheritance (Singh et al., 1991).

4 Traditional Breeding of Glandless Seed/Glanded Cotton Plants

Since a close relationship exists between gossypol content and pigment glands, tactics to control gossypol through selecting and controlling the gland trait have proven to be appropriate and effective. In cotton breeding, the gland trait has been used for two different purposes: to produce glands rich in gossypol or with high glanding (HG) or to produce glandless plants.

On the one hand, breeding and application of a variety with a high density of glands and high gossypol content can enhance cotton plant resistance to pests and diseases. Such lines and varieties with high gossypol are beneficial as they reduce the cost for pest control and therefore cause less environmental pollution from the use of chemicals (Calhoun and Jones, 1994; Calhoun, 1997; Lee, 1978; McCarty et al., 1996; Bourland and Benson, 2002; Li et al., 1996).

On the other hand, to make full use of cottonseed protein and oil, scientists bred the glandless cotton 23B, glandless both in seeds and plants, with no or very low gossypol content (McMichael, 1954, 1959, 1960). Subsequently, many more glandless varieties have been bred from the resources created by McMichael (Lusas and Jividen, 1987; Miravalle, 1972; Vaissayre and Hau, 1985). Cottonseed compares favourably to other traditional food sources as a source of protein in several human nutrition studies, and glandless cottonseed is safe and nutritious (Lusas and Jividen, 1987). However, glandless varieties have proven to be commercially unviable as they are more susceptibility to insect pests because of the systemic absence of glands that contain gossypol and other protective terpenoids (Sunilkumar et al., 2006). Hence, the best choice currently available is plants with glandless seeds and glanded foliage, and many scientists have attempted to breed such plants.

A new upland cotton (*G. hirsutum*) cultivar has been bred in China, Xiangmian 18, with glandless seed, which has very low seed gossypol and is a glanded plant. This was developed from Xiangmian 10 × Zhong 5655, and was released in China during 2000. The glandless traits of glandless Zhong 5655 originated from Hai 1 (*G. barbadense* L.) This new cultivar can be grown for the production of seed protein and oil as well as lint (Zhang X. et al., 2001).

To transfer the traits for glandless seeds and glanded plants – so-called "delayed pigment gland morphogenesis" or "delayed development of gossypol glands" – Australian species have been bred to cultivated species of *Gossypium* to produce tri-species hybrids. A series of hybrid seeds or materials with the traits for glandless seeds and glanded plants have been obtained, but these have not yet reached the potential for use in commercial cotton production because of many problems such as sterility, with wild traits and other disadvantages (Altman et al., 1987; Kulkarni et al., 2002; Mergeai, 1992; Mergeai et al., 1995, 1997; Dilday, 1986; McCarty et al., 1996; Vroh et al., 1999; Bi et al., 1998, 1999; Zhu et al., 1993, 2004, 2005; Bourland and Jones, 2006; Ahoton et al., 2003).

5 Terpenoid Aldehydes in Glands and the Biosynthetic Pathway of Gossypol and Associated Terpenoids

Lysigenous glands in cotton plants contain terpenoid aldehydes (Bell, 1969; Stipanovic et al., 1977). In cultivated cotton, glands in achlorophyllous plant parts of *G. hirsutum* predominantly contain the gossypol and its methyl and dimethyl ethers are found in *G. barbadense*. Glands in young green tissues of *G. hirsutum*, however, contain hemigossypolone as the predominant terpenoid aldehyde, and a new quinone, hemigossypolone-7-methyl ether occurs in *G. barbadense* (Bell et al., 1978; Benedict et al., 2004). A survey of terpenoid quinones and their heliocide derivates in wild *Gossypium* spp. and related genera in the *Gossypieae* showed considerable diversity. Several cadinane sesquiterpenoids and heliocides (sesterterpenoids) are deposited in the pigment glands of cotton plants, which function in pathogen and insect resistance (Stipanovic et al., 1999; Martin et al., 2003).

Based on the structure of gossypol, it was suggested that it might be formed metabolically from acetate via the isoprenoid pathway. Heinstein and colleagues examined the location of incorporated ^{14}C and studied the incorporation of mevalonate-2-^{14}C, a key intermediate in the isoprenoid pathway, into gossypol, as well as the distribution of radioactivity in the gossypol molecule (Heinstein et al., 1962, 1970).

The cotton terpenoid aldehydes and cadalene derivatives are sesquiterpenes (C15) derived from a cytosolic branch of terpenoid metabolism via the mevalonate pathway (Heinstein et al., 1962, 1970). Farnesyl diphosphate (FPP) is generated as the linear carbon skeleton of the sesquiterpenes in cotton (Essenberg et al., 1985; Stipanovic et al., 1986; Liu et al., 1999). The various sesquiterpene synthases cyclize FPP to form the molecular frameworks of different sesquiterpene types (Gershenzon and Croteau, 1993).

Gossypol is the sesquiterpenoid formed in cottonseed, with only traces of desoxyhemigossypol (dHG) and hemigossypol. In cotton foliage, hemigossypolone is formed from dHG (Stipanovic et al., 1999). The cadinene enzyme was first purified from a glandless cotton mutant by Davis and Essenberg (1995) as a soluble hydrophobic monomer with a molecular mass of 64 to 65 kD.

As reported by Martin et al. (2003), there are several cadinane sesquiterpenoids and heliocides (sesterterpenoids) deposited in pigment glands in cotton plants that function in pathogen and insect resistance (Stipanovic et al., 1999). A proposed pathway for the biosynthesis of these compounds is shown in Fig. 1. Infection of cotton stele tissue with conidia of *Verticillium dahliae* that induced the formation of sesquiterpenoid phytoalexins also induced 3-hydroxy-3-methylglutaryl-CoA reducterpenoid aldehydese (HMGR) mRNA and HMGR activity, demonstrating the important role of HMGR in the biosynthesis of the sesquiterpenoids (Bianchini et al., 1994). Studies with specifically labeled mevalonic acid (MVA) or acetate demonstrated the folding pattern of farnesyl diphosphate (FDP) required for gossypol formation. Subsequently, the enzymatic product of the cyclization of E, E-FDP in cotton extracts was identified as (+)-δ-cadinene (CDN) (Benedict et al., 2001; Davis and Essenberg, 1995; Chen Z.Y. et al., 1995). The enzymatic mechanism of CDN synthase for the formation of the cadinane structure of cotton sesquiterpenoids was shown to involve the isomerization of FDP to a nerolidyl intermediate, cyclization to a *cis*-germacradienyl cation, a 1, 3-hydride shift, cyclization to a cadinanyl cation and deprotonation to form (+)-δ-cadinene (Benedict et al., 2001). CDN synthase catalyses the committed step in the formation of the cadinane sesquiterpenoids from FDP at a branch point in the MVA pathway. Gossypol is the sesquiterpenoid formed in cottonseed with only traces of desoxyhemigossypol (dHG) and hemigossypol. In cotton foliage hemigossypolone is formed from dHG (Stipanovic et al., 1999). A Diels–Alder reaction accounts for the cycloaddition of myrcene or β-ocimene to hemigossypolone to form heliocides (Stipanovic, 1992). The monoterpenes or its precursors are synthesized by the 1-deoxy- -xylulose-5-phosphate (DOXP)

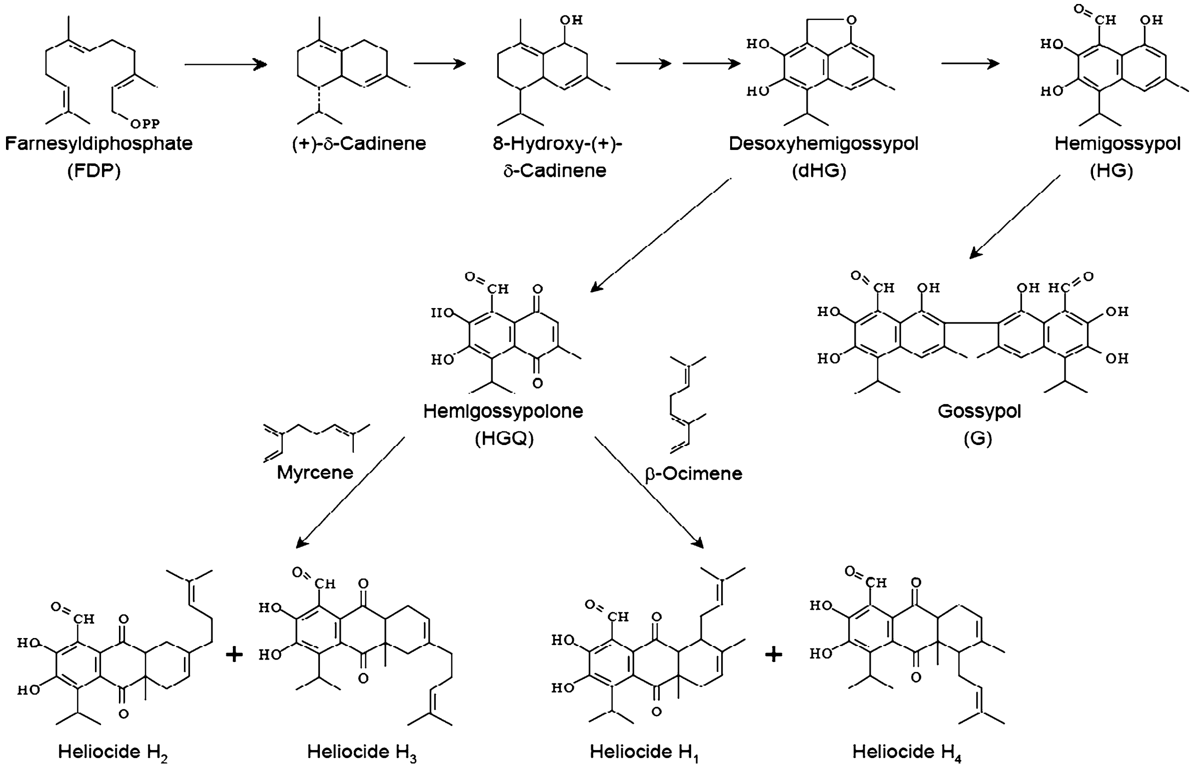

Fig. 1 The biosynthesis of gossypol in cottonseed and gossypol, hemigossypolene and heliocides$_{1-4}$ in cotton leaves (Modified from Martin et al., 2003)

pathway in the plastids (Martin et al., 2003; Essenberg et al., 1985; Bell, 1986; Stipanovic et al., 1986; Benedict et al., 1995; Davis and Essenberg, 1995; Alchanati et al., 1998).

In recent years, the biosynthetic pathway of gossypol and its derivates has been further elucidated. 8-Hydroxy-(+) d-cadinene is a precursor of hemigossypol in *G. hirsutum* (Yan-Hong wang et al., 2003). A (+) d-cadinene-8-hydroxylase, a cytochrome P450 mono-oxygenase of cotton sesquiterpene biosynthesis (CYP706B1), was expressed in aerial tissues of glanded cotton cultivars, but not or at an extremely low level in the aerial tissues of a glandless cultivar. The expression pattern of CYP706B1 and the position at which it hydroxylates (+) d-cadinene suggest that it catalyzes an early step in gossypol biosynthesis, and thus CYP706B1 holds good potential for manipulating gossypol levels in cottonseed via genetic engineering (Luo et al., 2001). Desoxyhemigossypol is a key intermediate in the biosynthesis of these compounds. A methyltransferase (S-adenosyl-L-Met: desoxyhemigossypol-6-O-methyltransferase) was isolated, purified, and characterized from cotton stele tissue infected with *V. dahliae*. Desoxyhemigossypol-6-methyl ether leads to the biosynthesis of methylated hemigossypol, gossypol, hemigossypolone, or the heliocides (Liu et al., 1999, 2005).

6 Molecular Cloning of Genes Associated with Gossypol and Glands

Based on elucidation of the terpenoid biosynthetic pathway, the related genes have also been cloned. The cadinene gene was first cloned and functionally characterized from the A-genome diploid cotton *G. arboreum* and comprise a large multigene family in cotton (Chen Z.Y. et al., 1995; Davis et al., 1996; Meng et al., 1999; Tan et al., 2000) similar to the terpene cyclase genes found in other plants (Facchini and Chappell, 1992; Back and Chappell, 1995). Several allelic and gene family variants of the cotton cadinen genes have since been isolated from both *G. arboreum* (Chen et al.,

Table 1 The cadinene genes and complementary DNA cloned in *Gossypium* in GenBank

Subfamily or gene name	Accession number	*Gossypium* species	Author	Submited year
cdn XC14	U23205	*G. arboreum*	Chen Z.Y. et al.	1995
cdn XC1	U23206	*G. arboreum*	Chen Z.Y. et al.	1995
cad1-b	X95323	*G. arboreum*	Chen X.Y. et al.	1996
cdn A	U27535	*G. arboreum*	Chen Z.Y. et al.	1995
cad1-A	Y18484	*G. arboreum*	Liang	1998
cad1-A	X96429	*G. arboreum*	Chen X.Y. et al.	1996
cad1-A	AF456410	*G. barbadense*	Cai	2002
cad XC14	AF453326	*G. hirsutum*	Cai	2001
cad1-C2	Y16432	*G. arboreum*	Meng et al.	1999
cad1-C1	AF174294	*G. arboreum*	Tan et al.	2000
cdn1	U88318	*G. hirsutum*	Davis	1997
cdn1-C4	AF270425	*G.hirsutum*	Townsend	2000
cdn1-C5	AY800106	*G. hirsutum*	Townsend	2004
cdn1-D1	AY800107	*G. hirsutum*	Townsend	2004

1996; Liu et al., 1999; Meng et al., 1999; Tan et al., 2000) and the allotetraploid (A + D genomes) species *G. hirsutum* (Davis et al., 1998) (Table 1).

The cadinene enzyme and transcripts are induced in cotton stems infected with *V. dahliae* (Benedict et al., 1995; Alchanati et al., 1998; Bianchini et al., 1999; Tan et al., 2000), cotton suspension cultures treated with *V. dahliae* elicitors (Chen Z.Y. et al., 1995; Chen X.Y. et al., 1996; Liu et al., 1999), and cotton cotyledons infected with Xcm (Davis and Essenberg, 1995; Davis et al., 1996). cadinene is also developmentally regulated and cadinen transcripts increase during seed development in association with the biosynthesis and deposition of gossypol in the lysigenous storage glands of the embryo (Meng et al., 1999; Martin et al., 2003). Two major subfamilies of the *Gossypium* cadinene multigene family, cdn1-A and cdn1-C, have been proposed based on sequence relatedness, and appear to be differentially regulated at the transcriptional level (Meng et al., 1999; Tan et al., 2000). A third subfamily, cdn1-B, is represented by a single genomic clone.

A phylogenetic tree of the cotton cadinene genes showed the relatedness of members within the cadinene subfamilies and the distance between the different subfamilies (Fig. 2). cdn1-D1 is sufficiently different from the other cadinene genes of cotton to suggest that it belongs to another subfamily, designated cdn1-D.

The genetic structure of cadinene genes is well conserved, including the number, positions, and sizes of exons and introns, and also corresponds well to the genomic clones of other terpene cyclase genes, such as tobacco (*Nicotiana tabacum* L.) 5-epi-aristolochene synthase. Approximately 2 kb of promoter regions from each cadinene genomic clone were sequenced and a very low level of sequence conservation was found between all cadinene promoter regions. We found discrete regions of similarity, mostly in the vicinity of the predicted TATA box and transcription start site, but the similarity did not extend appreciably upstream (Back and Chappell, 1995).

Luo et al. (2001) cloned and identified the function of a gene of (+) delta-cadinene-8-hydroxylase, a cytochrome P450 mono-oxygenase of cotton sesquiterpene biosynthesis (Luo et al., 2001). Liu et al. (2005) identified and cloned a 1.9 -kb P450 coding for a 522 amino acid protein that is 48% identical to a soybean cytochrome P450 82 A3 and contains a conserved heme-binding motif and a consensus oxygen-binding pocket sequence (Liu et al., 2005). This P450 is expressed in the leaves of the glanded cotton *G. hirsutum*, but not in the leaves of glandless cotton. The leaves of glandless plants are devoid of glands and the associated terpenoids. This suggests that this P450 enzyme is involved in the terpenoid biosynthetic pathway in cotton.

Studies on gland-morphogenesis-related genes, applying suppressive subtractive hybridization and other methods, have successfully constructed a subtractive library and a normalized cDNA library from a cotton mutant, Xiangmian 18. Some relevant genes, such as the RanBP2 zinc finger protein gene in upland cotton, have been cloned (Cai et al., 2003; Chang et al., 2007; Xie et al., 2007). In addition, the gene of desoxyhemigossypol-6-O-methyltransferase

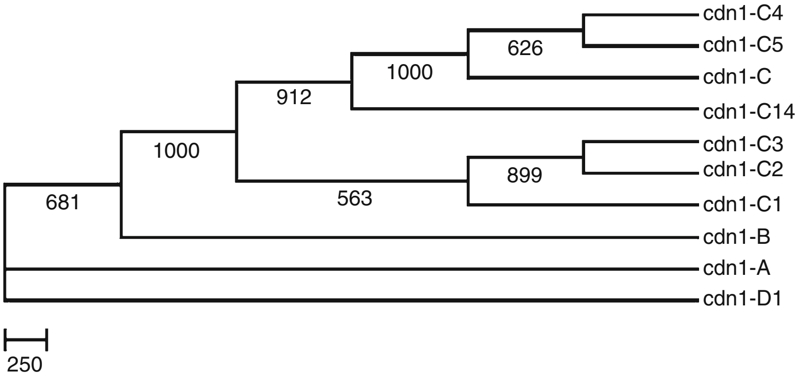

Fig. 2 Phylogenetic tree of cadinene protein sequences showing the subfamilies cdn1-A, cdn1-B, cdn1-C, and cdn1-D

from *G. barbadense* (Liu et al., 1999) and gene of cytochrome P450 related to glands have also been cloned and studied (Liu et al., 2005, 2007).

7 A Strategy for Eliminating Gossypol and Creating Glandless Seed-Glanded Cotton Plants

Traditional breeding is still the main approach in the breeding of cotton with glandless seed and glanded plant, but it is limited by, for example, long breeding cycles, frequent attachment to inappropriate genes, and especially, crosses between species. In practice, progress in traditional breeding to create glandless seed and glanded foliage cotton is very slow and large problems still exist. Genetic engineering can hopefully solve that. Advances in understanding the terpenoid biosynthetic pathway and cloning of the relevant genes provide tools to manipulate the biosynthesis of these defense terpenoids through genetic engineering creating glandless seed and glanded foliage cotton. Three aspects are involved in the strategy to eliminate the toxic cottonseed gossypol through genetic engineering: silencing gossypol-related genes in the seeds of glanded cotton using antisense and RNAi method; turning on related foliage genes in glandless cotton; and creating cotton only containing (+) gossypol, the toxicity of which is very low compared to (–) gossypol.

Antisense expression of cadinene genes was envisaged as a way to activate this silencing mechanism in cotton by blocking the cadinane-type sesquiterpene pathway and abolishing gossypol production in the transformants. An antisense construct of cdn1-Cl was introduced into cotton plants (Martin et al., 2003), and Southern analyses showed integration of antisense cdn1-C1 cDNA driven by the CaMV 35S promoter into the cotton genome. Northern blots demonstrated the appearance of cdn synthase mRNA preceding CDN synthase activity and the formation of gossypol in developing cottonseed. T2 cottonseed had reduced CDN synthase activity and up to a 70% reduction in gossypol. In T1 leaves, the accumulated gossypol, hemigossypolone, and heliocides were reduced by 92.4, 83.3, and 68.4%, respectively. These results demonstrate that the integration of antisense cdn1-C1 cDNA into the cotton genome leads to a reduction in CDN synthase activity and negatively impacts the biosynthesis of cadinane sesquiterpenoids and heliocides in cotton plants.

Another new cotton variant from the progeny of hemizygous *G. hirsutum* cv. Coker 312 transformed with antisense (+)- delta-cadinene synthase cDNA has reduced the TA levels (Benedict et al., 2004). The gossypol content in seeds of the variant was markedly lower than in seeds of T1 antisense plants. Eighty-nine percent of the variant seed had a 71.1% reduction in gossypol, and foliage of the variant plants had a 70% reduction in gossypol and a 31% reduction in heliocides. Compared to non-transformed plants, no reduction occurred in the number of lysigenous glands in the seed of the variant. The cotton variant showed uncoupling of TA synthesis and gland formation. In this case, the study assumed that the cotton variant may have resulted from somaclonal variation occurring in callus tissue during the transformation-regeneration process.

Constructs for the constitutive or seed-specific antisense suppression of cdn1-C4 were introduced into cotton by *Agrobacterium*-mediated transformation (Townsend et al., 2005). Gossypol levels were not reduced in the seeds of transformants with either construct, and the induction of cadinene expression did not affect stems of the constitutive antisense plants infected with *V. dahliae*. However, the induction of cadinene mRNA and protein in response to bacterial blight infection of cotyledons was completely blocked in the constitutive antisense plants. These results suggest that cdn1-C4 may be involved specifically in the bacterial blight response. Moreover, the cadinene multigene family may comprise a complex set of genes differing in their temporal and spatial regulation, and they may be responsible for different branches of the cotton sesquiterpene pathway. The antisense approach, however, has been limited or somewhat ambiguous (Martin et al., 2003; Townsend et al., 2005; Sunilkumar et al., 2006).

Recently, a study successfully used RNAi and a seed-specific promoter from the cotton a-globulin B gene to disrupt gossypol biosynthesis in cottonseed by interfering with expression of the cadinene synthase gene during seed development (Sunilkumar et al., 2006). The study demonstrated the possibility of significantly lowering cottonseed- gossypol levels in a stable and heritable manner. Results from enzyme activity and molecular analyses on developing transgenic embryos were consistent with the observed phenotype in mature seeds. Most importantly, the levels of gossypol and related terpenoids in foliage and floral parts were not diminished, and thus their potential function in plant defense against pests and diseases remained untouched. This research provides evidence for spatial and temporal confinement of RNAi-mediated suppression of the s-cadinene synthase gene in cottonseeds that contain the transgene. The results demonstrate the feasibility of a targeted RNAi-based approach to solve an age-old problem of cottonseed toxicity and provide an avenue to exploit the considerable quantities of protein and oil available in the global cottonseed output. The GL genes will be cloned and applied in genetic engineering to obtain glandless seed and glanded foliage cotton.

Since the (+) enantiomer shows little if any toxicity to nonruminant animals, cottonseed with high -protein content is underutilized due to toxic (–)- gossypol. The data from extracts of the Marie Galante cotton variety and the fact that the intact seed contain 95% (+) gossypol suggest a regio-stereoselective bimolecular coupling of hemigossypol to gossypol (Benedict et al., 2006). This can be used in future genetic engineering to eliminate the toxic (–) gossypol. As the (–) enantiomer has potential medicinal uses, cottonseed with >95% (–) gossypol could have biopharmaceutical applications and can also be created through genetic engineering.

8 Conclusion

With the increase in world population, decrease in available arable land, and need for more energy from plants, the world has been threatened by insufficient food. The desire to make cotton both a fiber and a food crop becomes more urgent. Peaple have bended themselve to this research for decades and have attempted many approaches to get rid of toxic gossypol and have made much progress as described above. In recent years, one successful research trend on glands and gossypol in cotton has focused on molecular cloning and genetic engineering to produce glandless seed and glanded foliage to abolish toxic seed gossypol beyond the immediate application of eliminating gossypol from cotton seeds, one can readily anticipate other application for the gene and its promoter. For example, the promoter could be used to introduce pesticides such as the BT toxin exclusively into the foliage (Liu et al., 2007). One could even use an exotic toxin, for example, from scorpions, since the green tissue special promoter would be directly expressed exclusively in the foliage. Obtaining such a promoter also provides a tool to manipulate the biosynthesis of HQG and heliocides through genetic engineering (Liu et al., 2007). The long-term goal is to elucidate the gene network mechanism controlling glands and gossypol while increasing the resistance of cotton to pests and pathogens, thus expanding utilization and the commercial value of cottonseed. Glandless seed and glanded foliage cotton will bring a revolutionary change in cotton breeding, cotton planting, and industrial processing of cotton seeds, even for use as a food supply. Cotton will become a crop that produces fiber, food, and oil simultaneously, which will help solve the problem of world food supply shortages.

At the same time, the research has also helped us to obtain a better understanding of the relationship between secondary compounds and storage organs (tissues) in plants and the molecular mechanisms controlling them. For example, artemesin and glands in southernwood (an antimalarial) have a similar relationship to gossypol and glands in cotton (Xu et al., 2003). The long-term goal of our research is to clarify the molecular mechanism (s) of the genetic control networks involved in secondary substances and storage organs in plants, and ultimately to control the biosynthesis of useful secondary compounds that will promote the sustainable development of agriculture and benefit humans.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (No. 30771311 and No. 30440032) and Natural Sciences Foundation of Chongqing cstc 2007 BB1328 of China and USDA and Congress of USA and Nan'an committee of Science and Technology of 2008 in Chongqing.

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Micronutrient-Efficient Genotypes for Crop Yield and Nutritional Quality in Sustainable Agriculture

Amir Hossein Khoshgoftarmanesh, Rainer Schulin, Rufus L. Chaney, Bahareh Daneshbakhsh, and Majid Afyuni

Contents

Abstract About 4 billion people will be added onto the present population by 2050. To meet further demand for food, agricultural production should increase on the existing land. Since the Green Revolution, higher crop production per unit area has resulted in greater depletion of soil phytoavailable micronutrients while less attention has been paid to micronutrients fertilization. Now, micronutrient deficiency has become a limiting factor for crop productivity in many agricultural lands worldwide. Furthermore, many food systems in developing countries can not provide sufficient micronutrient content to meet the demands of their citizens, especially low-income families. There are several solutions such as soil and foliar fertilization, crop systems, application of organic amendments to correct micronutrients deficiency and to increase their density in edible parts of plants. This review article presents (1) agronomic approaches to improve crop yield and micronutrient content of food crops, and (2) genotypic variation in uptake and accumulation of micronutrients. Considering ecological concerns, cultivation and breeding of micronutrient-efficient genotypes in combination with proper agronomic management practices appear as the most sustainable and cost-effective solution for alleviating food-chain micronutrient deficiency. Micronutrient-efficient genotypes could provide a number of benefits such as reductions in the use of fertilizers, improvements in seedling vigor, and resistance to biotic and abiotic stresses. Using bioavailable micronutrient–dense staple crop cultivars can also be used to improve the micronutrient nutritional status of human.

Keywords Micronutrients • Nutrient efficiency • Biofortification • Stress-tolerance indicators

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_13,

from Khoshgoftarmanesh et al., Agron. Sustain. Dev. 30 (2010) 83–107. DOI: 10.1051/agro:2009017

1 Introduction

The world's population is estimated to increase from 6 billion to about 10 billion by 2050. To meet the food demand of the growing world population, a large increase in food production is required. At the same time, the increases in world population will result in serious pressure on the existing agricultural land through urbanization and intensive cultivation (Alexandratos, 1995; Byrnes and Bumb, 1998). It has been estimated that to supply enough food for the world population in 2020, annual cereal production needs to increase by 40%, from 1773 billion tons in 1993 to nearly 2500 billion tons in 2020 (Rosegrant et al., 1999, 2001). About 85% of the increase in total cereal demand will occur in the developing countries.

There are two strategies to increase food production: (a) to expand the agricultural area and (b) to enhance crop yields per unit area. The expansion of agricultural area is limited due to lack of suitable lands, urbanization, degradation of soils, and increasing water scarcity (Brown, 1997). To meet the further demand for food, agricultural production must increase on the existing land. This means that more crop food production must be achieved per unit of the presently available arable land.

Since the "Green Revolution", intensive cropping, cultivation of high-yield genotypes, improved agricultural mechanization, production of macronutrient fertilizers with low impurities of trace elements, and using modern irrigation systems has resulted in higher crop production per unit area and greater depletion of soil phytoavailable micronutrients.

Losses of micronutrients through erosion, leaching, liming of acid soils, decreased proportions of farmyard manure compared to chemical fertilizers, and use of marginal lands for crop production are other factors that have increased the incidence of micronutrient deficiencies in agricultural soil worldwide (Fageria et al., 2002). Micronutrient deficiency problems are also exacerbated by the high demand of modern crop cultivars. Accordingly, low levels of micronutrients have been reported on many crops grown in different countries (Cakmak et al., 1996; Fageria, 2000a; Galrão, 1999; Graham et al., 1992; Grewal and Graham, 1999; Martens and Lindsay, 1990). Therefore, micronutrient deficiency has become a limiting factor for crop productivity in many agricultural soils. In order to obtain the genetic potential yields of crops, correcting micronutrients deficiencies is necessary.

On the other hand, agricultural practices have almost always targeted higher crop yield production while minimizing costs. In addition, nutrient output of farming systems has never been a goal of either agriculture or of public policy. Thus, the increase in crop yield in many agricultural systems as a result of chemical fertilizer application has been accompanied with reduced micronutrients concentrations in the edible parts of different crops. So that, already, many food systems in developing countries can not provide sufficient micronutrients concentrations to meet the demands of their people especially low-income families (ACC/SCN, 1992, 1997, 2000; Combs et al., 1996; Combs and Welch, 1998; McIntyre et al., 2001; Welch et al. 1997; Welch, 1998).

Recently, mid 1980s, the nutrition community reported the global significance of micronutrient malnutrition (Allen, 2000). In 2001, fighting micronutrient malnutrition was considered an integral component of three of the eight Millennium Development Goals (MDGs) of the General Assembly of the UN to alleviate the world's greatest health and poverty issues by 2015 (United Nations General Assembly, 2000). The focus of improving the micronutrient quality of crops notes the density of bioavailable micronutrients in crops as consumed in order to take into account crop factors which increase or decrease the bioavailability of crop micronutrients (see below). For example, phytate in grains is well known to inhibit absorption or utilization of crop Zn by humans and livestock, such that grain phytate must be considered whenever the quality of grain Zn for human use is considered.

Deficiencies of iron, zinc, vitamin A, and iodine not only compromise the immune system, but can irreversibly retard development in infancy. Deficiency of any of these in a pregnant or lactating woman can result in subclinical mental retardation in children. Such children may be less fit to control their environment and to provide for their own food security in later life, to compete for better education and for higher level jobs within their society. Vitamin A deficiency can lead to poor night vision, eye lesions and, in severe cases, permanent blindness; increased illness and death from infections. Iodine deficiency can cause goiter, mental retardation, brain damage and reproductive failure. Iron deficiency can cause nutritional anemia, problem pregnancies, stunted growth, and lower resistance to infections, long-term impairment in mental function, decreased productivity, and impaired neural development. Zinc deficiency can cause growth retardation, delayed skeletal and sexual maturity, dermatitis, diarrhoea, alopecia, and defects in immune function with resulting increase in susceptibility to infection.

Several approaches such as soil and foliar fertilization, improved crop systems, application of soil amendments and organic nutrient sources are possible to correct micronutrients deficiency and to increase their density in edible parts of plants. Considering ecological concerns, sustainable agriculture is looking for more environmental-friendly and cost effective approaches with low input of energy and chemicals. Among different strategies applied for correcting micronutrient deficiency in the food chain from plant to human, the most sustainable solution, particularly for developing countries, seems to be cultivation and breeding of micronutrient-efficient genotypes in combination with proper agronomic management practices.

Nutrient efficiency and nutrient-efficient genotypes have been defined in different ways (Stewart et al., 2005; Fageria et al., 2008). Definitions of nutrient efficiency generally can be divided into those emphasizing crop productivity and those emphasizing the internal plant nutrient requirement (Gourley et al., 1994). With regard to yield parameters, nutrient efficiency has been defined as the ability to produce a high plant yield in a soil that would otherwise limit the production of a standard genotype (Marschner, 1995). Other definitions of nutrient efficiency also referred to as 'agronomic efficiency', include the amount of shoot production per unit of nutrient applied (Caradus, 1990; Sauerbeck and Helal, 1990). Nutrient efficiency has also defined as yield response per unit of added nutrient or of nutrient in the crop shoots (Baligar et al., 1990; Blair, 1993; Thung, 1988). This paper will briefly discuss some aspects of using and developing micronutrient-efficient crop plant genotypes as a sustainable and cost-effective method for alleviating food-chain micronutrient deficiency malnutrition of humans.

2 Improving Crop Yield

Mineral nutrients have played an important role in enhancing crop production since the beginning of the green revolution. According to Borlaug and Dowswell (1993), nearly half of the increase in crop yields during the 20th century was due to chemical fertilizer application. Generally, improving the nutritional status of plants by applying fertilizers and maintaining soil fertility has been the critical step in doubling food production both in developed and developing countries (Loneragen, 1997). The increase in crop production has been mainly resulted from the application of N-P-K fertilizers (Brady and Weil, 2002). Since 1950, application of N-P-K fertilizers has been rapidly increased in agricultural lands while less attention has been paid to micronutrients fertilization. Further, higher crop production per unit area depleted greater amount of micronutrients from soils. Now, micronutrient deficiency is a limiting factor for attaining greater crop yields in many cultivated soils.

Accordingly, micronutrient deficiency has been reported widely in plant crops grown in different countries. For example, Fe deficiency in crops have been reported for various regions of Europe, east India, Bangladesh, Malta, Turkey, Zambia, Mexico (Sillanpaa, 1982), Indonesia (Katyal and Vlek, 1985; Welch et al., 1991), several North, Central and South American countries (Leon et al., 1985), Australia, and most Mediterranean and west African countries (Donald and Prescott, 1975).

Zinc-deficient soils are widespread in Mediterranean countries (Cakmak et al., 1997b; Khoshgoftarmanesh et al., 2004a), Australia (Donald and Prescott, 1975; Sillanpaa and Vlek, 1985), China (Takkar and Walker, 1993), Africa (Cottenie et al., 1981), Asia (Katyal and Vlek, 1985; Welch et al., 1991), and North and South America (Fageria, 2000b; Lopes and Cox, 1977). Some crops (maize, rice) are especially susceptible to Zn deficiency in most countries where they are grown.

Manganese deficiency has also been reported in soils of the United States (Reuter et al., 1988), Central and South America (Leon et al., 1985), Europe (Welch et al., 1991), semiarid regions of China, India, southeast and western Australia, and many African countries (Welch et al., 1991).

The importance of certain trace elements for optimum growth and development of plants has been reported for the last century (Barker and Pilbeam, 2007; Cakmak, 2002; Marschner, 1995). Up to now, several trace elements (B, Br, Cl, Co, Cu, F, Fe, I, Mn, Mo, Ni, Na, Rb, Si, V, and Zn) are known to be essential for certain plants. Several nutrients have been shown necessary only for a few species (Al, I, Na, Si, Rb, Ti, V, F, Br), and others are known to have stimulating effects on plant growth, although their functions are not yet recognized (Barker and Pilbeam, 2007). The essential micronutrients are those that their specific biochemical roles cannot be substituted by other elements. In the absence of adequate amounts of these elements inside plant tissues, crop growth and metabolic cycles will be impaired.

Several studies have reported the significant reduction in growth and yield of different plant species in soils deficient in Fe, Mn, Zn, Cu, B, and other micronutrients (Cakmak et al., 1998; Kalayci et al., 1999; Khoshgoftarmanesh et al., 2004a, b, 2006a, b, 2007; Khoshgoftar and HajiMozaffari, 2006). Therefore, correcting micronutrient deficiency through appropriate approaches is necessary to achieve higher crop yields.

3 Crop Quality and Human Health

The increase in food production to prevent starvation and malnutrition was the main goal driving the agricultural "Green Revolution" during the latter half of the 20th century (Welch and Graham, 1999). In parallel to increase in crop yield in many agricultural systems as a result of chemical fertilizer application and improved agronomic practices, micronutrients contents in the edible parts of different crops were reduced to levels lower than that of the traditional crops. Thus, many food systems in developing countries can not provide sufficient micronutrient contents to meet the demands of their people especially low-income families (ACC/SCN, 1992, 1997, 2000; Combs et al., 1996; Combs and Welch, 1998; McIntyre et al., 2001; Welch et al., 1997; Welch, 1998). Humans need trace elements in sufficient levels to sustain life. There are also some dietary substances such as phytate that affect nutrients bioavailability and as a result, human nutritional status (ACC/SCN, 1992, 1997, 2000; Combs et al., 1996; Combs and Welch, 1998; McGuire, 1993; Welch et al., 1997; Welch, 1998).

Micronutrient malnutrition or hidden hunger now affects more than 40% of the world's population (United Nations Administrative Committee on Coordination – Subcommittee on Nutrition, 1992). The consequences of micronutrient deficiencies are mostly found among women, infants and children from poor families in developing countries (Buyckx, 1993; Ramalingaswami, 1995; World Health Organization, 1992; Mason and Garcia, 1993). However, micronutrient malnutrition is not restricted to developing countries and is also widespread in well-developed industrialized countries (Cobiac and Baghurst, 1993; US Department of Health and Human Services, 1990).

There has been a huge increase in the number of people suffering from micronutrient malnutrition over the last four decades. For example, Fe deficiency anemia has grown from about 30% of the world's population in the 1960s to over 40% during the late 1990s. It has been estimated that over 3.7 and 1 billion people are at the risk of iron and iodine deficiency disorders, respectively. In addition, Zn, Se, vitamin A, vitamin C, vitamin D and folic acid deficiencies are widespread among populations especially in developing countries (Combs et al., 1996; World Health Organization, 1999). Many zinc nutrition specialists believe that zinc is as important as iron deficiency (Gibson, 1994; Gibson et al., 2008). Copper, boron, manganese, chromium and lithium are other micronutrients that are needed for nutrient balance.

3.1 Consequences of Micronutrient Deficiencies

Impaired immune function, increased mortality and morbidity rates, lower worker productivity, diminished intellectual performance, lower educational attainment, a lower livelihood, higher birth rates, lower standard of living performance, and increased rates of chronic diseases including coronary heart disease, cancer and diabetes are some consequences of micronutrient hidden hunger (Pinstrup-Andersen, 1999; Sanghvi, 1996; Welch and Graham, 2000).

Micronutrients malnutrition increase human susceptibility to infections and is the most important risk factor for the disease in developing countries (Brabin and Coulter, 2003; Food and Agriculture Organization of the United Nations, 2004; Murray and Lopez, 1997; Black, 2003). Anemia, problem pregnancies, stunted growth, lower resistance to infections, long term impairment in mental function, decreased productivity and impaired neural development are some consequences of iron deficiency. Zinc deficiency may result in growth retardation, delayed skeletal and sexual maturity, dermatitis, diarrhea, alopecia and defects in immune function with resulting increase in susceptibility to infection (Pinstrup-Andersen, 1999; Welch and Graham, 2000).

3.2 Nutrient Bioavailability

The nutritional quality of a diet can be determined based on the concentration of individual nutrients as well as interactions of other elements, promoters and antinutrients which affect bioavailability of micronutrients. In fact, the degree to which a nutrient is absorbed from the diet depends on antinutrients contents e.g., phytate, tannins and antivitamins (Graham et al., 2000). High levels of antinutrients including phytate and tannins in the diet reduce the bioavailability of micronutrients including Fe and Zn, while promoters such as vitamin A or β-carotene and vitamin C enhance micronutrients bioavailability in humans (Garcia-Casal et al., 1998). Phytate is an important antinutrient to zinc absorption. Phytate is especially high in cereal grains. Depending on the plant genotype, about 70 to 90 % of the total phosphorus in whole grain is in the form of phytate (O'Dell et al., 1972). Other antinutrients are of lesser importance in cereals.

Another factor affecting the bioavailability of micronutrients is interaction with other nutrients. For example, interactions between vitamin A (retinol) and zinc has been found in the late 20th century (Christian and West, 1998; Solomons and Russell, 1980). Synergistic and antagonistic interactions among chemically similar metal cations have been characterized in both plants and animals (Hill and Matrone, 1970). Such a synergistic effect strongly indicates that breeding for staples that are dense in both iron and zinc is required in order to effectively address iron-deficiency anemia. It has been reported that the anemia could be corrected using vitamin A but not iron supplementation (Hodges et al., 1978).

Cereal-based foods with low concentrations and reduced bioavailability of micronutrients have been considered a major reason for the widespread deficiencies of Zn and Fe in developing countries (Graham et al., 2001; Welch and Graham, 1999). Thus it is essential to find sustainable approaches to produce food supplies adequate in quantity and proper nutritional quality.

4 Correction of Micronutrient Deficiency

4.1 Fertilizer Application

Alleviation of micronutrient deficiencies to improve crop yields is difficult due to large temporal and spatial variation in phytoavailability of soil micronutrients (Brennan and Bolland 2006; Shaver et al., 2007). The easiest and most straightforward practice to correct micronutrient deficiency is to apply micronutrient fertilizers. Soil application of fertilizer is economically more affordable. However, in soils with high pH, most of soil-applied micronutrients will become unavailable for plant uptake within a few years (Martens and Westerman, 1991). The loss in residual effect of soil-applied nutrient requires adding more fertilizer to maintain a sufficient level of phytoavailable element, although total soil element contents are still high and actually increasing from application to application. Increased total soil metal may create problems of micronutrients losses or toxicity in the future, especially under conditions that mobilize soil metal such as soil acidification. Furthermore, soil application of fertilizers is not always a useful approach to increase micronutrients concentrations in cereal grain. Many studies reported minimal or no increases in grain micronutrient content, with increasing soil application of micronutrient fertilizers even when yield was increased.

Applied soluble micronutrients fertilizers become ineffective rather rapidly as the dissolved metals react with soil minerals and organic matter. In comparison with inorganic fertilizers, synthetic and natural chelates have the advantage of keeping the applied nutrient in solution in a less reactive form. Chelates are particularly appropriate for applications of Zn and Fe to alkaline and calcareous soils. Soil application of inorganic Fe fertilizers to Fe-deficient soils is usually ineffective because of rapid conversion of soluble Fe into plant-unavailable solid Fe(III) forms. In contrast, an application of synthetic Fe-chelates for correction of Fe deficiency is effective (Wallace and Wallace, 1982) for a longer period, or actually long enough to support one crop of tree fruits which are commonly fertilized with Fe-chelates.

The effectiveness of various synthetic and natural chelates has been widely investigated (Alvarez and Gonzalez, 2006; Gonzalez et al., 2007; Prasad and

Sinha, 1981). Despite their effectiveness, application of chelates is generally expensive (Wallace and Wallace, 1982), and is likely to be even more uneconomic if the aim is to increase micronutrient concentration in the grain rather than to increase yield. In addition, application of metal chelates may result in potential leaching risk because the more mobile the chelate, or the less biodegradable the carrier, the greater the risk for leaching (Gonzalez et al., 2007).

Foliar application can provide a rapid correction of severe deficiencies commonly found during the early stages of growth, and are temporary solutions to the problem. Foliar fertilization is more effective than soil application, but too expensive for resource-poor farmers. Low penetration rates in thick leaves, run-off from hydrophobic surfaces, wash off by rainfall, rapid drying of spray solution, limited translocation within the plant, and leaf damage are other problems related to foliar applications (Marschner, 1995). Most foliar-applied micronutrients are not efficiently transported toward roots, which may remain deficient. Therefore, application of micronutrients to either soil or foliage may be successful in increasing grain yield only in well-defined circumstances.

Another method to apply micronutrients to crop plants is the treatment of seeds with fertilizers, a technique called "seed priming". The most effective way to prime micronutrients in seeds is to grow the parent crop on a soil with high levels of phytoavailable Mn or Zn, while spraying high levels of Zn and Mn fertilizers on the plants during seed filling can also increase seed levels considerably. Mixing micronutrients with seeds must be limited to levels which do not harm the germinating seeds. It has been reported that seed priming with Zn sulphate resulted in a stronger increase in the grain yield of wheat grown on Zn-deficient soil than foliar Zn application after germination, but it did not significantly increase grain Zn concentration in contrast to foliar and soil application (Rengel and Graham, 1995; Yilmaz et al., 1997, 1998). 'On-farm' seed priming with water is an effective way to increase yield in maize and is important for resource-poor farmers as it is a low- or zero-cost technology and requires few external inputs (Harris et al., 2007). Recently, Harris et al. (2008) reported that priming seeds with $ZnSO_4$ significantly increased the grain Zn concentration and concluded that using $ZnSO_4$ to prime seeds was very cost-effective, with net benefit-to-cost ratios of 75 for wheat and 780 for chickpea.

Correction of micronutrients deficiency via fertilization, especially in calcareous soils, is not always successful and sustainable due to agronomic and economic factors such as reduced availability of micronutrient element due to topsoil drying, subsoil constraints, disease interactions, and cost of fertilizer in developing countries (Graham and Rengel, 1993).

Some chemical fertilizers, especially Zn and Fe fertilizer manufactured from industrial byproducts or mine wastes contain various contaminants such as cadmium and Pb (Afyuni et al., 2007). The potential risks to environmental quality posed by their repeated application to soil require careful evaluation (Mortvedt, 1985, 1996) especially for rice production (Chaney et al., 2004). Recently, preserving the environment is becoming a more important objective of agriculture to achieve sustainable agricultural goals (Cakmak, 2002; Tillman, 1999). To avoid potential future environmental risks, a more sustainable strategy is required than just adding chemical micronutrients fertilizers to the soil. Total amounts of some micronutrients such as Zn and Fe in agricultural soils are generally orders of magnitude higher than the crop demand. Thus, micronutrients deficiency in crops is a problem of insufficient phytoavailability in almost all cases and not of absolute lacking sufficient quantity of micronutrients.

4.2 Soil Amendments

Soil amendments are frequently used by farmers to improve some physical and chemical properties of soils (e.g. adjusting soil pH) in order to enhance plant growth. Limestone application is an effective and common practice to improve crop productivity of acidic soils (Foy, 1984, 1992; Kochian, 1995). Adding limestone may depress the uptake of Zn, Cu, Fe, and Co, and increase the uptake of Se and Mo by plants (Foy, 1984). The pH largely controls micronutrients solubility in soil and a small change in pH significantly affects solubility and uptake of metals such as Zn, Cu, Mn, and Fe. Considering increased pH of acidic soils by liming, micronutrient deficiencies become more severe after liming (Verma and Minhas, 1987). A high soil-pH favors the oxidation of reduced forms of Se such as Se^{-2} and SeO_3^{-2} to the more soluble and plant-available SeO_4^{-2} anion.

Several studies have examined the effects of combined fertilization with lime and P. Phosphorus causes changes in the extractability of Zn and, in almost all soils, further aggravates Zn deficiency in susceptible crops causing decreases in grain Zn concentration (Hylander, 1995). Thus, Zn fertilizers must often be applied to ameliorate Zn deficiency associated with limestone application (Tagwira, 1993).

In contrast to limestone, gypsum ($CaSO_4$) and elemental S are used to decrease the pH of alkaline soils as well as to amend sodic and saline-sodic soils. Application of acid-producing amendments on alkaline and calcareous soils could decrease soil pH and consequently increase plant-available Fe, Mn, Zn, Cu, and Co. Gypsum is used to exchange Ca for Na on the soil cation-exchange complex and to remove bicarbonate from the soil solution. Removal of bicarbonate from the soil solution can be highly beneficial for lowering the soil pH and increasing the availability of micronutrients (Singh et al., 1989). Singh et al. (1989) reported that gypsum application in a sodic soil increased the mean concentration of Mn, Zn and Cu in mesquite (*Prosopis julitlora*). Application of gypsum caused significant reductions in pH and electrical conductivity (Singh et al., 1989). The increase in soil availability of micronutrient as a result of gypsum application might be attributed to the decrease in soil pH and improved soil physical properties. Further research is necessary to ascertain whether benefits of applying gypsum to alkali, sodic soils are significant in terms of increasing micronutrient density in grain.

4.3 Organic Fertilizer Sources

Application of different organic materials e.g. manures, plant residues and waste materials is a recommended strategy to improve soil fertility and enhance nutrient use efficiency. Organic sources application is known as an effective management strategy to sustain high crop productivity in many cropping systems. Using organic sources may affect crop micronutrient nutrition through affecting soil physical and chemical properties, producing better root growth environment and adding some micronutrients to soil (Rengel et al., 1999).

Soil organic matter has a variety of direct and indirect influences on the phytoavailability of micronutrients in soil and their uptake by plants (Rengel et al., 1999). Binding of some metals such as Zn to organic matter reduces the concentration of free cation in solution, but if the metal-organic complexes are dissolved, then the increase in total dissolved ion may actually also increase effective metal phytoavailability at the root-rhizosphere interface, depending on the mobility and the dissociation kinetics of the metal-DOC complexes. Chelation of Zn and Fe by organic matter holds these nutrients in forms more accessible by roots within the rhizosphere, and retards formation of insoluble solid forms such as oxides and carbonates in soil (Schulin et al., 2009).

Adding organic resources such as crop residues, green manure, livestock manure, municipal biosolids or their composts or co-composts to soil has a number of beneficial effects on micronutrient nutrition including additional supply of some nutrients with the added organic matter, increase in ion exchange capacity and thus of the fractions of easily available nutrients, improved soil structure, increase in water storage capacity, improved drainage and aeration, increased accessibility of soil for plant roots, decreased salinity, stimulation of microbial exudation of organic ligands and of other microbial activities and as a result, accelerated release of nutrients from secondary soil matter, enhanced nutrient supply through mycorrhizae, and protection against root pathogens. All these effects promote plant growth, the development of the root system and thus also its capacity to acquire micronutrients (Schulin et al., 2009).

The formation of metal complexes with organic ligands in the soil solution is generally found to increase the mobility of soil micronutrients. On the other hand chelation can substantially reduce cellular metal uptake. Because microelements bound to the soil surfaces can only reach roots by diffusion, the mobilization effect of chelation generally seems to cause greater increase in plant uptake. In previous studies even strong chelates (Zn-EDDS, Zn-EDTA) still resulted in a net increase of Zn uptake (Novack et al., 2008). This can be explained by increased rate of movement of the microelement from soil surfaces to root uptake surfaces by movement of dissolved chelated elements (e.g., Wallace and Mueller, 1973). It has also to be considered that leaf-applied chelates might not be helpful for transport of micronutrients into grain (Cakmak, 2008; Novack et al., 2008).

Effects of different organic amendments on correcting micronutrients deficiencies have been widely investigated; however, very few controlled experiments have been done to determine which types of organic matter practices significantly enhance or depress the levels of micronutrients in edible portions of major food crops. Therefore, more research is needed to understand the impact of various types of organic amendments on crop nutritional quality.

4.4 Plant Residue

In many countries, especially arid and semi-arid regions, very little or no crop residue is left in the field; much is used for feeding animals or for fuel (Timsina and Connor, 2001). It is estimated that more than 1000 million tons of cereal residues are being annually produced in the developing countries (FAO, 1999). Rice, wheat, corn, soybean, barley, rapeseed, and potato are the major residue-producing crops that globally produced 2956 million tons of residues in 1998 (FAO, 1999).

Crop residues are considered as important sources of several micronutrients. Rice and wheat remove 96, 777, 745, 42, 55, and 4 $\mathrm{g\,ha^{-1}}$ of Zn, Fe, Mn, Cu, B, and Mo per ton, respectively. By estimating the total crop residue production of 105 million tons in India, and based on micronutrient contents of the residues, the micronutrient potential associated with crop residues would be about 35400 tons (Prasad, 1999). About 50 to 80% of Zn, Cu, and Mn taken up by rice and wheat crops can be recycled through residue incorporation (Prasad and Sinha, 1995b). Therefore, recycling of crop residues can help improve soil availability of micronutrients.

Crop residues addition to flooded soils stimulates microbial metabolism and thus increases soil solution Fe and Mn concentrations due to greater redox potential change (Katyal, 1977; Yodkeaw and De Datta, 1989; Atta et al., 1996). In calcareous soils, organic acids produced during crop residue decomposition may increase plant Zn uptake by dissolving Zn from the solid-phase pool to soil solution (Prasad and Sinha, 1995a). Chelating agents released from decomposing crop residues increase the concentration of total diffusible Zn and its diffusion coefficient (Singh et al., 2005). Rice straw application has been found to increase the Zn content of rice plants, possibly through its amelioration of soil pH and exchangeable sodium percentage (Singh et al., 2005). In contrast, crop residue application has been reported to decrease the labile Zn pools in an alkaline soil (Kang, 1988). Other researchers (Raj and Gupta, 1986) have also reported that application of rice or wheat straw decreased the phytoavailable Zn concentration in both flooded and upland soils.

Crop residues may also negatively affect crop production in the short term because of N immobilization and phytotoxic compounds release in allelopathic interactions. Plants may release chemical compounds which can either stimulate or inhibit the growth and development of another plant. These compounds are dispersed into the environment from root exudates, leaf leachates, or from dead and decaying plant parts (Rose et al., 1984; Huber and Abney, 1986). Nutrient uptake may be reduced by allelochemicals (Bhowmik and Doll, 1984; Rice, 1984; Barnes and Putnam, 1986). It has been suggested that sweet potato residues have an allelopathic effect (Walker and Jenkins, 1986). Sweet potato and cowpea indicator plants grown in sweet potato crop residues displayed symptoms resembling complex nutrient deficiencies. Abiotic factors, such as reduced soil pH, increased soil osmotic potential, reduced oxygen concentrations and low nutrient content of the residue, may also have caused growth inhibition and as a result, reduced plant nutrient uptake. Walker et al. (1989) reported that plant uptake of P, K, Ca, Mg, S, Zn, B and Cu were substantially lower in plants grown in soil with amendment by sweet potato root or vine residues than in plants grown in without sweet potato residue treatments. Successful integration of crop residue management strategies into cropping systems requires understanding of how crop residues influence cycling of nutrients from soil and fertilizers, as well as their effects on soil chemical, physical and biological properties, and crop production.

4.5 Animal Manure

Livestock manure is a good source of plant nutrients and its application in agricultural lands is a traditional nutrient management in many countries worldwide. The use of manures can also change plant-available micronutrients by adding micronutrients or by changing both the physical and biological characteristics of the soil (Stevenson, 1991, 1994). Eghball et al. (2004) found that applications of manure and compost not only improved soil physical properties but also provided nutrients for growing corn. The residual effects of manure on soil properties remained several years after application. Gao et al. (2000) found that manure was a better source of available Fe, Mn, and Zn compared to synthetic fertilizers, but manure accelerated the depletion of available Cu. Manure not only supplies large amounts of Zn to the soil, but also promotes biological and chemical reactions that result in the dissolution of non-available Zn (Wei et al., 2006). Wei et al. (2006) reported that manure application had no significant effect on available Cu. This might be attributed to the low amount of Cu in the manure used in this experiment. Copper could be bound to organic matter and thus be relatively unavailable to plants.

Zinc and Cu may be added to poultry feeds as a preventive for scour and to suppress bacterial action in the gut. Accordingly, Zn and Cu concentrations of some compound poultry and swine feeds are several times higher than that required by the animals for healthy development. In areas where animal manures have been applied for many years and where applications are expected to continue, high amounts of heavy metals (particularly Zn and Cu) may accumulate in soil (Nicholson et al., 1999). The micronutrient contents of farm manures largely depend on their concentrations in the feeds consumed and the efficiency of feed conversion by the animals. Fleming and Mordenti (1991) reported mean Zn concentrations of cattle manures in Belgium of 580 mg kg^{-1} DM. In Switzerland, cattle manures generally contain <200 mg Zn/kg DM (Menzi and Kessler, 1998). Webber and Webber 1983) reported cattle manure Zn concentrations in the range of 30 ± 225 mg kg^{-1} DM. The mean Cu concentration in cattle manures was reported to be 16 ± 62 mg kg^{-1} DM (Fleming and Mordenti, 1991; Menzi and Kessler, 1998). Sims and Wolf (1994) reported Zn concentrations ranging from non-detectable to 660 mg kg^{-1} DM in poultry manure and from non-detectable to 669 mg kg^{-1} DM in poultry litter. The maximum content of Cu in poultry manures and litters were 232 mg kg^{-1} DM and 1003 mg kg^{-1} DM, respectively.

Farm manures are a valuable source of major plant nutrients (N, P, K) and organic matter. However, care must be taken to ensure that manure applications containing elevated levels of heavy metals do not cause long-term soil contamination. Then, the typical amounts of heavy metals in solid manures have to be calculated before manure addition to soil. Excessive cumulative application of manure can cause accumulation of phosphate in surface soils, which increases the potential for runoff of phosphate which can cause adverse effects in streams. Thus applications of manures are being limited to the phosphate fertilizer requirement of crops rather than the N fertilizer requirement which applied much higher annual manure amounts. Several groups have developed models for application of major and micronutrients with all soil amendments for large areas, considering livestock manure, biosolids, phosphate and other fertilizers. These studies illustrate accumulation of Zn, Cu and Cd in soils when microelement rich manures are applied (Keller and Schulin, 2003). These issues are more important in areas with intensive animal production because feedstuffs are imported from distant farms, but the livestock residues are applied to the local farm of the livestock producer. In developing countries where livestock production is achieved with local inputs, accumulation of Zn and other elements in the soil is less of a concern.

4.6 Organic Amendments

Biosolids and composts are usually effective Zn and Cu fertilizers. Possibility of correcting micronutrient deficiency in different plant species by application of biosolids, municipal waste leachates, and compost has been reported (Khoshgoftarmanesh and Kalbasi, 2002). When soils need Zn or Cu fertilization, high quality biosolids and composts could be used to supply the required Zn and Cu. Such amendments are not normally able to replace B fertilizers, but mixing with coal combustion byproducts can supply adequate B for the mixture to serve as B fertilizer (Chaney and Ryan, 1993).

Because production of composts will save costs in both urban and agricultural areas, they can be used as inexpensive fertilizers. Although such benefits are possible from use of composts, these products must be safe for sustainable use in horticulture and agriculture for their use to be permitted by governments, and must reliably supply nutrient and organic matter benefits to become competitive products in the market place. The potential presence of pathogens, heavy metals, xenobiotic compounds, and possible element imbalance in composts has caused concern to compost application. Some believe that because the concentration of Zn or Cu in composts is higher than found in background soils, these materials must not be utilized on soils. However, researchers have used high quality organic matter/compost products for decades without adverse effects (Andersson, 1983; Chaney and Ryan, 1993; Woodbury, 1992). Composts prepared in developing countries often do not receive the contaminants which have caused concerns in developed countries. Over time boron use in glues and other products has declined such that municipal solid wastes which used to commonly contain phytotoxic levels of boron are only a useful B fertilizer today. As with biosolids, composts from off-farm sources should be analyzed for contaminants and nutrients so that these resources can be wisely managed on cropland.

4.7 Crop Rotation

Cropping systems practices influence micronutrient availability. A preceding crop may influence soil conditions that govern soil fertility in general and micronutrients phytoavailability to the successive crop in particular. These can result from residual effects of root litter and exudates on soil physical and chemical parameters as well as on soil microorganisms, generation of root channels, and other plant-soil interactions. Legumes may positively affect soil quality by improving soil physical properties. Improved physical properties of the soil provide a better root growth condition and as a result, enhance uptake of immobile micronutrients by roots. Crop rotation systems may also affect some soil chemical properties (i.e. pH). Alvey et al. (2001) found that a legume rotation system was more effective in increasing the pH of the rhizosphere as compared to continuous sorghum cropping system. The increase in pH was considered as an important mechanism of the plant to cope with acidity-induced nutrient deficiency. However, in the long term, N fixation increases soil acidity and must be counteracted by alkaline amendments. Khoshgoftarmanesh et al. (2007) reported that wheat plants grown after sunflower accumulated more Zn in their grains as compared to those planted after cotton. Liu et al. (2002) found that a rice-upland crop rotation promoted the reduction of Mn in the surface soil and accelerated the oxidation and accumulation of Mn in the subsoil. Bronick and Lal (2005) reported that crop rotation enhanced soil organic carbon accumulation. Diekow et al. (2005) reported that higher residue input associated with legume-based cropping systems significantly increased soil organic carbon. Mitchell and Entry (1998) found that long-term planting of legumes as a winter cover crop resulted in higher soil organic carbon levels compared with treatments that did not include a winter cover crop.

Wei et al. (2006) found that available Zn and Cu varied with different cropping systems. The amount of phytoavailable Cu in soil decreased in the order: continuous clover > crop–legume rotation > continuous wheat. Differences among cropping systems could probably be attributed to differences in the Cu uptake capacity of each crop. Long-term cultivation of leguminous crops may lead to a large increase in available Mn due to changes in the soil microenvironment by leguminous crops that result in the release of plant available Mn (Williams C.H. and David, 1976).

Gunes et al. (2007) found that intercropping of wheat and chickpea inoculated with a Rhizobium on a calcareous clay loam soil (pH 7.9 in water extract, 0.96% organic matter content) with a very low DTPA-extractable Zn concentration (0.11 mg kg^{-1}) increased the Zn concentrations of shoots and seeds in both species. Zuo and Zhang (2008) reported that Fe, Zn, and Cu concentrations of intercropped grown peanut increased significantly compared to those in monocropping both in the greenhouse and field. Systemic mechanisms may be involved in adaptation to nutrient stresses at the whole plant level. A reasonable intercropping system of nutrient efficient species should be considered to prevent or mitigate iron and zinc deficiency of plants in agricultural practice (Zuo and Zhang, 2009).

The effect of crop rotation on the available and total micronutrient contents in soils is somewhat different (Wei et al., 2006). Total nutrient content is affected mainly by crop uptake, and therefore the magnitude of the decrease depends on crop type and uptake intensity.

5 Developing Micronutrient-Efficient Genotypes: A New Strategy

5.1 Definition of Nutrient Efficiency

According to the Soil Science Society of America (1997), nutrient efficiency is the ability of a plant to absorb, translocate, or utilize more of a specific nutrient than other plants under conditions of relatively low nutrient availability in the soil or growth media. This is the agronomic focus rather than the food quality focus which will be discussed below. Graham (1984) defined plant nutrient efficiency as the ability of a genotype to produce a high yield under conditions in which a soil nutrient is growth-limiting. Blair (1993) defined plant nutrient efficiency as the ability of a plant to acquire nutrient from a growth medium and/or to utilize it in the production of shoot and root biomass or grain. According to Isfan (1993), efficient genotypes are those with high ability to absorb nutrients from soil and fertilizer, produce high grain yield per unit of absorbed nutrient and store relatively little nutrients in the straw. As defined by Clark (1990), an efficient genotype is one that produces more dry matter or has a greater increase in the harvested parts per unit time, area, or applied nutrient, has less deficiency symptoms, or has greater incremental increases and higher concentrations of mineral nutrients than other genotypes grown under similar conditions. Gourley et al. (1994) proposed that the nutrient requirement of an efficient germplasm is less than an inefficient germplasm. Fageria et al. (2008) defined a nutrient efficient genotype as a genotype that produces higher economic yield with a determined quantity of applied or absorbed nutrient than other genotypes under similar growing conditions.

Nutrient efficiency emphasizing utilization is generally defined as total plant biomass produced per unit nutrient absorbed, which is equivalent to the reciprocal of the nutrient concentration of the entire plant (Gourley et al., 1994). This often is called the ‘nutrient efficiency ratio’ and has been used extensively to describe the internal nutrient requirement, particularly of P, in many agronomic plant species (Baligar et al., 1990; Glass, 1989; Godwin and Blair, 1991). Using this definition, P-efficient alfalfa genotypes with higher shoot P concentration are selected to overcome P nutrition limitations in cattle (Miller et al., 1987).

The reciprocal of nutrient concentration does not consider the crop yield. Siddiqi and Glass (1981) suggested “utilization efficiency” defined as the product of yield times the reciprocal of nutrient concentration. The nutrient uptake per unit root length, surface area, or weight has also been defined as “uptake efficiency” (Buso and Bliss, 1988). In most studies, micronutrient efficiency has been calculated as the ratio of yield (shoot dry matter or grain yield) produced under micronutrient deficiency (−M) to yield produced with micronutrient fertilization (+M), as fllows (Graham, 1984):

$$\text{Micronutrient efficiency} = (\text{yield}-\text{M}/\text{yield}+\text{M}) \times 100$$

In this paper, the last definition is considered as the main definition of micronutrient-efficiency for separating micronutrient-efficient and inefficient genotypes. Such an index is used to select improved micronutrient-efficient genotypes in a breeding program, or to provide information on qualities of commercial cultivars for growers.

5.2 Micronutrient-Efficient Genotypes and Crop Productivity

Application of fertilizers is not a totally successful strategy in alleviating micronutrient deficiency because of agronomic, economic, and environmental (Mortvedt, 1994; Graham and Rengel, 1993; Hacisalihoglu, 2002).

A more efficient and sustainable solution to micronutrient deficiency limitations to crop production is the development and use of micronutrient-efficient plant genotypes that can more effectively grow on soil with low phytoavailable micronutrient contents, which would reduce fertilizer inputs and protect the environment as well. Selection of plant genotypes that can tolerate low nutrient supply may increase productivity on low fertility soils and reduce fertilizer requirements (Gourley et al., 1994).

One of the most important adaptive responses for crop plants involves their ability to deal with soil-mediated abiotic stresses involving deficient levels of micronutrients in the soil. Plant species vary significantly in tolerance to micronutrients deficiency stress; some are able to cope with low micronutrients availability, and thus, grow well even when other species or cultivars suffer reduced yield due to micronutrient deficiency (Graham and Rengel, 1993).

Exploiting genetic diversity of plants for enhanced productivity in poor fertility soils is a desirable and important objective in order to meet food demands for an increasing world population. Variation among plant germplasm in the ability to acquire nutrients from the soil has been investigated for decades (Godwin and Blair, 1991). There are several key mechanisms that could be involved in nutrient efficiency (Baligar et al., 2001; Fageria and Barbosa Filho, 2001; Fageria and Baligar, 2003; Khoshgoftarmanesh et al., 2004a, 2006a), including root processes that increase the bioavailability of soil nutrient for root uptake; enhanced root uptake and translocation of nutrients from the root to the shoot; altered subcellular compartmentation of nutrient in shoot cells; and more efficient biochemical utilization of nutrients in cells of the shoot. Many possible processes for control of nutrient efficiency have been investigated. In recent years, more attention has been paid to micronutrient efficiency.

Micronutrient efficiency is genetically controlled and the physiological and molecular mechanisms of micronutrient efficiency of plants are just beginning to be understood. Large ranges of genotypic variation in response to micronutrients deficiency stress have been reported in different plant species, particularly in cereals (Graham et al., 1992; Cakmak et al., 1997a, 1998). Such large variation is promising for developing plant genotypes that are more efficient in root uptake, translocation from root to shoots and/or internal utilization of micronutrients. Micronutrient-efficient genotypes may provide a number of other benefits, such as reductions in the use of fertilizers, improvements in seedling vigor, resistance to pathogens, and enhancement of grain nutritional quality (Graham and Rengel, 1993; Bouis, 1996; Graham and Welch, 1996).

Selecting and breeding staple food crops which are more efficient in the uptake of trace minerals from the soil and load more trace minerals into their seeds combines benefits both for agricultural productivity and human nutrition. This approach may have important applications for increasing farm productivity in developing countries in an environmental-friendly way (Cary et al., 1994; Kannenberg and Falk, 1995). Growing nutrient-efficient genotypes on soils with low nutrient availability would reduce land degradation by reducing the use of machinery (Thongbai et al., 1993) and by minimizing application of chemicals such as fertilizers on agricultural land (Rengel, 2001).

Beneficial effects of micronutrient-efficient crop genotypes for agricultural productivity result in extremely high cost/benefit ratios for investing in this type of micronutrient intervention. Furthermore, the adoption and spread of micronutrient enriched seeds by farmers can be driven by profit incentives because micronutrient enriched seeds increase crop productivity when planted in micronutrient-poor soils (Graham et al., 2001). The benefits can be disseminated widely and they are sustainable once developed unlike current micronutrient interventions that rely on supplementation or food fortification (Graham et al., 2001, 2000; Welch et al., 1997).

Developing plants with a high ability to extract transition metal nutrients from soils may be more useful for Fe and Mn. Soils which cause deficiency contain substantial levels of total Fe or Mn, but the minerals are not phytoavailable to traditional genotypes. Induced iron deficiency chlorosis is widespread and is a major concern for plants growing on calcareous or alkaline soils due to their high pH and low availability of iron (Welch et al., 1991; Marschner, 1995). In calcareous soils, Fe and Mn rapidly convert to immobile forms and generally several foliar applications are required to prevent deficiencies throughout the growing season. Selecting or breeding iron efficient genotypes of corn, soybean, sorghum and rice is considered the best way for correcting Fe deficiency stress on calcareous soils (Graham, 1984; Fageria and Baligar, 2003). Soil application of Cu, Zn, and Mo is more efficient than Mn and Fe fertilization, on most soils, but all transition metal nutrients are not readily translocated within plants on deficient soil (Nable and Webb, 1993; Graham and Webb, 1991).

Several studies have been done to select and breed cultivars with greater abilities to cope with adverse soil conditions. Micronutrient-efficient genotypes were described that have the ability to take up and utilize nutrients more efficiently under abiotic and biotic stresses (Pessarakli, 1999; Alam, 1999; Baligar et al., 2001) or that some micronutrient-efficient genotypes can tolerate abiotic and biotic stresses (Fageria et al., 2008; Khoshgoftarmanesh et al., 2004a, 2006a, b; Marschner, 1995). Plant crop yield under stress could be enhanced by selection or breeding of plants that have high micronutrient efficiency, and ability to interact effectively with environmental extremes (Fageria et al., 2008). For example, Khoshgoftarmanesh et al. (2004a, b, 2006a, b) and Khoshgoftar and HajiMozaffari (2006) reported that zinc application in a severely calcareous saline soil increased the salt tolerance of wheat genotypes. Zinc-efficient genotypes are reported to be more tolerant to salinity than Zn-inefficient genotypes (Table 1). Bagci et al. (2007) indicated that sensitivity to Zn deficiency stress became more pronounced when wheat plants were drought-stressed. The effect of irrigation on grain yield was maximized when Zn was adequately supplied. Close relationship between water use efficiency and Zn nutritional status of plants has also been suggested (Bagci et al., 2007).

Plant nutrition status may greatly affect predisposition of plants to attack by, or effect of pests and diseases (Fageria et al., 2008). Micronutrient deficiencies reduce the plant tolerance to diseases and insects (Marschner, 1995). The fungicidal effect of manganese (Mn), copper (Cu) and zinc (Zn) are well-known as they have been common constituents of fungicides (Streeter et al., 2001). Copper, B, and Mn are involved in the synthesis of lignin, and phenolic compounds. Silicon plays a role in producing physical barriers against pathogen penetration (Barker and Pilbeam, 2007; Graham and Webb, 1991).

Streeter et al. (2001) reported that Zn-sufficient plants are more tolerant to the effects of root pruning by the fungus than Zn-deficient plants probably due to fungitoxicity of Zn and the role of Zn in the integrity or stability of the host plant's membranes. The increased root exudation by Zn-deficient plants may accelerate the root-rot disease (Thongbai et al., 1993).

Table 1 Response of five wheat genotypes with different zinc efficiency to salinity and zinc application treatments (Khoshgoftamanesh et al., 2006a)

	Shoot dry matter yield (g pot^{-1})				
NaCl rate (mM)	Dur-3	Kavir	Falat	Rushan	Cross
Without Zn					
0	69.1	82.6	94.1	92.9	99.6
60	59.3	75.4	63.1	85.5	85.3
120	50.2	55.3	61.3	69.5	80.4
180	30.6	49.1	43.5	68.1	58.5
With Zn					
0	80.5	116.5	98.6	93.3	100.8
60	77.1	92.1	77.5	87.2	89.6
120	76.3	84.3	68.2	79.6	88.1
180	35.1	54.4	43.1	71.8	65.7

A relationship between Zn nutrition and severity of some root-rot diseases has been demonstrated in wheat (Thongbai et al., 1993) and medic plants (Streeter et al., 2001). Streeter et al. (2001) suggested that Zn application does not directly inhibit infection by *Rhizoctonia solani*, nor reduce its pathogenicity, but it does strongly increase root growth. Zinc nutrition is beneficial in reducing the occurrence of phyllody virus in white clover (Carr and Stoddart, 1963), take-all in wheat (Brennan, 1992), and charcoal rot development in maize (Pareek and Pareek, 1999).

Silicon applications reduce the severity of fungal diseases such as blast and sheath blight of rice, powdery mildew of barley and wheat, and vermin damage of rice by the plant hopper in the field (Barker and Pilbeam, 2007). Plant genotypes with higher ability to absorb and/or utilize micronutrients seem to be more resistant to disease than micronutrient-inefficient genotypes. The relationship between insect attacks and micronutrient-efficiency of plant genotypes needs further evaluation (Fageria et al., 2008).

Considerable progress has been made in breeding for Zn, Fe, and Mn efficient dicots and monocots and in identifying the genes involved (Marschner, 1995; Rengel, 2001). By comparing efficient and inefficient cultivars, some information on the genetic control of micronutrients efficiency has been obtained (Graham, 1984; Rengel, 2001). This information will accelerate development of more nutrient efficient crop plants by traditional and modern breeding techniques, and provide important information for genetic engineering.

5.3 Micronutrient-Efficient Genotypes, Crop Quality and Human Health

5.3.1 Total Concentration of Micronutrients in Plant Based Foods

Different traditional public health interventions have been used to improve micronutrient nutritional status of the target population; i.e. supplementation, food diversification, industrial fortification and biofortification. Supplementation and industrial fortification have been effective in reducing morbidity and mortality resulting from micronutrient deficiencies worldwide, but infrastructure, purchasing power, or access to markets and healthcare systems is required in these approaches, often not available to poor people particularly in rural areas (Mayer et al., 2008).

Supplementation with pharmaceutical micronutrient preparations e.g. vitamin A and Zn capsules can be a rapid and effective way to alleviate micronutrient-deficiency on an individual basis. However, this strategy has generally failed on a population level in developing countries, due to lack of adequate infrastructure and education (Graham et al., 2000; Stein, 2006). For example, vitamin A supplementation programs covered up to 58% of target population over the last decades in 103 priority countries with a goal of preventing blindness caused by vitamin-A deficiency (UNICEF, 2007). Iron supplementation had little effect on the anemia problem in India, because of mismanagement, underfunding, logistic problems, and poor compliance (Vijayaraghavan, 2002).

Food fortification is a strategy that can be applied rather rapidly at the national level without changes in the dietary habits of people. As a public health measure, wheat flour is commonly fortified with iron, margarine with vitamin A and D, and salt with iodine. However, Zn interventions with fortified foods or supplements are still largely experimental. Some countries also fortify wheat flour with zinc. Fortification of maize and wheat flours with Fe has been implemented in Mexico, Indonesia, and South Africa, but this approach still needs further evaluation (Gibson, 2006). Ahmed et al. (2008) concluded that fortification of whole wheat flour is feasible to alleviate Fe and Zn deficiency in vulnerable groups. A disadvantage of food fortification is that, like supplementation, its successful implementation into society requires safe delivery systems, stable policies, appropriate social infrastructures and continued financial support (White and Broadley, 2005; Gibson, 2006).

In the case that foods differ widely in available contents of micronutrients, dietary modification or diversification would seem a straightforward and sustainable way to combat micronutrients deficiency. But changes in dietary habits require individual and societal acceptance, as well as the availability of alternative foods at affordable prices. Poverty is another challenge that makes this strategy less applicable for poor countries.

Due to problems such as inadequate logistics, insufficient compliance, political instability and instability of funding, many of these interventions have failed. In particular, many of these programs never reached the majority of low-income women, infants and children in developing countries (Welch and Graham, 1999).

Using bioavailable micronutrient-dense staple crop cultivars is another approach that could be used to improve the micronutrient nutritional status of human (Bouis, 1996; Combs et al., 1996; Welch and Graham, 1999; Frossard et al., 2000; Welch, 2002). Cultivation of micronutrient-efficient crop genotypes holds great promise for making a significant, low-cost, and sustainable contribution to reducing micronutrient, particularly mineral deficiencies in humans.

This strategy, for which the term “biofortification” has been coined, has the potential to be sustainable as well as economical. Biofortification is based on the principle that health comes from the farm, not the pharmacy (Mayer et al., 2008). Like fortification in general, it does not require people to change their dietary habits. This approach is attractive for the farmer because of increased yields and reduced seedling losses and does not require special infrastructure. In addition, the output of this program can be distributed to the remote rural areas where poor populations are living at low cost (Graham et al., 2000; Yang et al., 2007).

Biofortification can be achieved (i) by breeding or genetic engineering crop plants with improved ability to accumulate target micronutrients in potentially bioavailable form in edible plant parts (Graham et al., 2000; Welch and Graham, 2004; White and Broadley, 2005), (ii) by increasing (bioavailable) micronutrient density in food plants by agricultural methods of crop cultivation, for example by fertilizer application or by amendments that increase the element’s bioavailability in the soil for plant uptake (Frossard et al., 2000; Graham et al., 2001; Welch, 2002).

Most modern cultivars of wheat and rice have a lower concentration of micronutrients in grain than traditional cultivars because breeders generally focused on increasing yield, with no attention to the micronutrient concentrations in grain. Breeding micronutrient-dense cultivars of staple foods is a powerful tool to combat micronutrient malnutrition. Recent findings show that it is possible to breed for enhanced levels of iron, zinc and provitamin A carotenoids in edible tissues of rice, wheat, maize, beans and cassava (Graham et al., 2001). Recently, the international genomes of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L. and *T. durum* Desf.), maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas*, L.) have been surveyed for high micronutrient density traits (Graham et al., 2001).

Large variation in the grain micronutrients concentration e.g. Fe and Zn in different wheat genotypes has been reported (Cakmak, 2002; Graham et al., 1999; Kalayci et al., 1999; Khoshgoftarmanesh et al., 2004a, 2006a, b). As shown in Table 2, different wheat genotypes vary greatly in grain Zn and Fe concentrations, ranging from 8.5 to 84 μg g^{-1} for Fe and 4.6 to 41.4 μg g^{-1} for Zn (Khoshgoftarmanesh et al., 2007). The large genetic variation found in Fe, Zn, Mn, and Se concentrations among geotypes in the major germplasm banks is sufficient to justify the possibility of developing micronutrient-efficient genotypes. Thus, it seems feasible for crop breeders to select for high micronutrients density traits in breeding programs.

Modern recombinant DNA technology can also be used to enhance the nutritional quality of food crops such as increasing the amount and bioavailability of micronutrients in plants (DellaPenna, 1999; Frossard et al., 2000; Goto et al., 1999, 2000; Lucca et al., 2001). For example, recombinant DNA technology was used to improve the provitamin A content of rice-grain endosperm in ‘Golden rice’ (Ye et al., 2000). Goto et al. (1999) transformed rice plants using a phytoferritin (a major protein storage form of Fe in plants) gene from soybean and a rice endosperm promoter gene to enrich Fe in the rice-grain endosperm. Lucca et al. (2001) also enriched the Fe concentration in rice-grain endosperm using the phytoferritin gene from pea plants.

The strategy of breeding for mineral and vitamin enhancement of staple foods has several complementary advantages. Micronutrient-dense crop genotypes deploy micronutrients to consumers through the traditional ways with no necessity for changes in consumers’ behavior (Mayer et al., 2008). The consistent daily consumption of large amounts of food staples by all family members is another benefit of this approach. Nevertheless, any intervention to improve micronutrient status must target women, children, and adolescents because of their elevated needs for minerals and vitamins (Nicklas, 1995).

Table 2 Range and mean concentrations of Fe and Zn in the shoot and grain of different wheat genotypes in the presence and absence of added Zn fertilizer (Khoshgoftarmanesh et al., 2007)

	Concentration (μg g^{-1})			
	Zn		Fe	
	Minus Zn	Plus Zn	Minus Zn	Plus Zn
Shoot				
Range	5.5–21.3	5.9–26.0	15.0–98.0	14.3–101.0
Mean	9.2	13.5	57.1	59.6
Grain				
Range	4.6–41.4	7.6–36.2	8.5–75.8	9.7–84.1
Mean	9.8	11.8	45.2	61.2

Biofortification programs for β-carotene, iron, and zinc have been estimated to be cost-effective (Neidecker-Gonzales et al., 2007; Stein et al., 2007). Investments in plant-breeding research are much less than those in supplementation and fortification programs because the increased value persists after development. In addition, supplementation and fortification programs must be sustained and thus, much more investment is needed in these programs. If investments are not sustained, benefits disappear. Development of iron- and zinc-dense cultivars of rice or wheat might cost as much as $10 million each over 10 years, including the costs of nutrition efficacy tests, the costs of dissemination in selected regions, and the costs of a nutrition and economic impact evaluation. Moreover, benefits are sustainable at low maintenance costs.

However, before beginning extensive breeding programs, it is essential to confirm that the micronutrient is sufficiently well absorbed and utilized by human consumers. In fact, further research is needed to determine if the edible portions of micronutrient dense crop grains still retain enriched levels of micronutrients after milling and processing, and if enriched levels of micronutrients in grain are bioavailable to target human populations.

Agronomic biofortification is required for optimizing and ensuring the success of genetic biofortification of cereal grains with Zn and Se (Broadley et al., 2006; Cakmak, 2008). In case of greater bioavailability of the grain Zn derived from foliar applications than from soil, agronomic biofortification would be a very attractive and useful strategy in solving Zn deficiency-related health problems globally and effectively (Cakmak, 2008). In fact, agronomic biofortification strategy appears to be essential in keeping sufficient amount of available Zn in soil solution and maintaining adequate Zn transport to the seeds during reproductive growth stage (Cakmak, 2008).

5.3.2 Bioavailability of Micronutrients in Food

Although increasing the concentration of micronutrients in plant foods is necessary, the absorption and utilization (i.e., bioavailability) of micronutrients in meals containing plant foods may be also improved (Graham and Welch, 1996). Plant foods contain various substances that interact with micronutrients. Some, called antinutrients, reduce whereas others, called promoters, enhance micronutrient bioavailability to humans (Graham et al., 2001). These substances can be also manipulated by agricultural practices (Graham et al., 2001) and by genetic manipulation (Lucca et al., 2001).

The nutritional value of a diet can not be determined solely from the total contents of individual nutrients. Concentrations of antinutrients and promoters as well as interactions between nutrients affect bioavailability, which is the amount of nutrient absorbed from the diet (Graham et al., 2000). In fact, the fraction of micronutrient content in food that is available for absorption by the human gastro-intestinal tract depends on the presence of other food ingredients. A number of nutrient promoters and antinutrients has been found. Research on the potential role of a variety of substances to act as promoters or antinutrients of some micronutrients such as Fe and Zn bioavailability to humans has been reviewed by House (1999). There is evidence for a promoter function of certain chelating low-molecular-weight organic acids as well as amino acids. Drakakaki et al. (2005) concluded that the expression of recombinant ferritin and phytase could help to increase iron availability and enhance the absorption of iron, particularly in cereal-based diets that lack other nutritional components. Phytate, tannins and other polyphenols, and some heavy metals act as antinutrients (Frossard et al., 2000; Graham et al., 2001).

Plant crop genotypes differ not only in grain micronutrient concentrations but also in the amounts of antinutrients and promoters in their seeds. For example, phytate concentration strongly depends on the plant genotype (Cakmak and Marschner, 1986; Khoshgoftarmanesh et al., 2007; Marschner, 1995; Torun et al., 2001). Erdal et al. (2002) found differences in phytate content of grain in 20 different wheat cultivars grown on a Zn-deficient calcareous soil (0.10 mg kg^{-1} DTPA-extractable Zn) in central Anatolia. Zinc fertilization (23 kg ha^{-1} Zn applied as $ZnSO_4$) increased seed Zn concentration in all cultivars and reduced seed phytate in the majority of the cultivars. The average increase in seed Zn was from 8.8 to 16.7 mg kg^{-1}, while phytate decreased in average from 10.7 to 10.0 mg g^{-1} grain dry weight. Raboy et al. (1991) showed the existence of an important variation in phytate-P among four wheat cultivars and their 60 F6 lines. Barrier-Guillot et al. (1996) reported that phytate-P in seeds of four wheat cultivars varied between 0.92 and 2.80 g kg^{-1} DW. The range and mean concentration of Zn and molar ratio of phytate-to-Zn in selected Iranian wheat genotypes are shown in Table 3. The genotypic variability in seed phytate concentration of wheat genotypes indicates that this variation can be exploited for breeding genotypes with low phytate concentrations to improve Zn bioavailability in cereal-based foods although attention should be paid to the fact that low phytate content increases the possibility of cancers. In addition, phytate is required for seed germination.

Table 3 The range and mean molar ratio of phytic acid to Zn in grains of selected wheat genotypes in the absence and presence of added Zn (Khoshgoftarmanesh et al., 2007)

	Phytic acid/Zn molar ratio			
	Whole grain		Bran-less flour	
	Minus Zn	Plus Zn	Minus Zn	Plus Zn
Range	39–88	43–76	27–49	18–37
Mean	48	31	29	26

In most cases, phytate-to-Zn molar ratios in foods are considered a predictor of Zn bioavailability (Gargari et al., 2007). This index has been used widely (Gibson et al., 2003; International Zinc Nutrition Consultative Group-IZiNCG, 2004), and it is considered as a good index for zinc bioavailability by the World Health Organization (World Health Organization, 1996) and International Zinc Nutrition Consultative Group (International Zinc Nutrition Consultative Group-IZiNCG, 2004).

5.4 *Combining the Benefits of High Yield and Better Nutritional Quality*

An important question in development of micronutrient-efficient genotypes is the possibility of combining high yield with better micronutrient nutritional quality. Previous studies showed that it is possible to combine micronutrient-rich traits with high yield. Both seedling vigor and nutritional quality can be improved through genetically modifying seeds with micronutrient enrichment traits. The highest micronutrient densities, which are approximately twice as high as those popular modern cultivars and indicating the existing genetic potential, can be successfully combined with high yield.

The combining of benefits for human nutrition and agricultural productivity, resulting from breeding staple food crops which are more efficient in the uptake of trace minerals from the soil and which load more trace minerals into their seeds, results in extremely high benefit-cost ratios for investments in agricultural research in this area. This approach would be more valuable and cost-effective by estimating the costs paid to heal micronutrient malnutrition in developing countries. Some adverse effects of Zn and Fe deficiency cannot be remediated by supplying adequate levels of Zn or Fe later in childhood, so prevention needs to be the focus of this seed improvement program.

High trace mineral density in seeds produces more viable and vigorous seedlings in the next generation, and efficiency in the uptake of trace minerals improves disease resistance, agronomic characteristics which improve plant nutrition and productivity in micronutrient deficient soils (Welch, 1999; Yilmaz et al., 1998). Adoption and spread of nutritionally-improved varieties by farmers can rely on profit incentives, either because of agronomic advantages on trace mineral deficient soils or incorporation of nutritional improvements in the most profitable varieties being released (Harris et al., 2008).

It has been shown that wheat plants grown from seed with high Zn content can achieve higher grain yields than those grown from the low-Zn seed when Zn was not applied to the soil (Yilmaz et al., 1998). Therefore, sowing seeds with higher Zn contents can be considered a practical solution to alleviate plant Zn deficiency especially under rainfed conditions, in spite of it being insufficient to completely overcome the problem (Yilmaz et al., 1998).

Mineral-packed seeds sell themselves to farmers because these trace minerals are essential in helping plants resist disease. More seedlings survive and initial growth is more rapid. Ultimately, yields are higher, particularly on trace mineral “deficient” soils in arid regions. Because roots extend more deeply into the soil and so can tap more subsoil moisture and nutrients, the mineral-efficient varieties are more drought-resistant and so require less irrigation. And because of their more efficient uptake of existing trace minerals, these varieties require lesser chemical inputs. Thus, the new seeds can be expected to be environmentally beneficial as well. It is conceivable that seed priming by spraying seed fields with Fe and Zn fertilizers during grain filling will provide enough additional yield benefit to justify additional seed price. But this approach is insufficient to improve the density of bioavailable micronutrients in grain of crops grown with “primed” seed. Several different commercial practices may be beneficial to improve plant production despite low levels of soil micronutrients in many nations.

5.5 Need to Limit Grain Cd and As While Improving Levels of Zn and Fe in Crops

In contrast with the essential nutrients Zn and Fe, cadmium (Cd) in soils can be accumulated to levels in rice and some other crops that are dangerous to consumers (Chaney et al., 2004). In areas of Asia where mine wastes contaminated rice paddy soils, rice accumulated Cd to levels over 0.4 mg kg^{-1}, the CODEX ALIMENTARIUS maximum recognized level safe for lifetime consumption by humans. In Japan, China and Thailand, mine-contaminated paddy soils have caused adverse effects in exposed subsistence farm families (Nogawa et al., 2004; Cai et al., 1998; Swaddiwudhipong et al., 2007). The nature of rice production and composition of polished rice grain contribute to the potential for this effect: (1) when flooded rice is drained at flowering, rapid drop in soil pH and transformation of CdS to more soluble forms allows rapid uptake of Cd to rice grain during grain filling; (2) polishing of rice removes much of the Zn, Fe and Ca in brown rice; levels present in polished grain are insufficient to support human health; and (3) subsistence farming individuals may consume home-grown rice for their lifetime, allowing accumulation in the kidney over time until injury occurs. Although other crops require consideration regarding transfer of soil Cd to humans, western populations have not been found to suffer Cd diseases, perhaps because few consume home-grown staple foodstuffs grown in contaminated soils for their lifetime. Further, most soil Cd is accompanied by 200-fold higher Zn, so higher Zn tends to limit Cd uptake into edible crop tissues (Chaney et al., 2004).

Two aspects of breeding Fe and Zn improved crops should consider the potential for Cd accumulation. First, cultivars vary in Cd accumulation due to genetic variation (Grant et al., 2008); normal breeding practices can produce higher Cd progenies by chance alone. In general, Cd has been found to enter plants on the root Zn-transporters (Hart et al., 2002). Thus breeding to increase Zn uptake may cause increased Cd accumulation as well. Transport to grain of Cd and Zn are somewhat independent, so it is possible to achieve both higher Zn and Cd uptake but only higher Zn movement to grain. Breeding programs to improve grain Zn and Fe should check to assure that Cd is not being increased along with Zn. In the same way, breeding to increase aluminum tolerance to allow production in strongly acidic soils will tend to increase uptake of Cd and other divalent cations (McLaughlin et al., 1999). Breeders of acid tolerant crops need to assure that their new cultivars are not also increased in Cd.

Arsenic (As) is usually independent of grain Fe and Zn, but recent issues of As in irrigation water and rice require similar effort to consider the potential for As accumulation in grain. Because rice is grown in flooded soils, and flooding produces arsenite which is more soluble, more phytotoxic, and more accumulated by rice, using high As waters to produce rice may produce grain with excessive As for lifetime consumption by humans (Meharg, 2004; Abedin et al., 2002). For rice cultivars to be grown on such As enriched soils, breeding lower As accumulating genotypes may be critical to the continued success of agriculture (Williams et al., 2005).

5.6 *Challenges in Programs for Selecting or Breeding Micronutrient-Efficient Genotypes*

Many different definitions for efficiency make the use of the term ambiguous. These differences in defining nutrient efficiency makes it difficult to compare results of different studies particularly by considering that nutrient efficiency is influenced by other factors, such as soil fertility status, climate, crop rotations, and management practices (Stewart et al., 2005). Gerloff and Gabelman (1983) proposed that genotypes differing in yield under nutrient stress should only be designated efficient or inefficient if they are normal in appearance and have similar yields when an optimal amount of the nutrient is available. To reduce the confounding effects that other factors have on nutrient efficiency, Gourley et al. (1994) proposed that equivalent yields of genotypes be demonstrated where nutrients are not limiting. This advise has not been widely followed by researchers; plant species and cultivars with significantly different genetic potentials have been compared for their nutrient efficiency. Figure 1 shows models of the response of three plant varieties with different potential yield to different soil nutrient levels. These plant varieties have different yields in the presence of an optimal amount of the nutrient. The plant variety 3 produced the highest yield under non-stress conditions while its nutrient efficiency, determined as commonly used efficiency definition, is less than other varieties. In contrast, the most nutrient-efficient variety 1 had the lowest yield when an optimal amount of the nutrient is available.

One of the major difficulties with breeding for resistance to nutrient deficiency is that the stress condition is frequently assessed based only on plant growth. However, growth is as a very complex character influenced by different inter-related processes. Therefore,

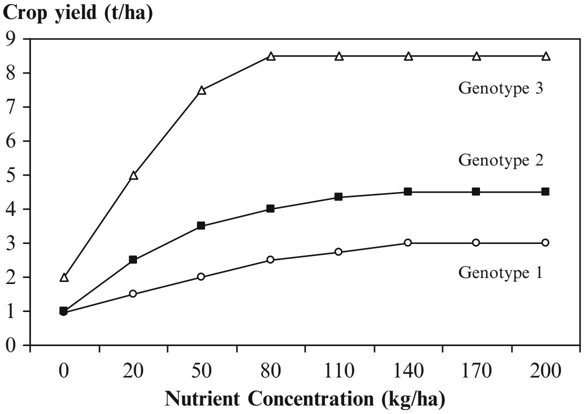

Fig. 1 Suggestive response of three different plant varieties to nutrient concentration in media. Genotype 3 produced the highest yield under non-stress conditions while its nutrient efficiency, determined as commonly used efficiency definition, is less than other genotypes. In contrast, the most nutrient-efficient Genotype 1 had the lowest yield when an optimal amount of the nutrient is available

growth is not a suitable parameter for distinguishing causes and effects of micronutrient deficiency stress.

Better screening techniques need to be developed by targeting specific processes that promote micronutrient deficiency, rather than those that appear as a consequence of it. It is expected that in the near future molecular methods will dominate selection for nutrient efficiency. Development of molecular markers for regions of the genome segregating with the trait of a particular nutrient efficiency will be crucial in that respect. However, molecular markers may be population-dependent, making them unsuitable for use in breeding programs. Even in screening based on molecular methods, seed nutrient content may need to be measured to assure the effectiveness of the program (Rengel, 2001).

Identification of genotypes with differing nutrient efficiencies, by whatever definition, generally includes investigation of potential morphological, physiological, and biochemical mechanisms involved. These mechanisms have been well reviewed (Caradus, 1990; Sauerbeck and Helal, 1990). However, it is often difficult to separate cause from effect when evaluating potential mechanisms of efficient nutrient uptake and utilization. Mechanisms conferring micronutrient efficiency are diverse and complex. Considering the close relationship between the root and shoot activities, differences in yield or nutrient accumulation by plants may incorrectly be attributed to differences in root morphology and function (Gourley et al., 1994).

Another challenge in breeding programs for assessment of a large number of genotypes is that field testings are time-consuming and expensive. Therefore, simple, fast and inexpensive techniques rather than expensive field experiments are required (Graham, 1984; Graham and Rengel, 1993). At this date, valid field screening is still needed before improved cultivars can be identified. Although genes may be identified in laboratory and greenhouse programs, incorporating micronutrient efficiency or density in grain with high yield and other agronomic performance measures will require field screening using traditional breeding approaches.

In most cases the crop yield has been considered as the main basis for separating micronutrient-efficient and inefficient genotypes (Clark, 1990; Stewart et al., 2005; Fageria et al., 2008). There is a concern whether or not micronutrient-efficient genotypes with higher tolerance to micronutrient deficiency can accumulate more nutrients in their edible parts. Although micronutrient-efficient genotypes possess higher uptake capacity, they do not necessarily have higher micronutrient concentrations in their leaves or grains (Graham et al., 1992). Micronutrient-efficient genotypes may even contain lower micronutrient concentrations in their edible parts than micronutrient-inefficient genotypes (Cakmak et al., 1997a, b, 1998). Enhanced micronutrient uptake by efficient genotypes under micronutrient deficiency stress improves dry matter production and often results in decreased tissue micronutrient concentration (dilution effect) to levels similar to those present in micronutrient-inefficient genotypes (Marschner, 1995). For example, Cakmak et al. (1997a) reported that in Zn-deficient soil, the most Zn-efficient rye had lower Zn concentration in its tissues than a Zn-inefficient durum wheat. Khoshgoftar et al. (2006a) reported that Zn-efficient wheat genotypes had lower shoot and grain Zn concentrations as compared to Zn-inefficient genotypes although the total amount of Zn per shoot or grain was higher in Zn-efficient genotypes due to more shoot dry matter yield.

Two important questions need to be answered in programs for selecting or breeding micronutrient-efficient cultivars. The first question is whether there is stability in response to nutrient efficiency of plant genotypes in different environments or how do the interactions of genotype by environment (G×E) affect response of genotypes to nutrient deficiency and fertilization condition? The second question regards relationships between crop productivity, mineral concentrations and other nutritional quality factors (e.g. concentrations of promoters and antinutrients) and the way that both crop productivity and grain quality aspects can be combined in developing new micronutrient-efficient genotypes.

Another important aspect that has to be considered in developing micronutrient-dense genotypes is that the bioavailability of micronutrients in enriched genotypes must be tested in humans to assure that they are of benefit to people preparing and eating them in traditional ways within normal household environments. Consumer acceptance must be tested (taste and cooking quality must be acceptable to household members) to assure maximum benefit to nutritional health.

5.7 Stress Tolerance Indicators for Selecting Micronutrient-Efficient Genotypes

Micronutrient deficiencies are abiotic stresses for plant production and most researchers have considered micronutrient efficiency synonymous with plant tolerance to micronutrient deficiency stress. A better understanding of the stress tolerance indicators is required for developing more reliable screening procedures to identify and select genotypes with high micronutrient efficiency. Breeders have used several yield stability analyses for identifying tolerant and sensitive genotypes to different environmental stresses (Fernandez et al., 1989). A relatively severe deficiency of an essential nutrient can be considered as a stress for plants (Fernandez, 1991). The field stress environment is characterized primarily by low inputs, suboptimal levels of irrigation, nutrients, temperature, and plant protection measures (Blum, 1988). Selection of genotypes adapted to both stress and non-stress environments have been the main objective of several yield trials.

Selection criteria are proposed to select genotypes based on their performance in stress and non-stress environments (Rosielle and Hamblin, 1981) who defined stress tolerance (TOL) as the difference in crop yield between the stress (Y_s) and non-stress environment (Y_p), and mean productivity (MP) as the average yield of Y_s and Y_p. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI), expressed by the following relationship:

$$\mathrm{SSI} = [1 - (Y_s/Y_p)]/\mathrm{SI} \tag{1}$$

where SI is the stress intensity and is estimated as $[1-(Y_s/Y_p)]$, and where Y_{s-} and Y_{p-} are the mean yields over all genotypes evaluated under stress and non-stress conditions. Rosielle and Hamblin (1981) evaluated the genotypic selection based on mean productivity (MP) and stress tolerance (TOL) and showed that selection of genotypes based on stress tolerance was efficient in improving yield under stress conditions, whereas the selected genotypes performed poorly under non-stress environments.

The heritability for yield is sometimes higher in the non-stress environment compared with the stress environment (Frey, 1964). Generally, evaluation in the non-stress environment allowed a better expression of genotypic potential, with higher heritability estimate yield and yield components than genotypes evaluated under the stress environments. Genotype effect and its interaction with environment are usually higher under favorable conditions compared to stress environments because the non-stress environmental conditions allow the genotypes to express their maximum genetic potential.

Genotypes can be categorized based on their performance in stress and non-stress environments into four groups: (A) genotypes that are not affected by stress and produce the same in both stress and non-stress environments; (B) genotypes that produce high yield only in non-stress environments; (C) genotypes with high yield in stress condition and low yield in non-stress environment; (D) and genotypes with low yield in both stress and non-stress environments. The optimum criterion should be separation of Group A from the other three groups. However, the stress tolerance indicators, mean productivity, stress tolerance, and SSI, failed to distinguish Group A genotypes from the other three groups. Fernandez (1991) presented a new stress tolerance index, STI, which can be used to identify genotypes that produce high yields under both non-stress and stress environments.

The stress intensity (SI) is defined as:

$$\mathrm{SI} = 1 - (Y_{s-}/Y_{p-}) \tag{2}$$

where Y_{p-} is the potential yield of a given genotype in a non-stress environment and Y_{s-} is the yield of a given genotype in a stress environment. The stress index can take values between 0 and 1. The larger value of SI indicates more severe stress conditions. Mean productivity (Eq. (3)) favors higher yield potential and lower stress tolerance. Rosielle and Hamblin (1981) showed that under most yield trials, the correlations between mean productivity and Y_p, as well as between mean productivity and Y_s, would be positive. Thus, selections based on mean productivity generally increase the average yield in both stress and non-stress environments. However, mean productivity can not distinguish between Group A and Group B genotypes.

$$\mathrm{MP} = (Y_s + Y_p)/2 \tag{3}$$

The tolerance index (TOL) is calculated by equation (4). A larger value of the tolerance index represents relatively more sensitivity to stress, thus a smaller value of tolerance index is favored. Selection based on tolerance index separates genotypes with low yield potential under non-stress conditions and high yield under stress conditions. The tolerance index fails to distinguish between Group C and Group A.

$$\mathrm{TOL} = \mathrm{Y_p} - \mathrm{Y_s} \qquad (4)$$

The stress susceptibility index (SSI) is another indicator to screen genotypes. Smaller values of SSI indicate greater tolerance to stress. Under most yield trials tolerance index and stress susceptibility index are positively correlated. Selection based on stress susceptibility index (SSI) favors genotypes with low yield under non-stress and high yield under stress conditions. Thus, SSI also fails to distinguish Group A from Group C.

Geometric mean productivity (GMP) is a better indicator than MP to separate Group A and Group C.

$$\mathrm{GMP} = \surd(\mathrm{Y_s} * \mathrm{Y_p}) \qquad (5)$$

Fernandez (1991) proposed stress tolerance index (STI) given by the following equation

$$\mathrm{STI} = (\mathrm{Y_p})(\mathrm{Y_s})/(\mathrm{Y_{p-}})^2. \qquad (6)$$

A high value of STI indicates great tolerance to stress and high potential yield. The stress intensity value is also incorporated in the estimation of STI. Thus, STI is expected to distinguish Group A from Group B and Group C.

Three-dimensional plots of $\mathrm{Y_s}$ (x-axis), $\mathrm{Y_p}$ (y-axis) and STI (z-axis) based on grain yield of different bread wheat genotypes grown under severely Zn-deficient soil (stress condition) and Zn fertilized soils (non-stress condition) is shown in Fig. 2. The method presented by Fernandez (1991) was used to show interrelationships among these three variables, to separate the Group A genotypes from the other groups (Groups B, C, D). To compare with the micronutrient efficiency index commonly used by plant nutritionists, a correlation was made between the STI and Zn efficiency of wheat genotypes. It seems that STI is a good selection criterion for identifying high-yield and stress-tolerant genotypes. The X-Y plane is divided into four regions by drawing intersecting lines through $\mathrm{Y_s}$ and $\mathrm{Y_p}$ and the four groups are marked as Group A to Group D (Fig. 2). Most of the Group A genotypes showed high STI and genotypes in group D showed low STI values. Analysis of variance for grain yield and stress tolerance indices for wheat grown in zinc stress and non stress conditions indicates that the STI indicator is less affected by the environment and its stability is more than other indices (Table 4). Table 5 presents the calculated stress tolerance indices and mean grain yield of studied wheat genotypes grown in zinc stress and non stress conditions. Correlation coefficient between stress tolerance indices and yield in zinc stress and non stress conditions is also shown in Table 6. The values of STI are positively correlated to the grain yield produced both in the presence and absence of added Zn. In addition, a very high and positive correlation was found between the STI and MP indicator while there was no significant relationship between the STI values and the amounts of Zn-efficiency calculated by the common formula ($\mathrm{Y_s/Y_p}$) used by several researchers. It has been previously mentioned that some Zn-efficient genotypes have lower potential yields (Kalayci et al., 1999; Khoshgoftarmanesh et al., 2004a).

The same results were achieved where a three-dimensional plot among $\mathrm{Y_s}$ (x-axis), $\mathrm{Y_p}$ (y-axis) and STI (z-axis) was drawn based on grain yield of different bread wheat genotypes grown in severely Fe-deficient (stress condition) and Fe-fertilized soils (non-stress condition) (Fig. 3). Higher values of STI were related to Genotypes in Group A (genotypes with higher Fe efficiency and greater potential yield both in the presence and absence of added Fe).

It seems using these stress indicators for separating proper micronutrient-efficient genotypes with higher potential yields would be useful in future studies. Additionally, developing new stress indicators through including other desirable criteria (i.e. concentrations of micronutrients as well as antinutrients and promoters in grains) is very useful in modern breeding programs. In this way, it would be possible to divide crop genotypes into different groups based on both qualitative and quantitative aspects.

6 Conclusion

We reviewed different possible approaches including soil and foliar fertilization, crop systems, application

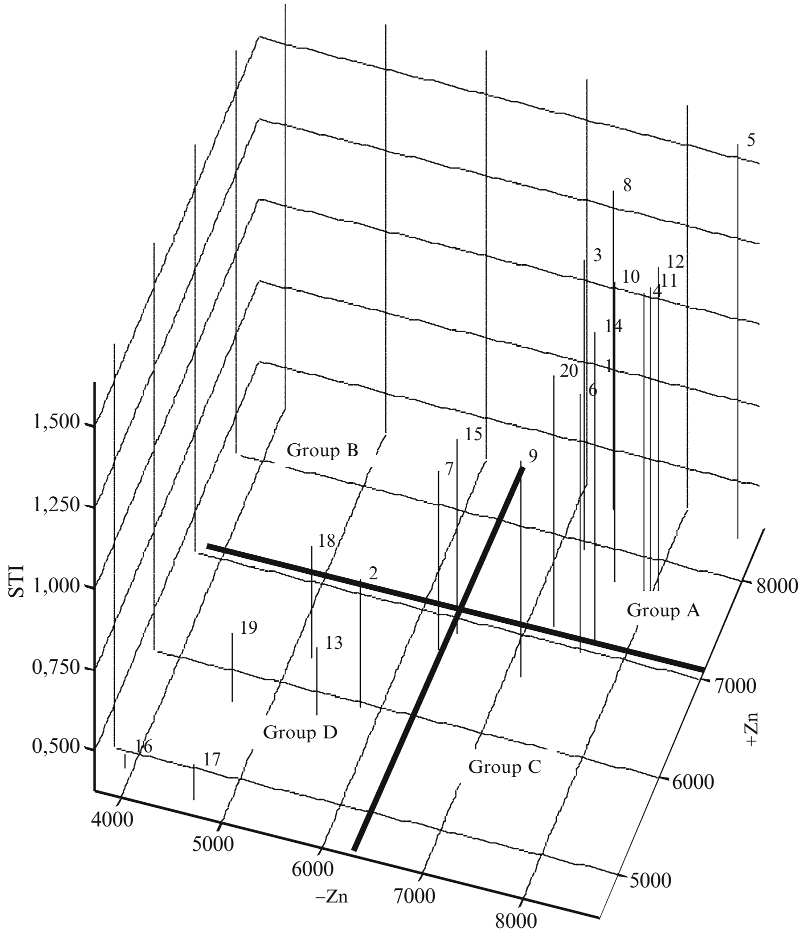

Fig. 2 The 3-D plot among salt tolerance index (STI), −Ze and +Ze condition for different winter wheat cultivars. Group A, genotypes that are not affected by stress and produce the same in both stress and non-stress environments; Group B, genotypes that produce higher yield only in non-stress environments; Group C, genotypes with higher yield in stress condition and lower yield in non-stress environment; and Group D, genotypes with low yield in both stress and non-stress environments. Mahdavi (1), Bezostaya (2), Navid (3), Alamoot (4), Alvand (5), Zarin (6), MV-17 (7), Gaspard (8), Gascogen (9), Sayson (10), Shahriar (11), Toos (12), Sabalan (13), Roshan Winter Back Cross (14), Shahi Cross (15), Shahpasand (16), Omid (17), Azar2 (18), Sardari (19) and C-81-10 (20) (Khoshgoftarmanesh, not published)

Table 4 Analysis of variance for grain yield and stress tolerance indices in wheat in zinc stress and non stress conditions. This table shows the effect of environment on different stress indices and their relative stability. GMP, STI, SSI, MP, TOL, EF, Y_p, and Y_s are geometric mean productivity, stress tolerance index, stress susceptibility index, mean productivity, tolerance index, zinc-efficiency, grain yield under with added Zn treatment, and grain yield under without added Zn treatment, respectively

GMP	STI	SSI	MP	TOL	EF	Y_p	Y_s	df	Source of variation
Pr > F									
<.0001	0.4948	0.7774	<.0001	0.9666	0.7154	<.0001	<.0001	1	Place
<.0001	<.0001	0.4551	<.0001	0.3119	0.3153	<.0001	<.0001	19	Genotype

of soil amendments and organic sources, and genetic and agronomic biofortification to correct micronutrients deficiency and to increase their density in edible parts of plants.

Soil application of fertilizer is the easiest way to correct micronutrient deficiency; however, it is not always successful and sustainable due to agronomic and economic factors such as reduced availability of mi-

Table 5 Means of yield and stress tolerance indices in zinc stress and non stress conditions in wheat. GMP, STI, SSI, MP, TOL, EF, Y_p, and Y_s are geometric mean productivity, stress tolerance index, stress susceptibility index, mean productivity, tolerance index, zinc-efficiency, grain yield under with added Zn treatment, and grain yield under without added Zn treatment, respectively

Cultivar	Y_p	Y_s	EF	TOL	MP	SSI	STI	GMP
Mahdavi	7112	7664	110	−552	7388	−27.9	1.16	7352
Bezostaya	5957	5812	99.5	146	5884	−21.8	0.734	5868
Navid	7928	7222	92	706	7575	119	1.20	7533
Alamoot	7566	7963	106	−397	7764	58.6	1.30	7750
Alvand	8389	8575	102	−186	8482	43	1.53	8470
Zarin	6973	7580	109	−607	7277	90	1.13	7250
MV-17	6647	6293	95.9	354	6470	−135	0.900	6453
Gaspard	8397	7332	88	1034	7850	−75.6	1.35	7814
Gascogen	6611	7131	108	−520	6871	−12.6	0.99	6853
Sayson	7703	7617	99.2	86.2	7660	−50.5	1.27	7646
Shahriar	7585	8029	107	−444	7807	−55.0	1.31	7786
Toos	7712	8054	108	−343	7883	66	1.34	7845
Sabalan	5500	5564	106	−63	5532	−60	0.651	5497
Roshan W.B.C	7384	7552	102	−168	7468	63	1.19	7454
Shahi Cross	6861	6410	94	451	6636	43.3	0.91	6580
Shahpasand	4838	3930	86	907	4384	30.5	0.392	4303
Omid	4706	4673	106	32.7	4689	159	0.464	4653
Azar2	6285	5182	90	1102	5734	−2.9	0.674	5646
Sardari	5710	4628	84	1083	5169	5.1	0.558	5107
C-81-10	7149	7236	103	−87	7192	−128	1.12	7163
LSD 5%	1272	812	21.5	1505	757	217	0.21	747

Table 6 Coefficient of correlation between stress tolerance indices and yield in zinc stress and non stress conditions in wheat. GMP, STI, SSI, MP, TOL, EF, Y_p, and Y_s are geometric mean productivity, stress tolerance index, stress susceptibility index, mean productivity, tolerance index, zinc-efficiency, grain yield under with added Zn treatment, and grain yield under without added Zn treatment, respectively

Trait	Y_s	Y_p	EF	TOL	MP	SSI	STI	GMP
Ys	1							
Yp	0.91**	1						
EF	0.16^{ns}	0.55*	1					
MP	-0.24^{ns}	−0.63**	−0.97**	1				
TOL	0.97**	0.98**	0.38^{ns}	−0.47*	1			
SSI	-0.12^{ns}	-0.06^{ns}	0.09^{ns}	-0.07^{ns}	-0.08^{ns}	1		
STI	0.96**	0.97**	0.38^{ns}	−0.46*	0.99**	-0.07^{ns}	1	
GMP	0.97**	0.98**	0.39^{ns}	−0.47*	1.00**	-0.08^{ns}	0.99**	1

* and **: significant at the 5% and 1% level of probability, respectively. ns: non significant.

cronutrient element due to topsoil drying, subsoil constraints, disease interactions, and cost of fertilizer in developing countries. Foliar fertilization is more effective than soil application, but too expensive for resource-poor farmers. Seed priming is also an effective way to increase yield and is important for resource-poor farmers. It seems a reasonable intercropping or rotation system of nutrient efficient species will help to prevent or mitigate micronutrient deficiency of plants in agricultural practice.

Selecting and breeding staple food crops which are more efficient in the uptake of trace minerals from the soil and which load more trace minerals into their seeds combines benefits both for agricultural productivity and human nutrition. Plant crop yield under stress could be enhanced by selection or breeding of plants that have high micronutrient efficiency, and ability to interact effectively with environmental extremes. Cultivation of micronutrient-efficient crop genotypes may have important applications for in-

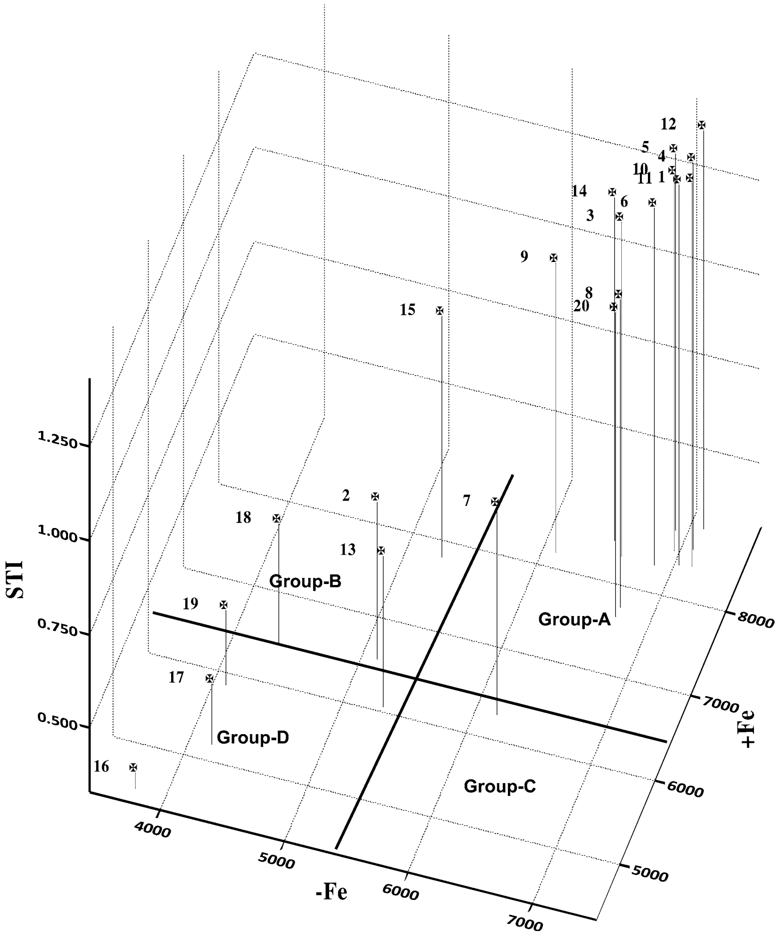

Fig. 3 The 3-D plot among stress tolerance index (STI), −Fe and +Fe condition for different winter wheat cultivars. Group A, genotypes that are not affected by stress and produce the same in both stress and non-stress environments; Group B, genotypes that produce higher yield only in non-stress environments; Group C, genotypes with higher yield in stress condition and lower yield in non-stress environment; and Group D, genotypes with low yield in both stress and non-stress environments. Mahdavi (1), Bezostaya (2), Navid (3), Alamoot (4), Alvand (5), Zarin (6), MV-17 (7), Gaspard (8), Gascogen (9), Sayson (10), Shahriar (11), Toos (12), Sabalan (13), Roshan Winter Back Cross (14), Shahi Cross (15), Shahpasand (16), Omid (17), Azar2 (18), Sardari (19) and C-81-10 (20) (Khoshgoftarmanesh et al., 2007)

creasing farm productivity in developing countries in an environmental-friendly way. This approach also holds great promise for making a significant, low-cost, and sustainable contribution to reducing micronutrient deficiencies in humans.

The combining of benefits for human nutrition and agricultural productivity, resulting from breeding improved staple food crops results in extremely high benefit-cost ratios for investments in agricultural research in this area. This approach would be more valuable and cost-effective by estimating the costs paid to heal micronutrient malnutrition in developing countries.

Despite beneficial effects of biofortified genotypes, we found the following major challenges in programs for selecting or breeding micronutrient-efficient genotypes: (1) The stability in response to nutrient efficiency in different environments and relationships between crop productivity, mineral concentrations, and other nutritional quality factors (e.g. concentrations of promoters and antinutrients) has less been considered. (2) There is no reliable indicator for identifying micronutrient-efficient genotypes with high grain yield. It seems that the stress tolerance index, STI, can be used to identify genotypes that produce high yields under both non-stress and stress environments. (3) In most cases, the crop yield has been considered as the main basis for separating micronutrient-efficient and inefficient genotypes while micronutrient-efficient genotypes may even contain lower micronutrient concentrations in their edible parts than micronutrient-inefficient genotypes. Therefore, combining crop productivity and grain quality aspects such as total content and bioavailability of nutrients in developing new micronutrient-efficient genotypes is necessary. (4) Breeding programs to improve grain micronutrient concentration should check to assure that a toxic metal such as Cd is not being increased along with micronutrients.

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Multi-Criteria Decision Models for Management of Tropical Coastal Fisheries

Merlina N. Andalecio

Contents

Abstract The crisis in the world's fisheries is attributed to excessive fishing pressure, long-term mismanagement, increased population growth, development and improvement of fishing technologies, uncertainty in global fisheries catch data, economic incentives and subsidies, and increasing demand for fish meal. For the coastal fisheries of developing countries, the problem is aggravated by coastal habitat degradation, widespread poverty in coastal communities, inshore encroachment of commercial fishing vessels, use of illegal and destructive fishing methods, resource use conflicts, pollution from uplands, and weak institutional arrangements. In response to these problems and in the hope of reversing their negative effects, fisheries management strategies have emerged for tropical coastal fisheries during at least the past 30 years. In fisheries, it is crucial to determine the outcomes of management strategies, especially when public money has to be accounted for. However, efforts to assess their impacts or measure progress are usually directed towards a single disciplinary approach, which fails to consider the multi-dimensionality of tropical fisheries including concomitant multi-level and conflicting goals and objectives. This article explores the utility of a multi-criteria type of evaluation as a potential analytical approach in impact evaluation for tropical fisheries management. The general framework of a multi-criteria evaluation method is a two-dimensional matrix composed of different choice possibilities including the set of criteria and indicators that will serve as bases in assessing these choice possibilities. The literature presents various criteria and indicators in fisheries management evaluation, the kinds and number of which would depend on stated goals and objectives of fisheries and the availability of resources to acquire the

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_14,

from Andalecio, Agron. Sustain. Dev. 30 (2010) 557–580. DOI: 10.1051/agro:2009051

information. The type of measurement, i.e., quantitative or qualitative, and the weighing of criteria and indicators are crucial in the evaluation process because they determine the multi-criteria aggregation approach to be used. Moreover, the participation of stakeholders and coastal resource users is crucial in complementing scientific information, in developing acceptable solutions, and in reducing conflicts and distrust in the evaluation and decision-making process. While many aggregation models in multi-criteria analysis in natural resource management exist, this article limits its review to only six models: the analytic hierarchy process, the weighted sum model, the ordination technique, concordance analysis, the regime method and Evamix; which are viewed to be applicable to the structure of decision-making in tropical fisheries management. This article also examines the performance of some of these models through a case study that determines the impacts of fisheries management strategies in San Miguel Bay, Philippines. The review reveals the following: (1) among the aggregation approaches, the analytic hierarchy process and ordination technique had the highest number of applications in fisheries while none was found for concordance analysis, the regime method or Evamix; (2) the application of hybrid models in multi-criteria analysis is increasing and found to be effective in many environmental decision problems including fisheries; (3) the application of multi-criteria decision models to fisheries management is relatively scarce during the last 10 years; only 26 papers were found in peer-reviewed journals; and (4) in the choice of model, its technical assumptions and limitations, its appropriateness for a specific decision-making problem, and its ability to handle the situation correctly vis-à-vis contextual, technical and political concerns should be considered.

Keywords Multi-criteria decision models • Fisheries management • Impact evaluation • Indicators • Criteria • Preference system • San Miguel Bay

Abbreviations

AHP	Analytic hierarchy process
BFARMCs	Barangay fisheries and aquatic resource management councils
CADS_TOOL	Cage aquaculture decision support tool
CI	Consistency index
CP	Compromise programing
CR	Consistency ratio
EBM	Ecosystem-based management
ELECTRE	Elimination and choice translating reality
Evamix	Mixed data evaluation method
FARMCs	Fisheries and aquatic resource management councils
FSP	Fisheries sector program
GIS	Geographic information systems
LGP	Lexicographic goal programing
MCA	Multi-criteria analysis
MCDM	Multiple criteria decision-making
MCE	Multi-criteria evaluation
MDS	Multidimensional scaling
MOP	Multiple objective programing
MPA	Marine protected area
MPAEM	Marine protected areas evaluation model
MUF	Multi-attribute utility function
Rapfish	Rapid appraisal technique for fisheries
RI	Random index
SIC	Sustainability index of criteria
WGP	Weighted goal programing
WSM	Weighted sum model

1 Introduction

Fish is an important protein source for over 2.9 billion people, providing a livelihood to an estimated 43.5 million people worldwide (FAO, 2009). However, due to excessive fishing pressure, the FAO (2009) reported that 80 percent of the world's fish stocks are fully exploited or overexploited, yielding less than their maximum potential or catching near maximum limits. The collapse of major international fish stocks such as the northern cod fishery and other ground fisheries is well documented in the literature (e.g., Myers and Quinn, 2002; Neis, 2000; Sinclair et al., 1999). This collapse did not happen overnight (Jensen, 2002) but is a result of overfishing, long-term mismanagement, increased population growth, development and improvement of fishing technologies, uncertainty in global fisheries' catch data, economic incentives and subsidies, increasing demand for fish meal, etc.

(Zeller and Pauly, 2005; Eagle and Thompson, 2003; Jensen, 2002; Bundy and Pauly, 2001; McManus et al., 1997; Caddy et al., 1998).

The management of complex and heterogeneous tropical coastal fisheries is difficult. For decades, interrelated problems including overfishing, habitat degradation, natural resource depletion, widespread poverty in coastal communities, encroachment of commercial fishing vessels inshore, use of illegal and destructive fishing methods, resource use conflicts, siltation and pollution from uplands, weak institutional arrangement and many others have received extensive attention. These problems are especially apparent in many tropical countries given the multispecies and multigear nature of coastal fisheries. In response to these growing problems and in the hope of reversing their negative effects, several fisheries management interventions, collectively termed as fisheries management strategy, have emerged for coastal fisheries. Fisheries management interventions include establishment of marine protected areas, area closures, mesh size regulations, gear restrictions, by-catch restrictions and controls, fishing effort restrictions, limited entry licensing, comprehensive monitoring and enforcement, temporal and spatial distribution of fisheries, and alternative employment (Lowry et al., 2009; Crawford et al., 2004; Wilson et al., 2002; Zeller and Reinert, 2004; Witherell et al., 2000; Pollnac et al., 2001; Allison and Ellis, 2001). Most of these fisheries management interventions were implemented in many developing countries' coastal fisheries during at least the past 30 years.

The universal goal of sustainable fisheries management is to improve the state of the fisheries for the benefit and enjoyment of the present generation and generations to come. These goals are specified in Charles (1989) and Cochrane (2000) to include resource conservation, food production, generation of economic wealth, generation of reasonable incomes for fishers, maintenance of employment for fishers, maintenance of the well-being and viability of fishing communities, sustainable utilization, economic efficiency, and equity in access to resources. These can be broadly categorized as ecological/biological, social, and economic objectives. Because of the diversity of the coastal fishery resources and interest groups, compromises in goals and objectives are often necessary (Mardle et al., 1997) because it is impossible to maximize different objectives simultaneously (Munda, 2005). This is the case in tropical developing countries where national goals and objectives of fisheries sometimes may not complement the needs of the local community. For example, conflicts often occur among attempts to conserve fish stocks, maximize efficiency and export earnings, and the desire to satisfy the needs of the fishing communities with respect to jobs and income (Khorsid and Morgan, 1990; Mardle et al., 1997; Whitmarsh, 1998). Conservation or preservation of biological diversity is often contrary to the aim of many fisheries organizations in maximizing production or economic efficiency (Agardy, 2000). In addition, increasing one objective such as regional benefits tends to decrease other goals such as national efficiency (Sylvia and Cai, 1995).

In fisheries, it is crucial to determine the outcomes of management strategy, especially when public money spent on projects and programs has to be accounted for. The success or failure of a management strategy has to be dealt with in an integrated manner that accounts for the multiple objectives of the fisheries. If the need is to regenerate, rehabilitate, conserve, protect and sustainably manage the fisheries resources, then the management of the coastal areas should be based on the understanding of the interrelationships of the various parts of the ecosystem (Griffis and Kimball, 1996; Legendre and Legendre, 1983) including human interactions. Many program evaluation reports or documents have failed to do away with mere descriptive summary of the findings or present an analytical framework to assess the performance of the fisheries management strategy.

Becker (2001) defined impact assessment or evaluation as "the process of identifying the future consequences of a current or proposed action". Fundamental to any evaluation is the method or tool to be used. In fisheries management, the importance of developing analytical and operational evaluation tools is critical for sound decision-making. Evaluation of fisheries management is often atomistic in nature which means that impact evaluation is frequently undertaken through a single-disciplinary approach (either a biological or an economic approach). Biological impacts are determined by abundance, biomass and sizes of fish, species composition, and diversity (Dinmore et al., 2003), whereas economic impacts are evaluated through monetary measures such as benefit-cost analysis, travel cost, hedonic pricing, input-output analysis, contingent valuation, etc. (Dalton, 2004; Hoehn, 1987; Propst and Gavrilis, 1987) as well as non-monetary techniques such as social well-being, psychophysical measures and attitude measures (Dixon et al., 1994; Gregory, 1987). One limitation of each approach is that the impacts are usually considered uni-dimensional, as demonstrated in the studies of Heen (1989) and Karydis and Coccosis (1990).

Because of the limited ability of current scientific methods to measure and understand with certainty the ecological processes and the high temporal and spatial variability and uncertainty in the biological components of the marine environment (Parr et al., 2003), it may be insufficient to consider only the biological impacts of management. What if scientific methods fail to detect the impacts on their underlying causality: does it mean that management has no effect at all? For example, McManus (1986) reported that in the Philippines, although a ban on commercial trawling had gradually been imposed on the fishery over several years, the degree of impact of the ban on the local species composition was not known. Many of the costs and benefits of management are difficult to quantify, and even if quantifiable, may be difficult to measure in monetary terms (Bingham et al., 1995). In addition, the social and institutional impacts of management (especially the distributional aspects) are not explicitly included in either biological or economic impact assessments. The social aspect is concerned with the survival of coastal communities dependent on the fisheries and can be evaluated as community participation and cooperation, employment, change in the degree of user conflicts, improved standard of living, etc. The institutional one deals with governance or the administrative and political aspects of management.

When the intention of the evaluation is to examine the multiple effects of management strategy, a single approach may no longer provide sufficient estimates of impacts because it precludes a meaningful evaluation of the complexities of fisheries and factor interaction. The current direction therefore is to consider the multidimensionality of fisheries (i.e., biological/ecological, economic, social and institutional). In which case, a multiple criteria (or multi-criteria) type of evaluation is a potentially useful analytical tool that can complement (Nijkamp et al., 1990) [not compete with or replace] and strengthen the existing approaches. Unlike any of the single approaches referred to earlier, multi-criteria methods deal with mixed information measured on varying scales – either quantitative (also known as *cardinal* – i.e. interval and ratio scales) or qualitative (i.e., ordinal or nominal/categorical scales) or both.

The purpose of this paper is to review the extent of the application of multi-criteria evaluation methods to tropical fisheries management. This paper is divided into sections. Section 1 introduces the problems and challenges related to the discipline-based evaluation of the impacts of management in tropical fisheries, and a rationale for the use of a multi-criteria type of evaluation. Section 2 presents the framework, structure and features of a multi-criteria evaluation method, with extensive discussion on the selection, measurement and weighting of criteria and indicators as well as stakeholders' participation. Section 3 reviews and examines some aggregation approaches usually applied in natural resource management and that show potential in tropical fisheries management. Section 4 presents a case study in fisheries impact evaluation and provides information on the number of applications of multi-criteria methods to fisheries management. The paper closes with a discussion on the limitations of multi-criteria methods and the need for a systematic evaluation in tropical fisheries management.

2 Fundamentals of Multi-Criteria Evaluation Method

Multiple criteria evaluation approaches may appear in the literature as multi-criteria analysis (MCA), multiple criteria decision-making (MCDM) or multi-criteria evaluation (MCE). Regardless of nomenclature, the intention is to examine a number of choice possibilities (e.g., alternative plans or strategies, management options, administrative zones or regions, etc.) through multiple criteria that measure the attainment of conflicting goals or objectives with the explicit inclusion of subjective weights. This allows different points of view to be identified and explored (Chesson et al., 1999; Mahmoud and Garcia, 2000; Van Delft and Nijkamp, 1977). Multi-criteria analysis appeared in the 1960s and 1970s as a decision analysis tool in response to the growing operations research in WWII (Hajkowicz and Collins, 2007). They have demonstrated their utility in many environmental issues that link economic, environmental, cultural and technical issues of management (Abu-Taleb, 2000; Makowski et al., 1996). The advantages of multi-criteria evaluation models are explicitly discussed in Hajkowicz (2008), Bryan and Crossman (2008), Hajkowicz and Collins (2007), Linkov et al. (2006), El-Gayar and Leung (2001), Brody et al. (2006), and Chesson et al. (1999) and are summarized as follows:

- useful in improving the decision-making process, leading to public acceptance of suggested options;
- applied in multi-stakeholder decisions providing a transparent, structured, rigorous and objective evaluation of options;
- dispute resolution tool that assesses progress of a particular objective by reducing the level of complexity of information;
- help balance conflicting demands of environmental conservation and development with regards to water allocation and coastal development.

The general structure of a multi-criteria evaluation method consists of at least a two-dimensional matrix, where one dimension expresses the various choice possibilities while the other dimension is composed of criteria that will evaluate these choice possibilities (Voogd, 1983). Munda et al. (1994) and Nijkamp et al. (1990) summarized the procedure as follows:

(a) structuring the problem (defines what is to be evaluated);
(b) generation and definition of choice possibilities (e.g., alternatives);
(c) choice of a set of evaluation criteria;
(d) determination of scores for each criterion and indicator per choice possibility (the value assigned to the choice possibility with respect to a criterion);
(e) identification of a preference system of decision-makers (criterion priorities have to be defined so that information can be combined); and
(f) selection of an aggregation procedure (choice of methods to come up with an analysis of the evaluation problem).

The outcome of the above procedure from (a) to (d) is an evaluation matrix composed of a list of criteria and the scores of the criteria for the choice possibilities. A number of existing multi-criteria methods have a similar structure of the evaluation matrix. Their only difference would be on how steps (d), (e) and (f) in the procedure will be handled. Prior to the discussion on the aggregation procedure, the primary components of the evaluation matrix in relation to fisheries management are presented below.

2.1 Choice of Evaluation Criteria and Indicators

The importance of developing evaluation criteria and performance indicators (structural and functional elements used to judge the success of management) for project appraisal (Van Pelt, 1993; Ware et al., 2009), habitat restoration (Pastorok et al., 1997), management programs (Anderson, 1989), ecosystem approach (Jennings, 2005), and sustainability assessment and management (Froese, 2004; Ehler, 2003; Garcia et al., 2000; Hardi et al., 1997) is well-recognized. The criteria and related indicators are often derived from goals and objectives of fisheries management and according to Bonzon (2000), the government and local authorities are likely to select them based on their own specific objectives.

The terms criteria and indicators are often used interchangeably in the literature. A criterion may be defined as a concept designed to specify the expected or desired outcomes of implementing a management strategy. It may not be an actual measure since there is no single exact measure for any of the criteria. Instead, one or more indicators or performance indicators measure a criterion. The kinds and number of criteria selected largely depend on the stated goals and objectives. When goals and objectives of fisheries are vaguely defined, more effort is needed to obtain specificity of the criteria; although sometimes vagueness of goals and objectives is inevitable to allow flexibility for changing program activities as future circumstances necessitate (Rutman, 1984).

Most criteria associated with fisheries sustainability and management that appear in the literature include feasibility (Yahaya, 1988; Yap, 2000), economic efficiency and benefit (Anderson, 1989; Bonzon, 2000; Tam et al., 1996; Whitmarsh, 1998), equity (Bonzon, 2000; Nickerson, 1999; Van Pelt, 1993; Yahaya, 1988), acceptability (Yahaya, 1988), ecosystem health and integrity (Linton and Warner, 2003; Wells, 2003), social welfare (Tam et al., 1996), effectiveness (Sumaila et al., 2000), enforceability of the management program (Maliao et al., 2004; Anderson, 1989; Yahaya, 1988), ecological accountability (Reynolds, 1993), institutional performance (Imperial, 1999), and biological diversity (Mawdsley and O'Malley, 2009). Each criterion may also be categorized under a broader classification. For example, Sutinen (1999) referred to biological, social, economic or administrative categories.

Concerns have been raised as to the number of criteria. If too many are used the process becomes unmanageable, or if too few, the evaluation process may become oversimplified. But how many are too many or too few, in fact, depends upon the availability of administrative resources or logistics to acquire the information. Even if an enormous number of criteria that incorporate biological, social, economic and administrative dimensions of managing a tropical fishery are identified, the choice still depends on whether they are "policy relevant, scientifically reliable and valid, simple, sensitive, possible to aggregate, affordable and feasible in terms of data collection" (Hanson, 2003). When baseline information with which to compare the current data is incomplete, existing documents that indicate standards, thresholds or reference points may be used. In a developing country, the validity of an evaluation is often challenged because baseline information is frequently insufficient and methods of data collection are inadequate to allow comparability of results (Pomeroy et al., 1997). The problem may not actually be incompleteness of data, but whether available data are reliable or not; and how to delineate those which are perceived useful to ensure a meaningful evaluation.

The sources of information may not only be those which were acquired through scientific means. If the knowledge base that we have is all that is available (Lane and Stephenson, 1995) then, Johannes' (1998) strong argument for a data-less management – "that is, management carried out in the absence of the data required for the parameterization and verification of models that predict effects of various management actions with useful confidence limits" justifies the inclusion of local knowledge (e.g., fishers' knowledge of the coastal waters and resources) in the evaluation process. He emphasized that management is not to be judged by its roots but by its fruits. Studies such as those of Berkes et al. (2001), Salas and Gaertner (2004), Ticheler et al. (1998), and White and Savina (1987) support the successful participation of fishers and local stakeholders in research, data collection and use of traditional knowledge in the formulation of scientific hypotheses. Thus, there should be no reason to question the participation of fishers in the evaluation process.

An indicator is a specific and straightforward measure. Bonzon (2000) characterized indicators as tools for measurements as value variables (either quantitative or qualitative), indices or pointers related to criteria of a given system. They are single measures of a resource element in an unaggregated form (Knuth and Nielsen, 1989) used to describe the state of the system and assess trends (Garcia et al., 2000). An indicator is not only a measurement tool, it is also a way of defining what is measured (Harte and Lonergan, 1995) and how will it best serve the users' needs (Rice, 2003). In general, indicators may be specific to particular uses and use contexts in both scale and content (Dahl, 2000); thus, they need not be generalized. Sustainability indicators are commonly categorized into discipline (e.g., indicators on biological, economic, social or cultural aspects), or according to whether they measure the factors that exert pressure, show the present system state or indicate responses of concerned groups to system changes or the pressure-state-response model (Adriaenssens et al., 2004; Willmann, 2000). Hundloe (2000) cautioned the use of an indicator that is only based on an economic measure because economy is only part of the environment and human system. Frequently employed ecological, social and economic indicators in fisheries are discussed in Garcia et al. (2000), Garcia and Staples, (2000) Kabuta and Laane (2003), Gislason et al. (2000), Pastorok et al. (1997), Staples (1997), and Vandermeulen (1998) among others.

Comprehensive listing of attributes and considerations in developing indicators are found in Ehler (2003), Kabuta and Laane (2003), Chong (2000), Vandermeulen (1998), Cairns et al. (1993), and Pastorok et al. (1997). These include responsive to change, supported by reliable and readily available data, relevant to the issue, unambiguously expressed, have broad relationships with existing evaluation tools, scientifically valid, national perspective, cost-effective, and if possible, predictive, easily understandable, simple and acceptable to intended users. The last attribute strengthened Staples' (1997) earlier point about the importance of considering the main users of the indicators in impact evaluation. Various resource users or decision-makers may make different choices of indicators. For example, scientists or people from academia would prefer indicators that are basic such as water quality parameters while fishers or local government officials would opt for applied indicators such as income, number of boats, etc. According to Bonzon (2000), indicators can reflect the needs of various entities and stakeholders (e.g., management authorities, producer associations, or the general public).

He further stated that in selecting indicators, information needed mainly for academic research must be distinguished from information directly related to strategic management planning. The choice of the number of indicators may also depend on the group of decision-makers; politicians may prefer a few simple indicators while technical experts would more likely include large numbers of indicators (Dahl, 2000). Indicators should be able to reduce the number of individual variables and data points while maintaining a sufficient level of understanding about coastal systems (Bowen and Riley, 2003). However, there are technical and methodological complexities that come with aggregation and weighting of mixed indicators (Dahl, 2000).

While Staples' (1997) and Vandermeulen's (1998) basic premise that indicators should be able to compare information with a standard, target, threshold or limit value has merit, this is often difficult to satisfy since standards and thresholds are not always established or available for many indicators. If this is the case, then the indicators can be classified as benefit or cost indicators. This means that the higher the value of the benefit indicators, the more they are preferred. Similarly, the lower values of cost indicators are more preferred. For example, the abundance of commercial fish catch is considered as a benefit indicator while unemployment rate is regarded as a cost indicator.

The amount of information generated for the criteria may also limit the number of indicators that can be measured to only a fraction of those possible (Cairns et al., 1993; Propst and Gavrilis 1987). Often, a criterion contains two or more indicators. Since many fisheries criteria and indicators are interrelated or interdependent, the choice of evaluation methods has to consider interdependence. Some evaluation methods have very limited assumptions; for instance, there are those which would only allow analysis if the criteria or indicators are independent of each other. However, it is less likely to find fisheries indicators that are unrelated, especially those belonging to the same criterion. For example, the indicators number of trainings and seminars conducted and level of awareness of resource users intended to measure the criterion acceptability of management may somehow be directly related. Our knowledge of which indicator would best measure a criterion is quite limited. Our assumption is that an infinite number of indicators contribute to the measure of a criterion and the probability of an overlap, redundancy or double counting of the indicators is bound to exist because of the inherent interrelationships among them.

While the development of performance criteria and indicators for fisheries management requires technical information, Chong, (2000) emphasized that the interest, willingness and commitment of the people or the community are also imperative in the sustainable management and conservation of fisheries and other coastal resources. Therefore, these same people have to be part of the development of the performance criteria and indicators for sustainable management.

2.2 Measurement of Criteria and Indicators

Deriving the values of the indicators, and subsequently, the criteria is critical in the final evaluation process. Usually both qualitative and quantitative information associated with several criteria need to be systematically considered when evaluating several decision alternatives (Wenger and Rong, 1987). Indicators as measures of criteria are categorized according to the four types of measurement scales, namely, nominal, ordinal, interval and ratio scales. Van Delft and Nijkamp (1977) and Vogt (1999) characterized these scales as follows: in the nominal scale the numerical operations are pointless because the numbers only represent names having no order or value while in the ordinal scale, the subjects are ranked in an order such that differences between rank orders have meaning. The interval scale does not have a fixed origin but it allows some numerical operations such as averaging, addition or subtraction. The ratio scale has a true zero point; thus, all standard operations can be carried out on this scale. Interval and ratio data can also be collectively called 'cardinal data'. Data measured on an interval or ratio scale are either continuous or discrete (discontinuous); continuous data are placed in a scale with an infinite range of points while discrete data are made up of distinct and separate units or categories (Vogt, 1999; Wheater and Cook, 2000).

In fisheries, a criterion is measured quantitatively or qualitatively using two or more indicators. But even if all indicators are measured using only one type of scale (e.g., quantitative), the units of measures may not be homogenous (e.g, hectares, currency, tons, percentages, etc.). This is a type of scaling problem that was resolved through transformation of values into a common order of 0 to 1 (Yakowitz, 1998) or normalization to obtain comparable scales because each function may have a different number of variables or mathematical relationships and corresponding maximum scores (Hruby, 1999). Although there are different kinds of normalization formula, the most commonly used are found in Pomerol and Barba-Romero (2000), Voogd (1983) and Hwang and Yoon (1981) and these are presented below:

(a) $e_{ij} = x_{ij}/\Sigma\, x_{ij}$
where e_{ij} = normalized indicator score
x_{ij} = score of the indicator
$\Sigma\, x_{ij}$ = sum of all indicator scores
(b) $e_{ij} = x_{ij}/x_{ij}^{max}$
where x_{ij}^{max} = maximum indicator score
(c) vector normalization:
$e_{ij} = x_{ij}/\sqrt{\sum x_{ij}^2}$
(d) linear scale transformation:
$e_{ij} = (x_{ij} - x_{ij}^{min})/(x_{ij}^{max} - x_{ij}^{min})$, for benefit criterion or,
$e_{ij} = (x_{ij}^{max} - x_{ij})/(x_{ij}^{max} - x_{ij}^{min})$, for cost criterion
where, x_{ij}^{min} = minimum indicator score;
x_{ij}^{max} = maximum indicator score.

When using vector normalization, all indicators are measured in dimensionless units, thus facilitating inter-indicator comparisons, but direct comparison is difficult because the minimum and maximum values of the measurement scale are not equal for each indicator. Linear scale transformation has the advantage of transforming results in a linear (proportional) way, making the relative magnitude of the outcomes equal (Hwang and Yoon, 1981). Also, if the nature of the indicators is different, the indicators are grouped as benefit indicators (larger x_j or value of the indicator is more preferred) or cost indicators (smaller x_j or value of the indicator is more preferred). Some authors (e.g., Nijkamp et al., 1990) recommended that if normalization is done, it is best to test the sensitivity of the outcome for the particular type of normalization.

The level of measurement imposes special conditions on the techniques to be used in further data manipulation. Smith and Theberge (1987) presented three aspects of measurement theory that will be useful in understanding the measurement of the indicators: (i) four basic scales of measurements (nominal, ordinal, interval and ratio) define the types of mathematical operation to be applied to the values; (ii) measuring environmental variables or subjective values; and (iii) uncertainty in measurements which affect both how measurement is done and the confidence that is placed in the values obtained. Works on multi-criteria methods rarely discuss how the measures of indicators were arrived at – e.g., uncertainties attached to the measurement. Because of the temporal and spatial differences associated with the collection of information for each indicator [data for the indicators and criteria may not be collected at the same time], it would be useful to standardize the methods of data collection to develop a meaningful database or information system.

2.3 Weights of Importance of Criteria and Indicators

The importance placed on the criteria and indicators is another consideration—importance may be modeled statistically by means of rank orders (Yakowitz, 1998), rating scales, paired comparisons or magnitude estimates. Some studies used multiple regression analyses to predict judgmental values as a function of various physical features of the environment while others applied multivariate techniques such as factor and cluster analyses to learn more about interrelationships among the indicators (Gregory, 1987; Petry, 1990). Prato (1999) noted that while there is no theoretical limit to the number of criteria (which he referred to as attributes), an individual's ability to assign weights to these criteria decreases with their number. One method I found in the literature that is useful to address this issue is the Analytic Hierarchy Process (AHP), developed by Thomas Saaty in the early 1970s (DiNardo et al., 1989; Leung et al., 1998). The AHP is a method of scaling ratios using the principal eigenvector of a positive pairwise comparison matrix (Saaty, 1980, 2001). Although a multi-criteria evaluation tool, the AHP can be used to just derive the weights of importance of criteria and indicators and not directly proceed with the comparison of choice possibilities. It structures a problem into a hierarchy then the weights of the criteria (and also indicators) are determined through pairwise comparisons (Saaty, 1980) according to preference, importance or likelihood (Peterson et al., 1994). Through pairwise comparisons, evaluators or decision-makers (e.g., fishers, coastal resource users) will not be overwhelmed with the amount of information that has to be processed mentally per unit time. To assess the scale ratio, Saaty (1980) provided a 9-point intensity scale of importance. One important feature of the AHP is the determination of consistency in judgments. This is done by computing the consistency ratio (CR), which is the ratio of the consistency index (CI) to the average random index (RI). Thus, $CR = CI/RI$, where $CI = (\lambda_{max} - n)/n - 1$ and RI is referred from a table of average random indices found in Saaty (1980). The λ_{max} is Σ (total normalized value of criterion z $\div$ column vector of priorities of criterion z). The consistency ratio measures the coherence of the pairwise comparisons and estimates the level of consistency with respect to the entire comparison process. A CR of 10% or less is considered acceptable. The consistency ratio measure allows AHP users to be aware of the seriousness of any inconsistent judgments (Leung et al., 1998).

Although weighting of the criteria is open to criticisms (Munda et al., 1994) because it involves human judgment, it is indispensable in impact evaluation work. Petry (1990) emphasized that as all the simplifications and approximations necessary for scientific analysis have some human value content, technical analysis and political decisions cannot be completely separated.

2.4 Stakeholders and Resource Users as Evaluators

The determination of the importance of criteria and indicators would have been simple and easy had preference among individuals been known to be similar. This objectifies value judgment and thus, a single evaluator would then be sufficient to represent preference of a population. But utility functions vary between individuals and preferences vary over time. The problem with a subjective judgment is how to validate whether the derived weighting is representative of the judgment of the population. Bodini and Giavelli (1992) resolved this problem through a survey technique that isolated viewpoints from the subjectivity of the planner and facilitated the involvement of local communities in the decision phases. However, their work failed to account for conflicts that may ensue in the final analysis – if stakeholders are categorized into groups, will each group's perception vary from each other? When Leung et al. (1998) applied the AHP approach, geometric means of the judgment from respondents were used to derive the overall and the respective group's priority, then, variation among individual judgments within and between groups was determined using analysis of variance. Grouping the individuals according to their respective stake in the fisheries would be more rational than combining all individuals with varied interests because of the problems of divergence in preference and domineering behavior of some individuals. The process that Stewart and Scott (1995) identified to re-homogenize individual groups is too tedious because every time a consensus is not achieved within a homogenous group, either multiple points of view or sub-criteria are created, or the group is subdivided into two or more groups representing the different interests. Srdjevic (2007) emphasized that the characteristic and size of the group may be critical in the application of multi-criteria methods because of the assumption of homogeneity.

In a top-down approach type of management, there are only a few individuals whose views are likely to influence the final decisions of the management process. However, when larger and varied groups of individuals are involved, identification of a preference system has to be approached differently. With an increased number of participants (or evaluators), the computational burden increases (Prato, 1999); however, the problem of computational overburden need not concern the evaluators – this is the responsibility of the analysts (who most likely will use computer software in data analysis). The institutional framework in which the entire decision-making process occurs determines the categories and number of evaluators. An institutional structure that is systematic and flexible is able to facilitate the integration of a wide range of viewpoints through interaction between and among diverse groups of individuals with varying stakes in the fisheries (Petry, 1990; Ridgley and Rijsberman, 1994). Although technical information is important, stakeholders' knowledge, experience and judgments are crucial in the application of a multi-criteria evaluation tool. This is evident in a complex system such as fisheries where incomplete information and understandings may exist (Adrianto et al., 2005). For example, Chong (2000) recognized the invaluable contribution fisherfolk may have in providing feedback of the condition of the resources and habitats. Similarly, the importance of different types of outcomes of management options to different stakeholders is essential in developing an acceptable solution (Pascoe et al., 2009). Linkov et al. (2006) observed that failure to incorporate stakeholders into decisions resulted in distrust and political tension.

The way the multi-criteria evaluation method is structured allows for the involvement of stakeholders in the decision-making and evaluation process. However, despite the wide recognition of the crucial participation of fishers in the management of fisheries resources (Clarke et al., 2002; Castilla and Fernandez, 1998; Ferrer et al., 1996; Gilman, 1997), the mechanism through which to include them in the formal evaluation process is not actually established. There are challenges to this which include quantifying uncertainty related to human input and overcoming the difficulty in negotiation when a large number of groups are responsible in decision-making and prioritizing actions (Ascough II et al., 2008).

3 Multi-Criteria Aggregation Approaches

Following the structure of an evaluation matrix, we are now able to construct an "impact evaluation matrix", characterized as an ex post evaluation (Nijkamp et al., 1990; Voogd, 1983) which deals with the analysis of the effects of management strategies that were already implemented. The multi-criteria evaluation method is usually named after the kind of aggregation procedure applied in the analysis. It often uses two kinds of input data: criterion scores and a set of political weights attached to these criterion scores. When input data are completed, the final step concerns the aggregation procedure to determine the progress of a choice possibility, whether based on an already established standard or in comparison with other choice possibilities.

Extensive discussion of multi-criteria evaluation methods and aggregation approaches is beyond the scope of this paper. For recent reviews of the applications of multi-criteria methods, readers are referred to Hajkowicz and Collins (2007), and Linkov et al. (2006). Since it is impractical to conduct a thorough survey of all the different methods, I narrowed the review to the model-based decision support tools usually applied in natural resource management, particularly coastal management. These are the analytic hierarchy process, the weighted sum model (WSM), the ordination technique, concordance analysis, the regime method and the mixed data evaluation method (or Evamix). I view them as applicable to the structure of decision-making in tropical fisheries management; specifically, in addressing the issue of performance evaluation of management strategies.

3.1 Analytic Hierarchy Process (AHP)

In Section 2.3, the analytic hierarchy process was used to elicit the weights of importance of criteria and indicators to attain a set goal or objective. Conversely, the AHP has also been extensively applied as a multi-criteria evaluation and decision support system in the last 20 years (Ho, 2008). The final result of the process is a numerical priority value for each choice possibility. The choice possibility with the highest score is considered the best one as determined by the decision process made explicit in the hierarchy and by the comparisons (Peterson et al., 1994). A more detailed analysis of the theoretical foundations and applications of the AHP appears in the papers of Vaidya and Kumar (2006), Saaty (2001), Triantaphyllou and Lin (1996), Ridgley and Rijsberman (1992) DiNardo et al. (1989), Khorramshahgol and Moustakis (1988), and Saaty (1980). The AHP uses the computer software expert choice to assist the evaluators in processing a large amount of information properly and performing sensitivity analysis.

The analytic hierarchy process has gained wide acceptance in the fields of urban and land development (Banai, 2005), water resource planning (Willet and Sharda, 1991), natural resource management and planning (Fernandes et al., 1999; Peterson et al., 1994; Schmoldt et al., 1994), restoration (Ridgley and Rijsberman, 1994), agriculture (Duke and Aull-Hyde, 2002), etc. The approach is highly dependent on the experience, knowledge and intuitive judgment of the evaluators. Ridgley and Rijsberman (1992) vouched for the use of this method as: (i) it does not demand independence of alternatives; (ii) it creates and is based on ratio scales rather than interval scales; (iii) it does not require that the range of criterion scores be known before comparison of choice possibilities relative to that criterion can be made; and (iv) it uses subjective assessments of preference intensity. These advantages may be apparent if the intention is to apply the AHP beyond weighting of the criteria and indicators to prioritizing of choice possibilities. This was demonstrated in the studies of Dey and Ramcharan (2008), Herath (2004), Leung et al. (1998), Schmoldt et al. (1994), Peterson et al. (1994) and Ridgley and Rijsberman (1992). Leung et al. (1998) and Varis (1989) found the AHP to be effective and robust in solving large, complex and evasive decision problems. Successful application of the AHP is demonstrated in Ridgley and Rijsberman's (1992) policy analysis for a Rhine estuary and Peterson et al.'s (1994) resource management plans. However, it is only the study of Peterson et al. (1994) which took note of the actual length of time i.e., two days' discussion, to implement and accomplish the process. Although the authors did not encounter any problem in reaching a consensus, they suggested that if this happens, a separate judgment can be aggregated using a geometric average. Contrary to the works of Ridgley and Rijsberman (1992) and Peterson et al. (1994) [who used consensus building among the evaluators in order to arrive at a final solution using the AHP], Leung et al. (1998) [after realizing that the group of decision-makers is large and diverse] employed a mail survey type of instrument. The results of their study showed that a mail survey is not an efficient technique to administer the AHP because of the low response rate. That is, only 52% of the 66 members of the Western Pacific Fishery Management Council returned usable surveys. Although the authors attributed such a low response rate primarily to the geographical distance and non-fisheries background of some Council members, the problem may also have been the lack of a venue for interaction. Respondents who are unfamiliar with the AHP may have had difficulty understanding its application without interacting with people who are more knowledgeable about the approach. The AHP has also been used in combination with other decision-making tools such as has been documented by Srdjevic (2007), Vaidya and Kumar (2006), and Bojórquez-Tapia et al. (2005).

There are only a few studies which applied the AHP to fisheries. Vaidya and Kumar (2006) provided a comprehensive review of 150 papers on the different applications of the AHP, none of which relate to fisheries. In the review of Mardle and Pascoe (1999), they only found four applications of the AHP in fisheries. These are the management of Maryland's river herring fishery (DiNardo et al., 1989), management of Kenai river Chinook salmon fishery (Merritt and Criddle, 1993), Eastern Finland's multispecies fishery (Kangas, 1995) and Hawaii pelagic fishery (Leung et al., 1998). Since the review of Mardle and Pascoe (1999), the present review was able to document 10 more applications of the AHP in fisheries management, summarized as follows:

(a) Pascoe et al. (2009) utilized the AHP to determine the impacts of different management options by assessing the objectives of fisheries management. The method was applied to three Australian fisheries and the results showed that the benefits and costs of management options or measures vary across fisheries.
(b) In developing a decision support tool for sustainable cage aquaculture, Halide et al. (2009) incorporated the AHP to select the best site for cage aquaculture. This was in combination with a decision support tool, CADS_TOOL (cage aquaculture decision support tool).
(c) Whitmarsh and Palmieri (2009) successfully applied the method to elicit the preferences of the public and stakeholders for the different objectives in salmon aquaculture. Their findings concurred with other research that social acceptability of aquaculture is closely linked to its perceived environmental impact. Although no remarks were made regarding the use of the AHP, their results demonstrated the importance of preferences of interest groups in influencing aquaculture policy.
(d) When Utne (2008) evaluated the performance of the Norwegian cod-fishing fleet, she found the AHP to be a useful performance measurement tool that incorporates performance indicators (e.g., accident risk, employment, profitability, quality of the fish meat, catch capacity (technical), and greenhouse gas (GHG) emissions/acidification) into the problem hierarchy. The process provided a basis for examining the stakeholders' involvement in fisheries management decision-making.
(e) Himes (2007) used the AHP to investigate preferences of stakeholder groups for performance indicators in evaluating marine protected area (MPA) management. They found the method to be strong, providing quantitative information on stakeholders' diverse attitudes and perceptions regarding MPA. It is also helpful in generating options for improved management and the possibility that stakeholders will accept and support the results.

(f) In the study of Ramos et al. (2006), reef diving choices and preferences were calculated using the AHP. This assisted the decision-makers to identify the most relevant criteria for diving i.e., the hope of seeing unusual aquatic organisms, and the benefits of updating diving skills.

(g) Nielsen and Mathiesen (2006) found the AHP to be a useful support tool for uncovering conflicts and eliciting preferences of various stakeholders involved in the management of the sand eel and Norway pout fisheries. The authors are optimistic that the preference process may improve accountability, openness and participation, and may contribute to good governance of said fisheries.

(h) Mardle et al. (2004) examined the utility of the AHP in measuring the importance of management objectives in achieving sustainable management in key interest groups of the UK English channel fisheries. Through the AHP, qualitative and quantitative criteria were incorporated into the framework of analysis. What is interesting is the usefulness of employing a personal survey (as opposed to a mail survey), which allows important interaction between the participants.

(i) Soma (2003) tested the performance of the AHP in the shrimp fishery sector in Trinidad and Tobago and found the method to be an "empowering, educating, focusing, facilitating and quantifying tool" in fisheries management. She concluded that the method is particularly useful in developing countries which are deficient in reliable quantitative data.

(j) Fernandes et al. (1999) evaluated coral reef management options and found that the AHP had been successfully utilized to make 'good decisions' consistent with the community's desires to preserve the social, ecological and economic benefits of park management.

3.2 Weighted Sum Model

The weighted sum model (also known as simple additive weightings) is the simplest and most commonly used method when all criteria are measured on cardinal scales, expressed in comparable units, and weights are assigned per criterion. This method is discussed in detail in Hwang and Yoon (1981), Nijkamp et al. (1990), and Triantaphyllou and Lin (1996). The criterion scores are standardized or normalized to be comparable and these normalized criterion scores are multiplied by their respective weights. The products are called the weighted scores and they are summed up over all criteria yielding a total weighted score or priority score for each choice possibility (Smith and Theberge, 1987). The choice possibility with the highest priority score is the one that performed well and is said to be the best choice possibility (P*). The best choice possibility (P*) is determined through the mathematical expression,

$$P^* = \max_{M \geqslant i \geqslant 1} \sum_{i=1}^{N} x_{ij} w_j, \text{ where } P^* \text{ is the priority score}$$

best of the choice possibility,

x_{ij} is the measure of performance of the ith choice possibility in terms of the jth criterion, and w_j is the weight of importance (Triantaphyllou and Lin, 1996). Usually the weights are normalized so that

$$\sum_{J=1}^{n} w_j = 1.$$

The method requires that criteria scores are both numerical (i.e., interval or ratio scales) and comparable because the regular arithmetical multiplication and addition measures are employed (Hwang and Yoon, 1981; Hajkowicz, 2008). The scores of the criteria have to be comparable because of the process of combining attributes such that a 'high' score for one criterion must receive about the same numerical values as 'high' scores of other criteria. There is a drawback in this kind of approach – it is difficult to interpret the multiplication of criterion values by weights. Consider, for example, criteria X and Y; the score of criterion X (0.8) multiplied by its weight (0.1) would yield the same product as that of criterion Y with a score and weight of 0.4 and 0.2, respectively. The problem lies with the tendency for the criteria to offset each other. Being based on additive utility assumptions, the WSM assumes transitivity of preferences and comparability of any pair of actions. Thus, it is more applicable to single-dimensional (all units of measurements are similar) than multidimensional (units of measurements are different) problems. Also, this method considers independence of criteria and indicators, when in reality they are complementary (excellence with regard to one criterion enhances the utility excellence with regard to the other criteria). It is a powerful tool as long as no important complementarities exist among the criteria (Hwang and Yoon, 1981). In the evaluation of fisheries management, the data available [especially ecological data] are likely to violate this assumption; therefore, the WSM may be of little use. Further, since all criteria are aggregated to obtain a single final result, the technique jeopardizes intermediate analysis (Petry, 1990; Triantaphyllou and Lin, 1996). According to Voogd (1982), one limitation of the WSM is that the outcomes will strongly depend on the (usually arbitrary) choice of the origins of the various measurement scales used. Chesson et al. (1999) applied the WSM technique to estimate the performance of the south-east fishery over time (i.e., 1993, 1994 and 1995) and examine the effects of fishing on two components, i.e. humans and the environment. In each component, quantitative indicators were identified. The weights of the components and indicators were determined from three groups of individuals: (1) individuals who value both economic and ecological components, (2) those who value short-term financial returns, and (3) those who reflect conservation preference. The output of the analysis is a trend over time showing the progress of said fishery towards ecologically sustainable development. One important aspect of the process is the aggregation of weighted scores of the components. The authors, however, noted that the level of aggregation can be used to reduce the information to manageable amounts without being misleading. When the WSM model was used together with the Borda Count method, Hajkowicz (2008) found that it helped stakeholders make group decisions despite strongly conflicting preferences. When the assumptions in the application of the WSM are ignored, Hajkowicz and Higgins (2008) presented two valid issues: (a) the results showing only very minor differences in utility may be insufficient to differentiate performance, and (b) incorrectly treating ordinal data as cardinal data.

3.3 Ordination Technique

Identified as a multivariate method, ordination techniques in ecological research are used to quantify the interrelationships among a large number of interdependent variables and to explain those variables in terms of a smaller set of underlying dimensions (e.g., components) (McGarigal et al., 2000). The type of ordination technique that is commonly used in a multiple criteria analysis is the geometric or multidimensional scaling (MDS) ordination technique. It is a qualitative evaluation approach based on the ideal point concept (Voogd, 1980) and where quantitative inferences can be drawn without violating the ordinal character of the input data (Voogd, 1982). However, when no ideal point is identified, the minimum and maximum criterion values may be used (Hajkowicz and Collins, 2007)

Multidimensional scaling is very useful when dealing with too many criteria, which in some cases are vague and unknown. It uses proximities of pairs of choice possibilities in constructing a multidimensional spatial representation. When the information is on a ratio scale, it is converted to rank order dis(similarities). Hwang and Yoon (1981) and Stalans (1995) described the detailed operation of non-metric MDS as an approach that looks for a configuration and this configuration gives the spatial representation of the patterns of proximities among objects. Choice possibilities or alternatives are represented as points in space where point distances correspond to rank order of dissimilarity judgments among choice possibilities. Points near each other are assumed to be close together in terms of preference. The evaluator locates his ideal point and the distance from the ideal point is measured (using Euclidean distance or another measure) in order to rank the choice possibilities in terms of preference. Interpretation of the relative positions of choice possibilities in space is associated with the characteristics of the criteria that were scaled. One way to determine which among the criteria contribute to the positioning in the configuration is through multiple linear regression – with the criterion as the dependent variable and the coordinates of the configuration as the independent variables. This would mean that the criteria are regressed over the coordinates of the configuration. The distance measure used to form the configuration assumes that the criteria are independent or non-complementary. The scores of the criteria may take any form since the scaling procedure produces numerical and comparable values of each resultant dimension. None of the dimensions correspond with the single criterion of the original matrix. This attribute is particularly useful when the number of criteria is large (around seven) and most criteria are expressed qualitatively (Hwang and Yoon, 1981). This approach, however, requires that the evaluation problem has enough degrees of freedom (Voogd, 1982).

Although not used directly as a multi-criteria method, non-metric MDS was useful in the study of Moriki and Karydis (1994) as a multivariate statistical tool to support the results of multi-criteria methods (i.e., concordance analysis, the numerical interpretation method, and the regime method). Multi-criteria methods yielded similar ranking for sampling sites along the coastal areas of Rhodes and Saronicos Gulf (Greece) according to nutrient and chlorophyll concentration. Three levels of nutrient loading characterized eutrophic, mesotrophic and oligotrophic waters. Non-metric MDS was applied to obtain a graphical representation of the ranked sampling sites. Moriki and Karydis (1994) only considered ecological indicator data. However, in the study of Moriki et al. (1996), ecological variables were integrated with socio-economic variables. They presented a case study of the island of Rhodes, Greece, which simultaneously analyzed economic, social and ecological data of the coastal system. The island was divided into five zones and the analysis was focused on the socio-economic characteristics of the zones, and chemical and biological parameters of the marine environment. Non-parametric MDS was one of the methods used to analyze coastal use conflicts, explore the development potential of the island and develop guidelines for future planning and decision-making. Similar to the other methods, MDS results showed a clear difference between intensity of land use and environmental quality, resulting in main aggregates, i.e., variables characterizing the quality of natural resources and the others characterizing the intensity of land use.

The ordination technique, MDS in particular, was applied in the development of Rapfish (rapid appraisal technique for fisheries), a multi-disciplinary rapid appraisal technique for evaluating the sustainability of fisheries with respect to ecological, economic, social, technological and ethical fields (see Pitcher, 1999). The technique constructs the best and worst possible fisheries from sets of scored attributes that were derived from fixed reference points (ideal points) or random reference points. MDS was able to generate ordination scores that provided a rating for a fishery from 0% (bad) to 100% (good). Rapfish has been applied in evaluating the status of fisheries globally such as 32 African lake fisheries (Preikshot et al., 1998), 42 Canadian fisheries (Pitcher, 1999), sustainability of fisheries in the North Atlantic (Alder et al., 2000), small pelagic fisheries (Pitcher and Preikshot, 2001), seven Tagus estuary fisheries (Baeta et al., 2005), 26 red sea fisheries (Tesfamichael and Pitcher, 2006), Basque trawl fisheries (Murillas et al., 2008) and ecosystem-based management (EBM) of fisheries in 33 countries (Pitcher et al., 2009). The marine protected areas evaluation model (MPAEM) (Alder et al., 2002) is another method which is based on the principle of Rapfish that explored the multidisciplinary approach of evaluating a single management intervention. Although both methods use multidimensional scaling as the analytical tool of the techniques, Rapfish evaluates the sustainability of fisheries while the MPAEM assesses the effectiveness of the management of existing MPAs. Six evaluation fields were defined in the MPAEM; namely, living (renewable) resources, non-living (non-renewable) resources, economic (market value), social, ecosystem functions and management (Alder et al., 2002). The model was tested in 20 MPAs in tropical, subtropical and temperate countries. While revisions of the model are imperative, it has shown potential for evaluating MPA management based on the evaluators' response. The most crucial part of the methods is the assignment of scores of the criteria per choice possibility which should be done by experts and well-experienced managers, researchers and stakeholders.

3.4 Concordance Analysis

Concordance analysis, also known as ELECTRE (eliminating and choice translating reality), is based on pairwise comparisons of several choice possibilities. It was originally introduced by Benayoun et al. in 1966 (Hwang and Yoon, 1981), and since then, the method has been modified in the works of Van Delft and Nijkamp (1977), Roy (1991), and Pomerol and Barba-Romero (2000). The method makes use of successive assessments producing ranking of choice possibilities rather than indicating the most preferred (Chung and Lee, 2009). It is an outranking technique where choice possibility that is dominated or outranked the most is eliminated. The important input is a set of weights, and the output is a set of outranking relationships (or partial orders). Scores that form the impact matrix are used to pairwise compare choice possibilities (Bodini and Giavelli, 1992).

Compared with the weighted sum model, concordance analysis is not based on utility theory. Utility functions are not used because of the substitutability property of the method where a bad outcome for a certain criterion can be compensated for by a good outcome for the other criteria (Hwang and Yoon, 1981). It is an evaluation method that can be used for cardinal data in the impact matrix and the weights vector. It examines both the degree to which the preference weights are in agreement with pairwise dominance relationships and the degree to which weighted evaluations differ from each other. The strength of this method is that it is able to underscore the two most important components of an evaluation matrix- the weighting and scores of the criteria. No assumption was made that the importance of the criteria is implicit in the evaluation scores; rather, weighting is explicitly considered. This method, however, assumes that the weighting and scores are derived quantitatively; although ordinal scores can also be used. Because the method clearly assumes that scores are cardinal in nature, the ordinal scores may be cardinalized to satisfy this assumption. This cardinalization of ordinal criteria or indicators is an indirect approach of transforming qualitative information into quantitative, and this is especially useful in mixed types of evaluation (Nijkamp and Vindigni, 1999). Then, all indicator scores are normalized to be comparable. One disadvantage of concordance analysis is the use of threshold values that can be selected arbitrarily. Because of this limitation, Van Delft and Nijkamp (1977) in Hwang and Yoon (1981) introduced the concept of net dominance relationships to complement the analysis. Two components of net dominance were presented – net concordance dominance value and net discordance dominance value. Elements in the concordance and discordance matrices are used to calculate the net concordance and net discordance values, respectively. The central point of the method is the determination of the concordance and discordance indices (Moriki and Karydis, 1994). Salient elements and detailed calculations of the method are found in Chung and Lee (2009), Hwang and Yoon (1981), and Nijkamp et al. (1990). Keeney and Wood (1977) remarked that with ELECTRE it is difficult to do sensitivity analyses to see just how much better one system is than another, but the latest version (i.e. ELECTRE IV) is able to incorporate sensitivity analysis. Moriki and Karydis (1994) found concordance analysis useful in identifying distinct areas of pollution according to nutrient characteristics of coastal waters.

3.5 Regime Method

The regime method is a generalized form of concordance analysis based on a pairwise comparison method whose point of departure is an ordinal evaluation matrix and an ordinal weight vector (Nijkamp et al., 1990; Nijkamp and Torrieri, 2000; Vreeker and Nijkamp, 2001; Hinloopen et al., 2004). Nijkamp and Torrieri (2000) discussed its operation and summarized as follows: A concordance index ($C_{ii'}$) is computed which is the sum of the weights of the criteria/indicators for which choice possibility i outperforms i′. The same procedure is also done when comparing choice possibility i′ with i ($C_{i'i}$). The difference between $C_{ii'}$ and $C_{i'i}$ yields the value of the index. Due to the ordinal nature of the information contained in the evaluation matrix, the magnitude of the difference between choice possibilities is disregarded. Unlike in concordance analysis, whose focal point is the concordance index, in the regime method, it is the sign of the difference for each pair of choice possibilities (Moriki and Karydis, 1994). The numerical size of the difference of the indicator for each pair of comparisons is ignored (Nijkamp and Torrieri, 2000). Pairwise comparisons are recorded in a table called a regime matrix composed of $Z\,(Z-1)$ comparisons where Z is the number of choice possibilities. Then, a net concordance dominance index ($\mu_{ii'}$) is calculated, where $\mu_{ii'} = c_{ii'} - c_{i'i}$. According to Nijkamp et al. (1990), the analysis aims to avoid the difficulty of having ambiguous results by partitioning the set of feasible weights, so that for each subset of weights a definite conclusion can be drawn about the sign of the index $\mu_{ii'}$. Because of the ordinal nature of $\mu_{ii'}$, its size is not the focus of the analysis but the sign. A positive (+) value of $\mu_{ii'}$ means that choice possibility i is preferred over i′ and negative (–) when the reverse is true (Moriki et al., 1996; Moriki and Karydis, 1994).

3.6 Mixed Data Evaluation (or Evamix)

A clear disadvantage of the qualitative methods (e.g., regime analysis) is that the available quantitative information is partially used (only the ordinal rank characteristics). Although the ordination technique can also be used to analyze mixed data, a better set of methods has been developed to deal with mixed qualitative-quantitative evaluation scores. Just like Concordance Analysis and the Regime Method, Mixed Data Evaluation, commonly called *Evamix*, is classified as a relative multi-criteria evaluation because there is no ideal value and the final appraisal score does not provide the absolute quality of a choice possibility; it only shows how different a certain choice possibility is with respect to the others (Voogd, 1982).

Evamix is based on the principle of analyzing information which has both quantitative and qualitative properties. It is also a generalized form of concordance analysis except that separate indices are constructed for qualitative (ordinal) criteria O and quantitative (cardinal) criteria C (Martel and Matarazzo, 2005). The difference between two choice possibilities can be expressed in a condensed way by means of two dominance measures, i.e. the ordinal dominance score ($o_{ii'}$) and cardinal dominance score.

Ordinal dominance score ($o_{ii'}$)

$$o_{ii'} = \left\{ \sum_{j \varepsilon O} [w_j \bullet \mathrm{sgn}(e_{ji} - e_{ji'})]^{\gamma} \right\}^{1/\gamma}$$

Cardinal dominance score ($c_{ii'}$)

$$c_{ii'} = \left\{ \sum_{j \varepsilon C} [w_j |e_{ji} - e_{ji'}|]^{\gamma} \right\}^{1/\gamma}$$

where

$e_{ji} =$ score of criterion j and choice possibility i

$w_j =$ weight attached to criterion j.

$$\mathrm{sgn}(e_{ji} - e_{ji'}) = +, \text{ if } e_{ji} > e_{ji'}$$
$$0, \text{ if } e_{ji} = e_{ji'}$$
$$-, \text{ if } e_{ji} < e_{ji'}$$

$\gamma =$ denotes an arbitrary scaling parameter which in this case will be assigned a value of 1.

In the determination of $o_{ii'}$ only the ordinal characteristics of e_{ij} variables are considered, while for $c_{ii'}$ the metric properties are also considered. Both measures are standardized to be comparable. Then, the total dominance score is computed as the weighted sum of the cardinal and ordinance dominance scores (Chung and Lee, 2009). The procedural framework of Evamix uses three approaches in order to come up with an appraisal score: the subtractive summation technique, subtracted shifted interval technique and additive interval technique. Detailed computation and application of the Evamix procedure are found among others in Voogd (1982, 1983), Nijkamp et al. (1990), Martel and Matarazzo (2005), Hajkowicz and Higgins (2008). However, Hinloopen et al. (2004) pointed out that the disadvantage of Evamix is that the separation of cardinal and ordinal information into two groups introduces a utility tree that is not based upon the preference structure of the decision-maker but on the level of information of the criteria. While published applications are rare compared with other multi-criteria analysis techniques (Hajkowicz and Higgins, 2008), the concepts and principles of Evamix are applicable to the structure of the problems in tropical fisheries management.

3.7 Summary of Multi-Criteria Aggregation Approaches

A vast and diverse number of aggregation techniques in multi-criteria analysis exist for different environmental decision-making problems. The choice of technique may actually depend on the nature and structure of the problem and information. It is important to make sure that the model is appropriate to the decision problem and complements the available data and not the other way around, i.e. forcing the data to fit the model. Thus, the limitations and assumptions of each model should be considered (see Table 1). The weighted sum model, analytic hierarchy process and Electre approaches rely on the provision of relative weightings of the criteria by stakeholders (Ascough II et al., 2008). When criteria or indicator scores and weights are both determined on a cardinal scale, then the simple weighted sum model is applied. This method, however, assumes that criteria/indicators are independent of each other, a condition unlikely to be satisfied in fisheries assessment as many criteria and indicators are interrelated. Non-metric multidimensional scaling is usually employed when standards or reference points

Table 1 Assumptions and limitations in using the multi-criteria evaluation methods

Evaluation method	Assumptions/Limitations
Analytic hierarchy process	– Highly dependent on the experience and knowledge of the evaluators/decision-makers
Weighted sum model	– All criteria are measured on cardinal scales and expressed in comparable units; weights are assigned per criterion; – Considers independence of criteria; – Assumes transitivity of preferences (additive utility); – Applicable to single-dimensional problem (all units are similar)
Ordination technique (e.g., multidimensional scaling)	– Useful when dealing with large amount of variables; – The goal is to quantify the relationships among interdependent variables
Concordance analysis	– Not based on utility theory; – Method can be used in cardinal data (impact matrix and weight vector); – Can be applied even if evaluation matrix is ordinal as long as the weight vector is cardinal
Regime method	– Useful when evaluation matrix and weight vector are both ordinals
Mixed data evaluation (Evamix)	– Deals with mixed qualitative-quantitative evaluation scores

for criteria or indicators are available. These reference points are considered 'ideal points' such that choice possibilities which highly deviate from the ideal points are located in space farther from the ideal points. In contrast, concordance analysis, though it does not assume independence of criteria and indicators, also requires both criteria scores and weights to be measured on a cardinal scale. Concordance analysis also provides that even if the evaluation matrix is ordinal, the concordance set can still be determined as long as the weight vector is cardinal. However, when both criteria scores and weights are ordinal, then the regime method is appropriate. The appraisal scores in concordance analysis and the regime method are derived through pairwise comparisons of choice possibilities.

This non-exhaustive literature review shows that among the aggregation approaches discussed here, the analytic hierarchy process and ordination technique had the highest number of applications in fisheries while none was recorded for concordance analysis, the regime method or Evamix.

Several studies not only make use of one method but a hybrid of methods. For example, Moriki and Karydis (1994) applied concordance analysis and the regime method to assess eutrophication levels in the coastal ecosystem. They found that both methods produced similar results in ranking alternative sampling locations. Moriki et al. (1996) realized that the regime method and multidimensional scaling [together with cluster analysis and principal component analysis] are applicable to a set of heterogenous data [measured on both ordinal and cardinal scales]. Ridgley and Rijsberman (1994) used the weighted sum model, concordance analysis and the analytic hierarchy process to elucidate the implications of using a set of impact assessments and preference evaluations. In order to analyze a set of criteria with varied units of measurements, Bodini and Giavelli (1992) applied weighted concordance analysis and Evamix. Scores for the concordance analysis were derived quantitatively then converted into qualitative data for the Evamix analysis. Other recent studies which combined multi-criteria evaluation models with other decision support tools include the following:

(a) Zhao and Yang (2009) combined a fuzzy assessment method and hierarchical analysis process to establish the integrative fuzzy hierarchical assessment model and applied it to the case study of Yong River in Ningbo City.

(b) Hossain et al. (2009), Buitrago et al. (2005), and Kitsiou et al. (2002) used multi-criteria evaluation in combination with geographic information systems (GIS) to identify suitable sites for carp farming, oyster aquaculture, and to rank coastal areas for development, respectively.

(c) Brody et al. (2006) applied multiple-criteria decision-making and spatial decision support systems in developing an evaluation approach that will identify locations for oil and gas activities in the coastal margin of Texas. The approach was also supported by the use of GIS.

(d) Adrianto et al. (2005) used a multi-criteria approach to assess the local sustainability of fisheries system in Yoron Island, Kagoshima. They developed a mixed-method approach which used a modified weighting scale of Saaty, a sustainability index of criteria (SIC), and a cognitive mapping technique.

(e) Leung et al. (2001) used a multi-objective programing model (decision maps, compromise programing and an I-O model) to examine tradeoffs between various conflicting objectives (regional employment, income and economic rent) of the North Norwegian cod fisheries in the Barents sea.

4 Application of Multi-Criteria Evaluation Methods to Tropical Fisheries Management

In order to examine the performance of the methods discussed in this paper and to determine their level of agreement in a tropical fisheries situation, a case study is presented. The aim of the case study is to determine the impacts of fisheries management strategies in a Philippine Bay.

4.1 Case Study: Evaluation of Fisheries Management Strategies in San Miguel Bay, Philippines

4.1.1 Study Area

San Miguel Bay is considered as one of the most productive fishing grounds in the Philippines (Mines, 1982). However, similar to any rich fishing area, it has been subjected to increased in-migration of people, intensive fishing, use of destructive fishing methods such as dynamite and cyanide, habitat destruction, and intense conflicts among various users, pollution, etc. The bay is situated at the southeastern part of Luzon between longitudes 122°59'E and 123°20'E and latitudes 13°40'N and 14°09'N (Cinco et al., 1995; Sia III and Luna, 1992). It is bordered by the provinces of Camarines Norte on the northwest and Camarines Sur on the south and east borders with a total area of about 1 115 km^2 and a coastline of 188 km (Garces et al., 1995). Seven coastal municipalities surround the bay; namely, Cabusao, Calabanga, Sipocot, Siruma, Tinambac, Basud and Mercedes.

The bay harbors a variety of finfishes and invertebrates. According to Pauly (1982), because of the bay's estuarine environment, 91 euryhaline marine species which tolerate freshwater and/or brackish water were found. He recorded that from 1868 to 1981, 188 species of fish belonging to 71 families were found in the bay. Such diverse fauna became the target of overexploitation as early as the 1970s, as noted by Simpson (1978) cited in Mines (1982). The fishers of the bay use a wide range of traditional fishing gears and methods and these are documented in Pauly et al. (1982) with three additional methods (i.e., the use of explosives in fishing, use of cyanide and other poisons in fishing, and the use of Danish seines (buli-buli)) reported by Silvestre and Cinco (1992). The bay is considered as a traditional ground for trawlers because of its wide area of soft bottoms (Silvestre and Cinco, 1992). During the 1970s, conflicts transpired between gillnetters and commercial trawlers, leading to the banning of commercial trawling within the bay's municipal waters (Mines, 1982).

Multi-disciplinary research in the 1980s already recommended management interventions. However, nothing was done then because of the government's thrust to modernize the fishing industry in the country. In the early part of the 1990s, the bay was revisited through the implementation of the fisheries sector program (FSP) of the Department of Agriculture. The program not only gathered technical information on the status of the bay's resources and users but also implemented resource management interventions that would lead to the bay's recovery. Aside from the programs and projects of the national government, there were also management interventions initiated and implemented by the local government.

4.1.2 Data Collection

The information required were collected through survey interviews and review of secondary sources including records from government offices and outputs of major fisheries research programs and projects in the Philippines. The literature guided the identification and definition of criteria and indicators.

4.1.3 Definition of Fisheries Management Strategies

A fisheries management strategy is composed of several management interventions. In this case study, a coastal municipality (composed of management interventions) represents a particular management strategy, as follows:

Basud- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment, marine fishery reserves/fish sanctuaries

Cabusao- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment

Calabanga- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment, marine fishery reserves/fish sanctuaries

Mercedes- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment, marine fishery reserves/fish sanctuaries

Sipocot- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, artificial reef deployment

Siruma- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment, marine fishery reserves/fish sanctuaries

Tinambac- ban on commercial fishing, gear and vessel restrictions, modification of licensing system, implementation of livelihood, mangrove reforestation, artificial reef deployment

4.1.4 Weights and Scores of Criteria and Indicators

There are 24 indicators which belong to five multiple criteria; namely, acceptability, biotic diversity, economic performance, enforceability and equity Table 2. The indicators can be categorized as *cardinal* or *ordinal* indicators. The cardinal weights of the criteria and indicators were elicited from the different sectors of coastal resource users in San Miguel Bay, specifically the institutionalized group known as fisheries and aquatic resource management councils (FARMCs) using the analytic hierarchy process. FARMCs are created in all coastal cities/municipalities of the country pursuant to the provisions of the Republic Act No. 8550, also known as the Philippine Fisheries Code of 1998.

The measures of the indicators are called indicator scores. They were derived from existing data (e.g. databank of the Philippine Fisheries Sector Program) and direct assessment by resource users. The indicator scores were determined at two time periods – before and after implementation of fisheries management strategies. The scores of the 12 ordinal indicators were determined from the members of the Barangay fisheries and aquatic resource management councils (BFARMCs) in each coastal municipality of San Miguel Bay. The 211 BFARMC representatives are knowledgeable, experienced and have lived in the coastal site for years, thus making them a good choice. About 98% of them attended school; 49% had an average of 6 years experience in coastal resource management; and 60% have lived in the municipality since birth. The scores of the 12 cardinal indicators were determined from secondary sources. Most secondary information available for San Miguel Bay is aggregated; thus, one of the challenges in measuring these indicators is the process of disaggregating them so that each municipality would have its own indicator information. All indicators are benefit indicators except for the number of commercial fishing boats and banned fishing gears; employment structure of small-scale fishers; and profit distribution among different fishing gears, which are considered cost indicators.

The 12 ordinal indicators specified the maximum and minimum values at +10 and –10, respectively. And as for cardinal indicators, the maximum and minimum values vary per indicator and would depend on the municipalities. The final output is an impact evaluation matrix showing the degree or level of change. The linear scale transformation was used to normalize the data. It considers maximum and minimum values and these values can be determined in two ways: (a) specify the possible maximum and minimum values of the indicators (values are constant); and (b) use the highest and lowest values from among the

Table 2 Measurement of criteria and indicators

Criteria	Indicators	Unit of measurement	Type of measurement
Acceptability	(a) Resource users' participation in the fisheries management process	0–10	Ordinal
	(b) Level of awareness of resource users in fisheries resource management	0–10	Ordinal
	(c) Number of fishers who belong to an organization	Total number	Cardinal
	(d) Change in the level of intra-sectoral conflicts	0–10	Ordinal
	(e) Change in the level of inter-sectoral conflicts	0–10	Ordinal
Biotic diversity	(f) Abundance of reef fishes	Total frequency of reef fish	Cardinal
	(g) Abundance of commercial fish catch fish catch	Weight in tons of commercial	Cardinal
	(h) Species richness of reef fish	Species richness (H')	Cardinal
	(i) Extent of mangrove areas	Hectares	Cardinal
	(j) Status of coral reef resources	Percentage live coral cover	Cardinal
Economic performance	(k) Number of commercial fishing boats and banned fishing gears	Total number	Cardinal
	(l) Fisherfolk gross revenue from fishing	Total value of fish catch (PhP)	Cardinal
	(m) Assessment of fisherfolk gross revenue from fishing	0–10	Ordinal
	(n) Employment structure of small-scale fisheries	Ratio of full-time to part-time fishers	Cardinal
Enforceability	(o) Presence of comprehensible laws and regulations related to management	0–10	Ordinal
	(p) Frequency of information dissemination about the management	0–10	Ordinal
	(q) Perception of suitability of enforcement techniques	0–10	Ordinal
	(r) Performance assessment of fisheries law enforcers	0–10	Ordinal
	(s) Financial support for fisheries law	Budget estimates (PhP) enforcement	Cardinal
	(t) Assessment of the allocated financial support for enforcement	0–10	Ordinal
Equity	(u) Profit distribution among different fishing gears	Percentage contribution of major fishing gears to the total gross profits	Cardinal
	(v) Amount of financial support for additional livelihood	Budget (PhP)	Cardinal
	(w) Assessment of the success of additional livelihood implemented	0–10	Ordinal
	(x) Inclusion of women in the management process	0–10	Ordinal

seven coastal municipalities being compared (values are variable). The normalized values of the indicators ranged between 0 and 1.0. A particular management strategy (represented by a municipality) is said to perform better with respect to a given indicator when its normalized value is highest or equal to 1.0. Table 3 shows the normalized impact evaluation matrix.

Table 3 Normalized impact evaluation matrix

Indicators	Basud	Cabusao	Calabanga	Mercedes	Sipocot	Siruma	Tinambac
(a) Resource users' participation in the management process	0.73	0.7	0.75	0.74	0.69	0.85	0.74
(b) Level of awareness of resource users in resource management	0.85	0.69	0.76	0.78	0.8	0.79	0.74
(c) Number of fishers who belong to an organization	0.01	0.01	0	0.01	0.01	1	0.04
(d) Change in the level of intra-sectoral conflicts	0.51	0.49	0.54	0.77	0.41	0.39	0.58
(e) Change in the level of inter-sectoral conflicts	0.38	0.3	0.39	0.58	0.3	0.22	0.35
(f) Abundance of reef fish				1		0	
(g) Abundance of commercial fish catch	0.58	0.23	0.68	0.75	0.38	0.42	0.76
(h) Species richness of reef fish				1		0	
(i) Extent of mangrove areas	0.07	0.27	0.72	0.07		0.03	0.33
(j) Status of coral reef resources				0.42		0.35	
(k) Number of commercial fishing boats & banned fishing gears	0.9	0.63	0.18	1	0.98	0	0.6
(l) Fisherfolk gross revenue from fishing	0.7	0.22	0.36	0.95	0.27	0.38	0.62
(m) Assessment of fisherfolk gross revenue from fishing	0.1	0.15	0.25	0.22	0.18	0.17	0.2
(l) Employment structure of small-scale fishers		0	1				
(n) Presence of comprehensible laws and regulations	0.62	0.64	0.75	0.75	0.65	0.78	0.7
(p) Frequency of information dissemination about the management	0.72	0.63	0.78	0.79	0.62	0.65	0.63
(q) Perception of the suitability of enforcement techniques	0.89	0.9	0.74	0.83	0.95	0.9	0.84
(r) Performance assessment of law enforcers	0.53	0.43	0.38	0.56	0.51	0.48	0.52
(s) Financial support for enforcement		0.29	0.9	0.78			0.64
(t) Assessment of the allocated financial support for enforcement	0.51	0.52	0.6	0.68	0.52	0.57	0.54
(u) Profit distribution among different fishing gears	0	0.97	0.88	0.79	1	0.8	0.94
(v) Financial support for additional livelihood implemented		0.7	1	0			0.22
(w) Assessment of the success of additional livelihood implemented	0.7	0.66	0.68	0.63	0.67	0.71	0.73
(x) Inclusion of women in the management process	0.76	0.72	0.67	0.72	0.76	0.76	0.7

4.1.5 Results

The impacts of fisheries management strategies (represented by the coastal municipalities) were evaluated using concordance analysis, the regime method, Evamix and non-metric multidimensional scaling (MDS). A final outranking of the municipalities was created. Although there may be variability in the ranking for some municipalities, Table 4 shows that the municipality of Mercedes has the optimal fisheries management strategy in all three methods. This means that the management strategy in Mercedes performs the best on the greatest number of indicators. The other municipalities that are not outranked are Siruma and Calabanga. The results show that all three municipalities have marine reserves/fish sanctuaries as a form of management interventions. These are not found in the municipalities of Cabusao, Sipocot and

Table 4 Results of concordance analysis, regime method and Evamix

Fisheries management strategies/Coastal municipalities	Concordance analysis	Regime	Evamix	
			Subtractive summation technique	Subtracted shifted interval technique
Basud	–2.28	+++	–0.43	–0.04
Cabusao	–0.94	++	–0.86	–1.18
Calabanga	2.75	++	0.29	0.25
Mercedes	4.90	++++++	0.57	0.41
Sipocot	–3.43	++++	–0.14	0.10
Siruma	0.34	+++	0.43	0.26
Tinambac	–1.34	+	0.14	0.19

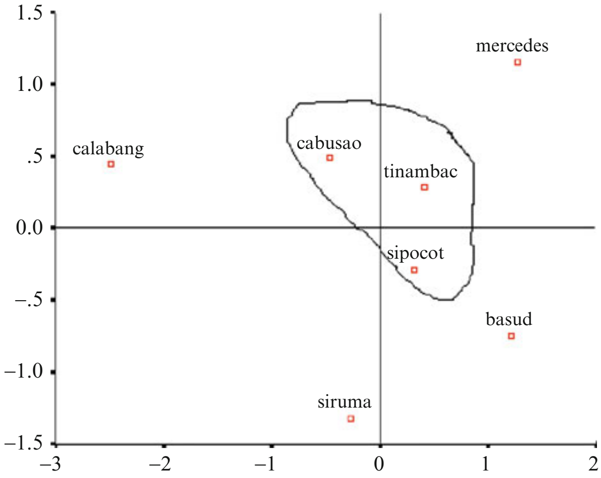

Fig. 1 Configuration derived in two dimensions

Tinambac. The results of concordance analysis, the regime method and Evamix are comparable, at least for the top three municipalities.

When the data were further examined using non-metric MDS through SPSS version 11, the results grouped the municipalities into a two-dimensional space. Figure 1 shows that Cabusao, Tinambac and Sipocot are closer to each other in the configuration. In order to determine which indicators influence such association, linear multiple regression was done. The indicator as a dependent variable was regressed over the independent variables which are the coordinates of the configuration. The indicators which provided significant regression results ($p < 0.05$) include extent of mangrove areas; number of commercial fishing boats & banned fishing gears; employment structure of small-scale fishers; and inclusion of women in the management process. The stress in the configuration is 2.8%, a somewhat good goodness of fit, and thus there is no need for higher dimensionality.

4.1.6 Discussion

The case study applied more than one technique in order to evaluate the impacts of fisheries management strategies. The cardinal weight vector was derived using the analytic hierarchy process while the aggregation procedure applied concordance analysis, the regime method and Evamix. Important issues have to be considered in choosing the appropriate combination of techniques in fisheries impact evaluation. Only a few studies have explored the use of the AHP in fisheries management, maybe because in many impact evaluation studies, coastal resource users' assessment is seldom considered in a formal evaluation process. Many researchers are quite apprehensive about integrating subjective judgments into the process of impact assessment because judgment varies among individuals and with time. This may be one major limitation in terms of integrating resource users into the evaluation process. However, due to the uncertainty and incompleteness of technical information, coastal resource users' experience and local knowledge are critical in providing balance to the entire evaluation process.

Most fisheries criteria (e.g., equity, economic efficiency, ecological sustainability) are broadly defined, and thus would require measurement indicators. Measurement of a criterion is rarely possible with only a single indicator because other indicators may also contribute to the measure of a criterion. There will be instances when the number of indicators is not equal for all criteria (e.g., five indicators for biological diversity versus four indicators for economic efficiency). If this is the case, Voogd (1983) suggested aggregating indicators per criterion. The simplest form of aggregation is to take the mean of the normalized indicators. Thus, the mean is now considered as the normalized criterion score. Another important consideration is the possibility of finding heterogenous (measured on varying scales) indicators in each criterion. For example, indicator scores for the criterion economic efficiency criterion may be a combination of ordinal, ratio or interval data. In the Evamix method, the criteria or indicators measured quantitatively are separated from those measured qualitatively. Thus, concern as to the validity of combining mixed information is resolved in this method. Its most important feature is a separate calculation of standard dominance scores for ordinal and cardinal criteria/indicators. When the impact evaluation matrix is composed of several criteria/indicators measured on varying scales, Evamix may be a useful aggregating approach. Since Evamix does not specify the minimum number of criteria/indicators to use, then it is possible to use all of them. Voogd (1983) suggested, however, that in any impact evaluation the number of criteria/indicators should be delimited to approximately seven or eight.

4.2 Extent of Application of Multi-Criteria Methods to Tropical Fisheries Management

If possible, the evaluation of the impacts of fisheries management should relate to the goals or objectives of fisheries. These objectives are most likely stated in a management plan, inferred from general policy statements or sometimes proposed by the authors (Chesson et al., 1999). The evaluation process, however, becomes very difficult if management objectives are not clearly specified (McAllister et al., 1999). The gaps, difficulties and challenges in developing and implementing fisheries management systems, recognized in an ICES Symposium held on November 16–19, 1998, set out important challenges in the development of a multi-criteria evaluation method for fisheries. The salient points raised during the Symposium, found in Stokes et al. (1999), are summarized as follows:

a) Evaluation and management of fisheries systems require sound decision-making despite uncertainty. Fisheries management systems must develop techniques to account for these uncertainties;
b) The slow pace of fisheries management to recognize the need to implement formal and rigorous decision-making. The existing case studies and techniques and approaches from the fields of operation research and management science may be useful;
c) The need for collaborative efforts among stakeholders (e.g., management agencies, scientists, industry, etc.) to articulate objectives for fisheries management to be consistent with international fishery conventions and standards;
d) There is a change in the governance for fisheries, from a single discipline to a multi-discipline approach involving the socio-economic context of the fishery; and
e) The relevance of formal evaluation and management procedures and system performance in providing information upon which credible management decisions can be based.

The outcome of the conference articulated the need for and lack of an evaluation instrument for fisheries management systems. The direction is no longer towards a single disciplinary approach but is multi-disciplinary with respect to what is to be evaluated and who will participate in the evaluation.

While multi-criteria methods have been increasing in application to natural resource management decision problems, their application to fisheries management appears to have been slow. The best-known examples are found in the reviews of Romero and Rehman (1987) and Mardle and Pascoe (1999). Romero and Rehman (1987) reviewed 13 case studies on how multi-criteria decision models [i.e., multi-objective programing (MOP), lexicographic goal programing (LGP), weighted goal programing (WGP) and multi-attribute utility function (MUF)] were applied in fisheries. They found that more than half focused on the establishment of the optimum structure of the fishing fleet in particular geographical areas. Mardle and Pascoe (1999) found 30 case studies relating to management schemes for a particular fishery; fleet structure analysis, requirements and composition; policy and development plans; catch quota policies; resource

Table 5 Multi-criteria evaluation method applications in fisheries

Model	Decision problem	Authors
Analytic hierarchy process (AHP)	Evaluate fisheries management options	Fernandes et al. (1999); Nielsen and Mathiesen (2006); Pascoe et al. (2009); Soma (2003)
	Measure importance of management objectives and performance indicators	Himes (2007); Mardle et al. (2004); Utne (2008); Whitmarsh and Palmieri (2009)
	Site selection	Ramos et al. (2006)
AHP + CADS_TOOL (cage aquaculture decision support tool)	Evaluate cage aquaculture site	Halide et al. (2009)
Multiple objective programing (MOP), compromise programing (CP), weighted goal programing (WGP)	Planning for regional aquaculture development	El-Gayar and Leung (2001)
Fuzzy logic model of expert knowledge	Evaluate ecosystem performance of the South African sardine *Sardinops sagax* fishery	Paterson et al. (2007)
MCA, modified AHP, sustainability index of criteria (SIC), cognitive mapping technique	Assess local sustainability of fisheries system in Yoron Island, Kagoshima	Adrianto et al. (2005)
Multi-objective programing model (decision maps, compromise programing, I-O model)	Tradeoffs between various conflicting objectives of the North Norwegian cod fisheries in the Barents sea	Leung et al. (2001)
Multi-criteria evaluation (MCE) + geographical information systems (GIS)	Identify suitable sites for aquaculture species carp farming in Chittagong, Bangladesh	Hossain et al. (2009); Buitrago et al. (2005)
Weighted sum model	Fisheries management evaluation	Chesson et al. (1999)
Ordination technique (marine protected area evaluation model (MPAEM) and rapid appraisal technique for fisheries (Rapfish))	Evaluate the status of fisheries globally	Alder et al. (2002); Alder et al. (2000); Baeta et al. (2005); Murillas et al. (2008); Pitcher and Preikshot (2001); Pitcher et al. (2009); Pitcher (1999); Preikshot et al. (1998); Tesfamichael and Pitcher (2006)

allocation; fishing effort input; optimal harvesting; commercial sampling schemes; fishing sites; and limiting entry. These case studies applied linear goal programing, generating methods, non-linear programing, multi-attribute utility theory, the analytic hierarchy process and multi-level Programing. Based on the present review of papers drawn from peer-reviewed scientific journals, there are 26 papers in fisheries that have applied multi-criteria evaluation methods during the past 10 years and these are summarized in Table 5.

5 Conclusion

This paper reveals the relatively few applications of multi-criteria decision models to tropical fisheries management. While they show potential because of their ability to: (a) provide a balance and integrate the various components of fisheries encompassing ecological, biological, social, economic and institutional objectives; (b) incorporate judgments of the various stakeholders in fisheries; (c) handle mixed information; and (d) allow interactions between the objective and subjective measures of the criteria and indicators, they are not extensively used. The main reason for such non-extensive application is because fisheries management evaluation has always been treated according to discipline, and not in an integrated manner. To measure the outcomes of management in a holistic or integrated manner is often problematic and therefore, only fragments of change are usually dealt with (Hanson, 2003) because there are restrictions and limitations in the measurement of change. Contributing to the limitations is the sense that each discipline (e.g., ecological/biological, social, political)

Table 6 Factors limiting the application of multi-criteria evaluation models in tropical fisheries management

Factors	Limitations
Data management	– too laborious unless a computer program is developed to make computation easier; – although not seen as a crucial factor, complete data for the indicators are ideal to increase the reliability of results; – supplied data may not contain all the information that the stakeholders/decision-makers conveyed
Acceptability of the approach	– new in the field of fisheries management, therefore the approach has to evolve into a more simplified one (i.e., the basic rule is that it should be easily understood)
Capability and willingness of stakeholders/resource users to apply the methods	– resource users with technical and analytical knowledge may be able to apply the multi-criteria approach but they would require extensive training; – sometimes resource users/stakeholders may not be willing to participate, especially when the process requires considerable time
Technical and financial constraints	– robustness and reliability of the method; – multi-criteria evaluation is quite expensive because it involves participation of different stakeholders and extensive data collection; thus, this should be part of a major coastal project or program

has its own assumptions that need to be satisfied. For example, Hruby (1999) indicated that statistical properties of decision-making models are different from those of ecological models, i.e., statistical approaches based on analysis of variance and normality of data are not appropriate when mixed qualitative and quantitative data or subjective judgments are incorporated.

Often, political leaders or decision-makers are faced with the problem of what to do with the types of information and how to integrate them so they can become usable for policy-making purposes. The issue of integration, though essential, is quite problematic in fisheries management because of alleged insufficiency in analytical methods. This may be resolved by further examining existing multi-criteria models in the field of operational research that may have potential use in evaluating tropical fisheries management. However, the limits of their application should also be recognized (see Table 6). In general, the choice of method would depend on whether it is appropriate for a particular decision-making problem or it is able to handle the situation correctly considering contextual, technical and political concerns. This is highly important in developing countries wherein cost associated with impact evaluation is a major constraint.

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Part III
Sociology and Economics

Farmer Responses to Climate Change and Sustainable Agriculture

Aysha Fleming and Frank Vanclay

Contents

Abstract Climate change is a major issue for agricultural sustainability, and changes in farming practices will be necessary both to reduce emissions and to adapt to a changing climate and to new social expectations. A complicating factor is that the processes of behaviour change are complex and can be slow to occur. Discourse analysis is useful in understanding how the discourses farmers are embedded in contribute to resistance to change. Discourses are particular ways of using language in particular situations. They have wide ranging effects on beliefs, values and behaviours. Interviews were conducted in 2008 with 63 respondents, including 22 apple growers, 29 dairy farmers and 12 agricultural consultants in Tasmania, Australia. In undertaking a discourse analysis of the transcripts of these interviews utilising N-Vivo, four specific discourses were identified as being important in shaping farmers' perspectives of climate change and sustainability: Money, Earth, Human responsibility and Questioning. Each discourse contributes to resistance to changing behaviour in particular ways. An understanding of these discourses offers a new approach to facilitating behaviour change.

Keywords Climate change • Agriculture • Sustainability • Discourse analysis • Discourses • Behaviour change • Resistance • Barriers to adoption • Tasmania

1 Introduction

'What's sustainable? You've got to look at our world as we know it. We're not in a sustainable position at the moment. That's why I say what is sustainable – I don't know.' Interviewee.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_15,

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Sustainability is a concept that is relatively easy to understand but difficult to define in practice. At a simplistic level, it means good environmental management and farming communities that are profitable and prosperous, or put another way, that are viable and vital. But what this means in practice and what specific management practices it infers is open to much debate (Vanclay and Lawrence, 1994, 1995). While sustainability has typically been conceived in terms of land degradation issues, over time sustainable agriculture has had to address a range of other issues including energy use, artificial inputs such as fertilizers and agricultural chemicals, and now climate change.

Climate change is increasingly acknowledged and accepted in science and political arenas. The emerging reality of climate change potentially increases the level of concern about issues of sustainability generally. Many agricultural industries will be impacted by climate change, and agriculture in Australia is projected to be especially affected (ABARE, 2007). Agriculture is likely to face considerable pressure to change its practices to become more sustainable for climate change, both in terms of mitigating emissions of carbon dioxide, nitrous oxide and methane, as well as adapting to the consequences of changes in climate already set in place. The consequences of climate change will be wide ranging, including physical changes to the landscape as well as expected changes in government requirements and market demands. There is a perceived urgency for agriculture to change to mitigate current greenhouse gas emissions and to prepare for future climate scenarios (e.g. CSIRO, 2008; Garnaut, 2008).

Despite the demand for action, few in the agricultural community are actually changing their farming practices because of climate change, at least in Australia (Milne et al., 2008). Potential reasons for inaction are diverse – doubt, complexity, avoidance, and the belief that others are responsible are just some of the likely responses that can limit action. Understanding the causes of this wide variety of responses from a social perspective can reveal new and potentially beneficial insights into behaviour (Potter and Oster, 2008). Therefore, more social research into understanding the factors that limit action for climate change, and how to overcome them, is needed (Trumbo and Shanahan, 2000; Moser and Dilling, 2007).

Climate change occurs on a global scale and over a period that is so long that many people find it difficult to relate to. Thus, the actions needed to influence the climate may be difficult to accept (Flannery, 2005). Further, people have different interactions with climate and understand it to be different things, varying from the expected weather, to the number of extreme events, to the level of carbon dioxide in the atmosphere. Climate change is socially constructed differently in different contexts by different social groups based on the different understandings (Pettenger, 2007). Advocates of change need to be aware of the perceptions of climate change that are prevalent in their particular target groups, and need to be able to deal with a diversity of responses, because the ways in which problems are framed and perceived are crucial factors in determining what solutions are seen to be possible (Irwin, 2001). Yet, when it comes to issues of sustainability and climate change, how farmers' social understandings are constructed is not well understood (Lowe et al., 2006). Knowledge about how social responses are generated in agriculture offers a new perspective in how to create alternative, more positive responses and hence facilitate change (Vanclay, 1992, 2004; Vanclay et al., 2009b).

This paper aims to contribute to the social understanding of climate change by demonstrating how the discourses that all social groups are embedded in are crucially linked to the behaviours that are able to be enacted in that group. Discourses fundamentally shape how all concepts are spoken about, and thought about, and thus able to be acted on (cf. Foucault, 1972). Therefore, discourses provide important knowledge of the forces that shape public perceptions and reveal the processes by which climate change is socially constructed. Awareness of discourse is a practical approach because, if the ways that environmental problems are socially constructed are better understood, a range of solutions can be tailored to fit. This paper aims to link the literature advocating action for climate change with the theory around discourses, and argues for a more socially aware understanding of agriculture and farming. It is hoped that this will offer a new, more successful method for promoting change in farming practices towards sustainable agriculture (see also Fleming and Vanclay, 2009a, b).

2 Discourses

The concept of discourse was introduced in the 1960s by the French philosopher, Michel Foucault. Foucault (1972) maintained that the way language is used has consequences for a whole range of things that go beyond the level of individuals or disciplines, to the very structures of society that shape and limit how people are able to speak, think, and act, and to the social structures that are developed accordingly. Discourses are particular ways of using language in particular situations. They exist at the level of a social group and serve to transmit and construct culture, pass on traditions, question the world, and are fundamentally important in the way we construct our identities.

'We speak with the voices of our communities and to the extent that we have individual voices, we fashion them out of the social voices already available to us, appropriating the words of others to speak a word of our own' (Lemke, 1995, 24–25).

Discourses shape the way we use language. Exposure to particular discourses over time creates perceptions about what is right and wrong, normal or abnormal, and thus significantly shapes how we think and act. This means that discourses are influential social constructions that should be examined, particularly in relation to behaviour change. While the study of discourse is a growing component of many academic fields including environmental studies (Harrison et al., 1996; Darier, 1999; Dryzek, 1997; Hajer, 1995; Carvalho, 2007; Kurz et al., 2005; Bäckstrand and Lövbrand, 2007), the potential for studying discourses as a practical approach to facilitating behaviour change is yet to be realized.

Discourses work toward normalisation and act in opposition to other, competing discourses, and therefore are dynamic and in a state of constant change (Wetherell et al., 2001). However, discourses can be actively changed because the constraints that discourses impose are open to challenge (Darier, 1999). Conflict between discourses creates a point of opportunity for developing new discourses. With an awareness of how a discourse is operating, it becomes possible to conceive how that discourse might be different, or to consider that a different discourse should be adopted, or even to create a new discourse altogether. Therefore, resistance in discourses is a site for agency and transformation. An analysis of resistance in discourses can offer useful insights into behaviour change and it can help to demonstrate the points where new discourses, with new actions and possibilities, might begin.

3 Methods

In 2008, interviews were conducted with 63 individuals from the apple, dairy and agricultural consultant communities in Tasmania, Australia. Sourced through personal contact with industry leaders, the interviews were conducted on-farm, or in-office, taking an average of 40 minutes. The interviews were conducted in a semi-structured manner suitable for recording people's feelings and perceptions. Indicative questions included: What do you think about climate change? What do you think are the causes? What are you doing about climate change on your farm? What do you think should be done by others? What is sustainable agriculture? How are you sustainable? What is your biggest risk? What do you think of the carbon pollution reduction scheme? What else would you like to see the government do?

The questions were intentionally open-ended to allow responses to be freely given and to limit the input (and potential bias) of the interviewer. The interviews were transcribed, entered on an N-Vivo database and examined using a constructivist grounded theory approach (Charmaz, 2006) and a discourse analysis methodology (Wetherell et al., 2001). The discourse analysis involved searching for themes relating to resistance to action for climate change. The analysis of these interviews and a corresponding literature survey are the basis of this paper.

4 Farmers' Thoughts About Climate Change

Most interviewees thought that climate change was occurring, and about half believed that they had made personal observations of landscape change, or change on their farm, that they linked to climate change. Yet despite accepting that climate change was occurring, only some thought it was anthropogenic in origin and many were undecided about the cause. Only some believed that it is necessary to mitigate the causes of climate change and/or are willing to do so. They believed that others had more responsibility to act. Some believed that the major responsibility for action lay with government, and that it is pointless for individuals to act without government leadership. Many were concerned about the inclusion of agriculture in the proposed carbon pollution reduction scheme. They were particularly distrustful of various intended government actions which they saw as penalizing farmers.

A small number were confused about the concepts of greenhouse gases, ozone depletion and weather, often conflating these with climate. This group was unable to name any greenhouse gases, and believed the hole in the ozone layer was related to climate change.

Most saw opportunities for Tasmania in a changed climate. They expected Tasmania to be sheltered from the worst effects of climate change and, therefore, compared to the rest of the nation and the rest of the world, to be relatively benefited. More broadly, however, many were worried about the future of the world for their children and grandchildren.

5 Four Discourses of Climate Change

Our analysis of the transcripts through subsequent levels from codes and categories to themes and discourses (Fleming and Vanclay, 2009b; Strauss and Corbin, 1998) resulted in four discourses being identified. Each of the four discourses shows a distinctly different way of talking and thinking about climate change. These are discussed below and, following these descriptions, are contrasted with each other in Table 1.

5.1 The Discourse of Money

In the discourse of money, nature is understood as a resource to be monitored, controlled and maximized, and sustainability is about continuing productivity and profit. The main concerns of climate change are about the ongoing viability of business and consistency of action at an international level. Climate change, in the form of a major disruption, is not a concern because physical changes are assumed to be gradual and are expected to be overcome through adaptation. Therefore, how people, governments and other countries act is more important than how the environment changes. There is concern about the equity of actions that might be taken to address climate change. Fairness would demand that everyone acts equally, yet this is not practical as everyone has different capacities and responsibilities for action. The government is not trusted to manage these different capacities and responsibilities effectively, especially in terms of the financial restrictions or taxes they will impose. Finally, individual actions are not accepted as important enough to be contributing to the problem and therefore it is pointless for individuals to act alone.

The discourse of money is characterized by a focus on maximizing profit, maintaining economic growth, supporting technological and financial market fixes, and orchestrating opportunities to maintain competitive advantage into the future. This discourse sees climate change as being able to be overcome through current cultural and social structures, namely market forces and innovation. As solutions to climate change are assumed to be possible, climate change is only perceived as a threat in terms of what decisions are made to inhibit production or penalise agriculture. The solutions are also assumed to be primarily technological and, therefore, agriculture as an industry needs to be especially careful to stay in line with what others do in order to remain competitive. Locally, Tasmania is seen to be particularly sheltered from major environmental impacts, and therefore there may be potential to benefit from climate change, if the only difference is a few degrees increase in temperature.

In the discourse of money, desirable farming practices centre on concerns about effectiveness, efficiency, market relationships, and industry positioning. Farmers' capacities to act for climate change are hindered because costs are perceived as being high, the effectiveness of action unproven, and action is seen as being detrimental to competitive ability. A wait and see approach is favoured, because how others act is crucial for positioning. These views inhibit those in this discourse from acting now, or in supporting Australia to act independently, despite arguments that the costs of inaction are likely to be greater than the costs of action (Garnaut, 2008), that Australia is likely to be particularly affected by climate change, that Australian farmers are particularly likely to suffer this burden financially (ABARE, 2007), and that adaptation is a finite process and unlikely to be sufficient to respond to climate change (Howden et al., 2009).

Table 1 Comparison of the four discourses that are influencing Tasmanian farmers

	Money	Earth	Human responsibility	Questioning
What is nature?	A resource to be monitored, controlled and maximized	A gift to be left untouched and respected	A system that is fragile and needs protection	A system that is infinitely complex, but potentially knowable
What is sustainability?	Continuing productivity and profit	Preserving the purity of nature	Protecting nature for future generations	A better future made possible by ongoing technological and scientific progress
What is climate?	Expected conditions for production	Natural cycles	The experience of weather over time	A scientific understanding based on models and historical records
What is drought?	Exceptional circumstances, business risk	Unpredictable natural event	Reason for better land management	A separate, but compounding issue; climate change is not climate variability
What is climate change?	Future business risk, unfair financial cost in relation to mitigation and adaptation	Natural event	A justification for calling for major change	A complex process, which appears to be taking place. More knowledge needed
What is the cause of climate change?	Multiple causes. Anthropogenic causes often seen to be accelerating a natural trend	Natural process	Anthropogenic is typically accepted but not a defining feature of this discourse	Anthropogenic causes are cautiously accepted, with the complexity of multiple causes emphasised
What is climate change in Tasmania?	Sheltered from extremes, less affected than elsewhere	No different to anywhere else	An opportunity to lead and set an example	Uncertain; current information is inadequate and more detail is needed
What is farming?	A way for earning financial reward through hard work	A life close to nature	Providing an essential service	An application of skill, knowledge and technology
Who has responsibility for solutions to climate change?	Government, corporations, industry bodies, consumers	Gaia, God, the cosmos	All people equally	Future researchers will have a major role when we learn more; current research contributes to the knowledge base
How is science perceived?	Potentially useful, can be complex and may need translation to be practical	Often irrelevant	Science has a role in creating solutions	Science is useful, and skepticism is an important scientific trait
How are global concerns e.g. terrorism, financial crisis, perceived?	Increase business impacts and opportunities	Shows failure to respect nature	Shows need for social transformation	Adds another layer of complexity

5.2 The Discourse of Earth

This discourse focuses on the earth and has as a key feature, ‘Mother Nature’, a divine metaphorical personification that embodies creative and restorative power. While there is concern for the negative effects on the environment that humans are causing, there is a belief that these are sufficiently insignificant to have any real effect and that the world will persist relatively unchanged. In other words, the earth has the power to endure. There is a strong sense that humans do not have dominion over the earth but that the earth has dominion over humans. There is also the sense that the earth is vast and beyond human comprehension.

In the discourse of earth there is a sense that because climate change is controlled by an external force, it might be part of a divine purpose and therefore not of any great concern. In this view, climate change will potentially provoke natural evolution of humans and other species, or humans may be wiped out but the earth will endure albeit in a different state, as occurred with the extinction of the dinosaurs and other major events.

Desirable farming practices in this discourse centre around respect for nature. Farmer's capacities to act for climate change are hindered because humans are not perceived to be able to influence the state of the planet, and the earth has a considerable capacity to withstand change, or homeostatic capacity. Climate change is seen as one aspect of 'the category of environmental insults deriving from industrial society' (Bulkeley, 2000, 319). Equally important problems are degradation, pollution, extinction and the use of environmentally-unfriendly products. While all of these problems are undesirable and even immoral, they are nevertheless not actually able to affect the earth's equilibrium. There is sufficient mystery and trust in the incomprehensible workings of the planet that human attempts to direct the future are naïve and inconsequential.

5.3 The Discourse of Human Responsibility

This discourse demonstrates a fundamental difference from the two discourses already discussed because, instead of financial or environmental concerns, it focuses on social action. The discourse of human responsibility is about the necessity of acting for climate change and working together to communicate, collaborate and participate. This discourse has a strong sense of agency and responsibility for action. While it is positive and focused on social action, it is held back by a lack of clear direction in what actions to take.

This discourse is primarily about achieving more public engagement with climate change and about creating more equitable and desirable government policies and even a better world order. However, this is a grand plan and climate change can get lost amidst the focus on transformations of social structures that are demanded. Society is seen to be the problem in this discourse and the tools to change society are identified as being people collaborating and working together, demanding what needs to occur using the power of democracy, yet this process is not actually achieving the major changes required to allow all the actions that are yearned for.

Desirable farming practices in this discourse centre on concerns over the capacity of farmers to meet their responsibility to feed the world's increasing population. The ability of farmers to act for climate change is hindered because actions are inhibited by the need to continually increase output and because of the limitations of current infrastructure, social systems and social norms. Changing consumption patterns and environmental values are seen as being essential in this discourse, but this requires system level transformation, which at this stage is still only being talked about and not yet incorporated into action (see also Harrison et al., 1996).

5.4 The Discourse of Questioning

The discourse of questioning is created through the interplay of the hegemonic power science has, which is propagated by the media, but moderated by public opinion. This discourse is focused on issues of fact, truth, knowledge, information and trust. It emphasises aspects of uncertainty or incomplete knowledge, and the complexity of the issue. Vested interests are seen as being likely to exaggerate climate change, and while there is probably some element of truth in how humans cause negative environmental impacts, the extent to which this occurs and how these are best addressed is still unknown and unable to be discerned until the emotional hype has subsided.

The discourse of questioning has doubt and the quest for more knowledge as its key features. Controversial or emotional information is likely to be distrusted and rejected. In this discourse, nothing about climate change is black and white, and everything is arguable and contested. Information is likely to have been found too confusing, too complex, too distant, too tainted, or too difficult to understand. In this discourse, further attempts to engage with finding more information, talking about the issue, or thinking reflexively about it are avoided until such a time as the answer is made sufficiently clear and legitimated by more scientific endeavor. This involves waiting for others to synthesise the information and come up with a position that is generally accepted and supported. At the moment, the most easily adopted positions are either total rejection, or sitting on the fence.

Desirable farming practices in this discourse centre on specialist knowledge and skillful application of technology. Farmers' capacities to act for climate change are hindered because climate change is too uncertain to be actionable, too controversial to be entirely true, and the required changes too radical to be trusted. In this discourse, trust is a particularly important issue, especially trust in whose knowledge and whether that knowledge relates to personal contexts (see also Carolan, 2006).

6 Discussion

The general perceptions about climate change of Tasmanian farmers as identified in this research are generally consistent with those found by other studies into public responses to climate change (e.g. Moser and Dilling, 2007; Milne et al., 2008; Lorenzoni et al., 2007; Doulton and Brown, 2009). However, very little research has examined farmers' discourses surrounding climate change for us to compare with our results. This paper seeks to emphasise that responses to climate change are a product of social, rather than individual processes, and therefore more research that takes account of the operation of discourses should be undertaken. We believe that many of the root causes for inaction in the face of climate change are social and discursive. Only a wider account of these social discourses can explain behaviour and thus, resistance.

Many studies of public responses to climate change choose to focus on problems with information, or individual psychology, which are often named the 'barriers' to action (e.g. Bord et al., 2000; Stamm et al., 2000; Stoll-Kleeman et al., 2001; Leiserowitz, 2007; Bostrom and Lashof, 2007; Kollmuss and Agyeman, 2002). While this literature offers important insights into understandings of climate change at an individual level, we believe that there is no such thing as a barrier to change, only legitimate reasons not to change (Vanclay, 1992, 2004). The processes by which these reasons are deemed legitimate or otherwise can be made apparent through analysis of discourses. We argue that a social focus on behaviour change is more useful in relation to facilitating action for climate change than a focus on the specific barriers to change, because it is only through a social approach that the 'practical and discursive constraints of context, both locally and nationally' (Harrison et al., 1996, 215) can be properly addressed.

The many explanations as to why people do not change behaviour that are discussed in the literature can be generally categorized into groupings around conceptual, practical and information barriers. These are summarised below in order to restate our belief that it is not as useful to find out the barriers to action as it is to properly understand the legitimate reasons for inaction. By describing the large number of individual barriers below, we hope to show how overwhelming change can be, if each barrier is to be addressed individually. Instead, we advocate a focus on discourse that offers a more holistic and thus more effective way of understanding and addressing inaction and resistance.

6.1 Conceptual Barriers to Climate Change Action

This grouping comprises the many arguments given as to why people can not comprehend climate change due to its complexity. In these arguments, climate is perceived to be a complex science created by multiple interactions between the oceans, land masses and the atmosphere. There are complex effects of climate on the environment, including, but not limited to, the weather. These effects occur over long time scales of years, decades and centuries, so cause and effect connections are difficult to establish and cycles are not often experienced by individuals and/or not accurately remembered. Climate systems and climate cycles are created on a scale that make it seem too distant and too abstract, or too vast and unalterable (Moser and Dilling, 2004). Public understanding of climate change is reliant on science to discover, monitor and potentially solve the problem (Demeritt, 1998), and the media is seen as the conduit for this information transfer from science to the public.

Conflicts between science and the media and the public about truth, values, knowledge, power, responsibility for action, and agency have been the focus of many studies about climate change and environmental sustainability (Potter and Oster, 2008; Boykoff, 2008;

Carvalho, 2007; Lorenzoni et al., 2007; Kurz et al., 2005; Sarewitz 2004; Jasanoff, 2004; Clover, 2003; Princen et al., 2002; Dryzek, 1997; Hajer, 1995; Ungar, 1992; Litfin, 1994). While some of this research does use discourse, it is our belief that this has so far been insufficient.

6.2 Practical Barriers

Another category of barriers to change can be conceived as relating to the practical dimensions of the posited solutions. In the conventional diffusion of innovations literature, these include available time, money and social infrastructure, as well as considerations of convenience, ease, flexibility, divisibility, referring to the breakdown of a change in behaviour into the required steps (Rogers, 1983; Vanclay, 1992, 2004; McKenzie-Mohr and Smith, 1999; Pannell et al., 2006). The individual states of motivation, risk, resources, support, individual character traits and skills also play a part. In relation to climate change, Moser and Dilling (2007) have outlined similar barriers to action.

Some scholars advocate a different, more social level approach (Potter and Oster, 2008; Lorenzoni et al., 2007) to change the social structures that limit these behaviours, and to create social mores to normalise the desired behaviours (Griskevicius et al., 2008). We see these as being complementary aspects of the broader concepts of changing discourse. Discourses influence the language used to talk about issues, the types of institutions needed in society and the way these institutions are used (Phillips and Jorgensen, 2002). Therefore, discourses are fundamental in understanding behaviour.

6.3 Information Barriers

The final category of barriers is problems of information and its communication. While the critique of the view that the provision of information alone does lead to behaviour change has been well-established since the rise of what is called the 'information deficit model' (see Potter and Oster for a review) some, for example, Sturgis and Allum (2004) still believe that the provision of information will change behaviour. They are not alone in this view as it is widely shared by many scientists.

In the view of those who think information will solve the problem, they see 'information' in simplistic, 'objective' terms, and not in its social context. The barriers to change that these people consider are the lack of information, the lack of access to information, problems in the targeting of information, and the lack of ability of people in understanding the information. There is the view, too, that in a society which is potentially overloaded with information, many people lack the ability to find the information they need, or lack the necessary tools and intellectual resources to evaluate the competing information that is on offer.

In our view, behaviours are embedded within different contexts and situations, and are linked to institutions, social networks and the contexts of place. These must all be considered and adapted or transformed with the adoption of even seemingly simple behaviour changes. We consider that 'problems' or 'barriers' should not be addressed individually, but should be included in the overall account of the reasons for behaviour which we see as being discursive. Changing behaviour on an individual scale is slow and likely to be resisted at many points: 'behavior change is not a one-by-one persuasion task, but a social challenge' (Tribbia, 2007, 248). This is a challenge achievable through working to change the discourses that currently limit behaviour.

7 Opportunities for Change

From the four discourses we have found operating in agricultural circles in Tasmania, there are points of opportunity for change. By understanding the way issues are framed and understood in particular discourses, the ways forward can be framed in a corresponding fashion. This can minimize misunderstandings and tap into existing motivations for action. In this way, an understanding of which discourse is operating in which context can provide a social insight into farmers' characters and positions.

The discourse of money sees resistance to changing practices for climate change as the best way to avoid costs, to focus on other more important problems and to allow time to learn more and therefore increase the likelihood of being more competitive when implementing actions later. However, those who are influenced by this discourse can be motivated to support action by stimulating their need to maintain competitiveness and to be involved with climate solutions developed by industry. To increase the desire for, and adoption of, actions in this discourse, the potential financial benefits and future costs need to be clearly identified. Information about how others are responding, especially at government, industry and consumer levels, is especially important. In this discourse, emphasis on the human responsibility for the environment and emotionally-laden tactics are unlikely to be successful unless tightly connected to issues of financial concern. Therefore, explicitly highlighting the connections between financial problems and climate change, for example reducing input costs by addressing climate change, is of more use than describing other general impacts, no matter how catastrophic, that have financial consequences merely implied.

The discourse of earth resists action for climate change because it sees any action humans can take as being too small and/or irrelevant to make a difference. This discourse sees that other forces are in ultimate control of the earth, and humans cannot affect the outcome of the future path of the planet, or the future of the species. However, this discourse supports action by wanting to promote the value, goodness and wonder of nature and caring for it in a way that is properly respectful and grateful. The best way forward for increasing action in this discourse is by highlighting the multiple environmental benefits of climate change action and emphasising the cultural shift toward sustainability, that is, respect for the earth, that is required. Promotion of the anthropogenic origins of climate change will not be an effective, or necessary, way to promote action in this discourse.

The discourse of human responsibility resists action for climate change because there is confusion about what to do and how to practically implement actions, especially because of concerns about major barriers that are seen to be impossible to fix, like social and governmental structures. However, there is a great deal of willingness to act and therefore significant untapped potential for action is demonstrated in this discourse. If given the resources, those in this discourse are likely to take action up quickly and this is also the most likely discourse to create new forms of action. In this discourse, emphasising the practical information about climate change actions, particularly those involving community or group interaction is the best way forward. Highlighting the reasons for action, or the urgency of action, will not be effective in this discourse as the desire to act is already present and further emphasis can overwhelm. Instead, demonstration of practical ways forward and providing social contacts and the framework for networks will be the most beneficial.

The discourse of questioning resists action for climate change because of distrust or dissatisfaction with information or perceptions of the inability of people to understand or relate to information. This discourse advocates avoidance, denial and delay. However, it has the potential to support action because it accepts that some knowledge is already available for how to proceed. It trusts scientific pursuit to eventually provide the answers and accepts that progress is achieved incrementally, so some action is advisable now. In this discourse, information about the potential benefits of actions needs to be highlighted, rather than information aimed at overcoming scepticism about the causes. In effect, showing this discourse that they too have a vested interest in acting on climate change, and a role to play in producing relevant, ‘on-the-ground’ knowledge about action for climate change, is the best way forward.

8 Conclusion

Each of the discourses identified through our interviews with members of the Tasmanian agricultural community shows a distinctly different way of framing the issues of climate change and sustainability. These are: as an issue of business viability; as an environmental concern; as a call for social action; or as a problem of trust and information. Knowledge of which of these discourses is at work within different social settings allows for different approaches for facilitating behaviour change to be implemented. Each of the discourses provides points of opportunity for action by focusing on the particular aspects that are central to the discourse and that would therefore motivate change by highlighting issues of financial benefit, environmentalism, social action or trusted knowledge respectively.

Climate change means that sustainability is more important than ever, but still understood in a diverse range of ways. Australian agriculture is going to be under significant pressure to implement a wide range of changes in practice for adaptation, mitigation and social responsibility. A social consideration of the agricultural community's behaviours and perspectives is now even more important to consider. More effective approaches to understanding behaviour change are needed because of the urgency of action for climate change. The diversity of social understandings and responses to climate change and sustainability mean that a new method for facilitating change is required. In order to cope with diversity, this new method needs to be focused on a social level of change in order to have a meaningful and significant effect. Awareness of discourse is the most appropriate tool for achieving this level of change.

Acknowledgements Aysha Fleming is supported by a Top-Up Scholarship from CSIRO's Climate Adaptation Flagship and from the Climate Futures for Tasmania project. Ethics approval for the project was granted by Tasmanian Social Sciences Human Research Ethics Committee (H10168). Sincere thanks to the many farmers and agricultural industry spokespersons who agreed to be interviewed, to Claire Hiller for help with the analysis and to Shaun Lisson for commenting on drafts.

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The Use of the *Marasha* Ard Plough for Conservation Agriculture in Northern Ethiopia

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Ken Sayre, and Jozef Deckers

Contents

Abstract Indigenous tillage systems are often undervalued in conservation agriculture (CA). In Ethiopia, since the 1970s there have been several attempts to develop and implement often major modifications to the *marasha*, the traditional ox-drawn ard plough, with the main aim of creating various types of surface depressions. The establishment of furrows and ridges increases soil moisture and grain yield and reduces soil loss. Dissemination of the modified tools, however, remains limited. Recent tendencies are towards testing relatively simple conservation agriculture tools. Major challenges remain, however; the need for capacity building and problems in marketing the tools. From experimental plots, often worked with exotic tools, there is a long road to real adoption by farmers. Rather than developing yet another CA tool, we investigate whether CA-based resource-conserving technologies might be achieved successfully with simple changes to the use of the *marasha*. On-farm observations on traditional conservation techniques were carried out throughout the northern Ethiopian highlands, and experiments were conducted involving resource-conserving technologies. Farmers traditionally use the *marasha* ard plough for various types of in situ soil and water conservation by creating surface depressions, either at the moment of sowing (*terwah*, *derdero*) or after crop emergence (*shilshalo*). Building upon this indigenous knowledge, we further developed resource-conserving technologies into a system named *derdero*+, whereby the traditional ard plough was found suitable for a "bed-and-furrow" system. From the socio-economic point of view, implementation of permanent beds and retention of stubble leads to decreased oxen (and straw) requirements, but also to an increased need for weeding in the first years.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_16,

from Nyssen et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010014

To overcome that problem, we introduced glyphosate herbicide into the tillage system. The decreased runoff (−51%) and soil loss (−81%) allow protection of the downslope areas from flooding, but soil nutrient build-up and soil structure improvement are slow processes, and hence the full benefit of the permanent bed system can only be expected after some years. Overall, this type of resource-conserving technology can be part of the ongoing intensification process which includes physical soil and water conservation, slope reforestation and irrigation development. It has, however, its own niche: the cropped land sensu stricto, i.e. the most important part of the land, both for the farmer and for a nation that is striving for long-term food security.

Keywords Animal traction • Ard plough • Conservation tillage • Conservation agriculture • *Derdero+* • *Marasha* • Indigenous knowledge • Permanent beds • Raised beds • Resource-conserving technology • Soil and water conservation

1 Introduction

In the northern Ethiopian highlands, smallholder agriculture is largely dominant and tillage takes place with a pair of oxen pulling the ard plough or *marasha* (Simoons, 1958; Nyssen et al., 2000; Solomon et al., 2006) (Fig. 1), a tool similar to that of the eastern Mediterranean and large parts of the Arab world (Audebeau and Mosséri, 1916; Varisco, 1982, 2004). The main conservation approach involves terracing by stone bunds, which is very efficient for soil and water retention within the plot. Crop yield and farmers' income increased on average by 10% (Desta et al., 2005). However, the effect of the stone bunds on crop production is concentrated along the bunds (Fig. 2) (Vancampenhout et al., 2006). Agricultural land surface management, and particularly the creation of minor closed depressions, is needed to have a conservation effect over the whole farmland. This fits into a conservation agriculture (CA) approach to enhance production, preserve natural resources and reduce poverty (Aune et al., 2001), through (a) in situ conservation of moisture, reducing runoff and evaporation; (b) reductions in erosion within farmland; (c) crop management practices that meet the above goals and increase labour efficiency and reduce the oxen requirement, leading to reductions in the demand for crop residues as livestock feed, and (d) enhanced soil fertility, to make efficient use of increased retention of rain.

Conservation agriculture (CA) is based on 3 principles (Bradford and Peterson, 2000; Verhulst et al., 2010): (1) reduction in tillage – the objective is to achieve zero tillage, but the system may involve controlled tillage seeding systems that normally do not disturb more than 20–25% of the soil surface; (2) retention of adequate levels of crop residues and surface cover on the soil surface to protect it from water and wind erosion, to reduce water runoff and evaporation, to improve water productivity and to enhance soil physical, chemical and biological properties associated

Fig. 1 The first ever photograph of an Ethiopian farmer with his oxen and *marasha*, near Senkata (Tigray) in March 1868 (© Royal Engineers of the British Army, reprinted with permission of the King's Own Museum, Lancaster, UK) and detail of the metal tine with wooden "ears" (Photo Leigh Vial)

Fig. 2 A sorghum and maize farm in the May Zegzeg catchment, Tigray, October 2005. The implementation of stone bunds for soil and water conservation has led to strongly improved yields in the vicinity of the bunds (arrow shows strong maize growth). In the foreground, *terwah* furrows remain visible in the harvested land

with long-term sustainable productivity, and (3) use of crop rotations for the beneficial effects of some crops on soil conditions and on the productivity of subsequent crops, and to mitigate possible weed, disease and pest problems related to crop residue retention. These CA principles are applicable to a wide range of crop production systems from low-yielding, dry, rainfed conditions to high-yielding, irrigated conditions. However, the techniques to apply the principles of CA will be very different in different situations, and will vary with biophysical and system management conditions and farmer circumstances (Verhulst et al., 2010). Applying CA also means altering generations of traditional farming practices and implement use. As such, the movement towards CA-based technologies is normally comprised of a sequence of step-wise changes in cropping system management to improve productivity and sustainability, implementing resource-conserving technologies in line with the CA principles.

Particularly, bed planting provides an opportunity to reduce compaction by favouring traffic to the furrow bottoms. The next logical step to increase the sustainability of beds is to make them permanent, reducing or avoiding tillage and retaining and distributing crop residues on the surface, reshaping the beds only as needed (Sayre, 2004). Introducing permanent beds, reduced tillage and residue preservation is expected to conserve water and as such will allow sowing early and cultivating crops and varieties with a longer growing period.

In an experimental setup with conservation agriculture-based resource conservation technologies in the North Wollo region of Ethiopia, McHugh et al. (2007) found that ridges significantly increased soil moisture and grain yield and reduced soil loss, whereas no-till minimised soil loss, but reduced yield. Yet, from experimental plots, often worked with exotic tools, there is a long road to real adoption by farmers. Leeuwis and Van den Ban (2004) demonstrated that innovations in farming may only spread spontaneously when they are embedded within the local socio-economic and cultural context. Rajaram et al. (1991) further mention that indigenous tillage systems are often undervalued. Hence, rather than developing yet another CA tool, we investigate here whether CA-based resource-conserving technologies might be achieved successfully with simple changes to the use of the *marasha*. Simplicity is a key concept for CA-based resource-conserving technologies. Hence our working hypothesis has been that further adaptations of the *marasha* can and will be made, but wholesale changes are probably unnecessary to the ox-drawn implement. After all, the farmers of Ethiopia have been adjusting this tool for thousands of years so as to meet their requirements under Ethiopian soil conditions.

We have chosen a step-by-step, empirical approach that involves intimate farmer participation throughout the initial research on possible strategies/technologies, the testing and modification of the most relevant possibilities, and finally, the extension of the final products.

2 Tillage in the Agricultural System of the Northern Ethiopian Highlands

The tropical highlands of Ethiopia are densely populated and have been cropped intensively for centuries (Nyssen et al., 2004). Agricultural sustainability problems related to soil erosion and fertility decline have arisen throughout this agro-ecological zone. The agricultural system is under stress due to the shrinking cultivated area per household, reduced fodder availability and land degradation (Aune et al., 2001).

This study concerns the Tigray and northern Amhara (Lasta) regions in the northern Ethiopian

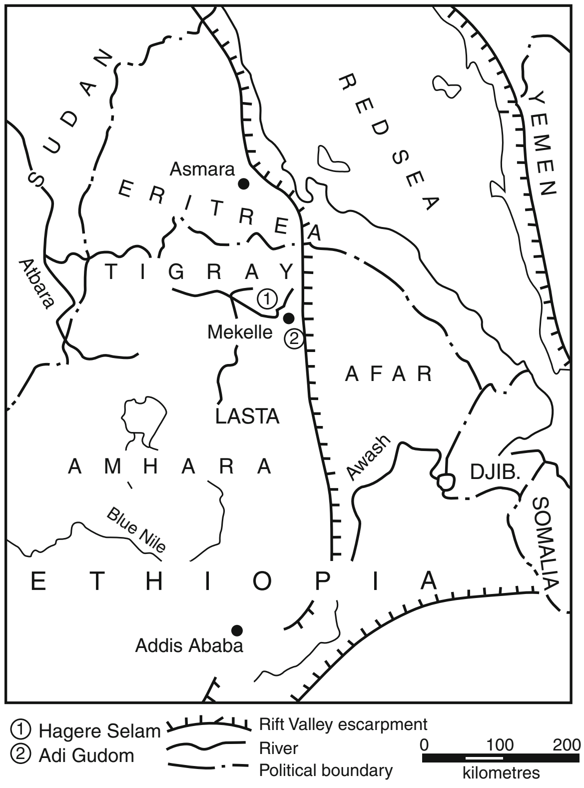

Fig. 3 The target area

highlands (Fig. 3). The main rainy season (>80% of total rainfall) extends from June to September but is sometimes preceded by three months of dispersed less intense rains. Average yearly precipitation is 500–800 mm. Agriculture in the northern Ethiopian highlands consists exclusively of smallholder farming activities, with landholdings approximately equal in size. Each household crops two or three parcels of land, with a combined area of between 0.5 and 1 ha (Nyssen et al., 2008). The agricultural system in the northern Ethiopian highlands has been characterised as a “grain-plough complex” with annually cropped fields. The main crops are barley (*Hordeum vulgare*), wheat (*Triticum* sp.), sorghum (*Sorghum bicolor*), maize (*Zea mays*) and tef (*Eragrostis tef*), an endemic cereal crop. Different species of pulses are also an important part of the crop rotation (Nyssen et al., 2008). As rainfall is inadequate and unpredictable, crop production is threatened by chronic soil moisture stress. Precipitation is often intense, leading to high runoff and temporary waterlogging. Cereal grain yields are low (<2 t ha^{-1}). Moreover, crops are often weedy and nitrogen-deficient, soil structure is poor, and soil erosion is widespread (Tadesse et al., 1996; Asefa et al., 2004; Spaccini et al., 2006; Nyssen et al., 2008).

Soil tillage with the *marasha* is commonly used to prepare flat seedbeds. The tool, pulled by a pair of oxen, breaks, but does not turn, the soil to provide weed-free seedbeds at sowing and to enhance infiltration (Melesse et al., 2008). The farmers adjust the tillage depth by modifying the length of the ploughshare and its angle and by the downward pressure on the handle. The amount of land a pair of oxen can plough in one day varies with oxen strength and with soil type and is estimated at about 0.25 ha day^{-1}. Multiple tillage operations are carried out in order to prepare a fine seedbed, particularly for tef, with 3–5 operations. This leads to poor physical soil properties, as expressed by very hard topsoils and difficult tillage conditions when the next crop needs to be sown. After harvesting, stubble grazing is widespread, followed by several tillage operations.

Oxen are of vital importance for assuring livelihood security as they can be sold when the household experiences a stress situation such as drought. There is also a high social prestige associated with ox ownership (Aune et al., 2001). The productivity of the Ethiopian livestock system is declining as an increasing part of the biomass resources are consumed by oxen and other livestock. A change in the livestock population and productivity will not occur unless the tillage system is modified and overall crop productivity improved.

Two sites, both on Vertisols, were selected for experimental studies on land preparation techniques (Fig. 3): (1) the May Zegzeg catchment near Hagere Selam (2600 m a.s.l.), where catchment management including zero grazing has been implemented since January 2004 (Nyssen et al., 2009) and (2) the Gum Salasa area near Adi Gudom (2090 m a.s.l.) (Tewodros et al., 2009; Tesfay et al., 2010; Tigist et al., 2010). Vertisols are challenging for land preparation: they are very hard when dry, and in the rainy season, when their upper part gets moistened, clay swelling leads to soil sealing, poor infiltration and waterlogging. Due to its resistance to waterlogging, tef is a preferred crop on this soil type, but the repeated ploughing that is done to prepare its fine seedbed leads to collapse of the soil structure and hence leaves behind a compacted topsoil.

3 Introduction of Implements

In the past, researchers and organisations repeatedly attempted to replace or modify the *marasha* plough, for CA operations among others. The typical trajectory of adoption is similar to that of the steel mouldboard ploughs introduced by the Italians in the 1930s (Fig. 4). This was unsuccessful at smallholder level due to its heavy weight, the need for complicated adjustments and the higher power requirements than those of the Ethiopian ard, especially in soils with high clay contents (Solomon et al., 2006). Since the 1970s there have been several attempts to develop and implement a broad bed and furrow maker in Ethiopia (Jutzi et al., 1987). However, like other implements, dissemination of broad bed makers was very limited (Solomon et al., 2006). Recent tendencies are towards testing relatively simple CA-based resource-conserving technology tools (Melesse et al., 2009). Major challenges remain, however; the need for capacity building and problems in marketing the tools (Rockström et al., 2009).

On the other hand, research on the raised bed and furrow making in central Ethiopia mainly focused on draining excess water for the optimum production in Vertisol-dominated areas where soil water is generally not a limiting factor (Abiye et al., 2002).

As preparing beds and furrows with hand tools is not an option, except sometimes in irrigated land, our research has been oriented towards the use of the non-modified *marasha* for land preparation in a raised-bed CA-based resource conservation system.

4 Indigenous Use of the *Marasha* for In Situ Soil and Water Conservation

On-farm observations were carried out at various locations throughout the Tigray and Lasta highlands, and interviews with farmers and agricultural extension workers allowed characterising the prevailing in situ soil and water conservation techniques using the *marasha*: *shilshalo*, *terwah* and *derdero* (Table 1).

4.1 The Terwah Technique

The *terwah* technique, a traditional tillage system (Table 1; Fig. 2), is especially used in tef, where the farmers prepare furrows on the contour at regular intervals (approx. 1.5 m) to conserve water. This technique differs from the guide furrows which one can see in many fields, by the fact that (1) the furrows are deeper and wider, and (2) the *marasha* is adjusted to prepare these furrows, whereby specially prepared *doogri* (wings) are fitted on it. As a consequence, part of the runoff is trapped in the *terwah* and allowed to infiltrate. At these places the crop will grow particularly well. This technique is applied especially in tef, because this crop is sown on the surface after seedbed preparation without more ploughing (Deckers et al., 2001), allowing the farmer to also sow inside the *terwah*, which avoids the perception that the *terwah* is occupying valuable land. Using the *terwah* system for CA-based resource-conserving technologies with other

Fig. 4 Italian mouldboard ploughs imported into Ethiopia (near Mekelle, in 1938, photo Guidotti). "Gift of H.E. the Head of State to the inhabitants of Tigray" states the original legend of this photograph obtained from the Istituto Agronomico per l'Oltremare (Florence, Italy). As for other, more recent metal tools, the technology was not adopted by the Ethiopian smallholder farmers, especially due to excessive draught requirements and problems in maintenance

Table 1 Overview of traditional in situ soil and water conservation in Ethiopia and modified conservation agriculture-based resource-conserving technologies, all using the *marasha*

		Tillage system	
	Moment of implementation	Broad-spaced, deeper furrows on contour	Narrow-spaced, deeper furrows on contour
Traditional in situ conservation tillage	Yearly, after emergence		*Shilshalo* (on sorghum and maize only) (Sect. 4.2; Fig. 5)
	Yearly, at last tillage (at sowing)	*Terwah* (on tef only) (Sect. 4.1; Fig. 2)	*Derdero* (on all crops) (Sect. 4.3; Fig. 6)
Modified resource-conserving tillage	Yearly, at last tillage (at sowing)	*Terwah*, retaining stubble (on all crops) (5.3; Fig. 9)	Semi-permanent beds = *derdero*, retaining stubble (5.2; Fig. 8)
	Permanent; yearly, refreshed at sowing		*Derdero*+ = permanent beds, retaining stubble and using non-selective herbicides (before furrow refreshment and sowing) (5.1; Fig. 7)

crops than tef exists as a concept in Ethiopia but meets resistance. A senior agricultural officer in Tigray explained that agricultural experts prefer to focus on complicated implements such as the tied ridger while neglecting traditional techniques.

The *terwah* technique differs from *michdad* or *feses*, which are graded furrows to evacuate excess water from waterlogged areas or from whole farm fields (Erkossa et al., 2005), especially in high rainfall areas where it may lead to rill erosion (Woldeamlak and Sterk, 2003). However, in most of northern Ethiopia, moisture is an important limiting factor for agricultural production, hence in situ moisture retention is important. The improvement in crop performance in the vicinity of the bunds and terraces constructed along the contour (Vancampenhout et al., 2006) is a good indicator for the need to make contour furrows in the farm fields. An experimental site equipped with lined runoff and sediment collectors (Tewodros et al., 2009; Tesfay et al., 2010; Tigist et al., 2010) was used in 2005, 2006 and 2007 to compare the practice of ploughing and broadcast sowing on plain seedbeds with the *terwah* system, among other things. Here, the technique was shown to be effective for in situ soil and water conservation, reducing runoff on average by 11% and soil loss by 36% (Table 2). An important result of these runoff plot measurements, however, is that the *terwah* technique is efficient especially at the beginning of the rainy season (when soil moisture is most needed by the plants), whereas during the second part of the rainy season the furrows get filled with sediment; the land behaves from then onwards in a similar way to the plain tilled land (Tewodros et al., 2009).

Currently, the *terwah* method is promoted by the Agricultural Office extension services, who additionally advise lifting the plough from time to time (every 4 or 5 m) when preparing the *terwah*. In this way, the furrow is interrupted like a tied ridge; hence lateral drainage is avoided or slowed down.

4.2 *Shilshalo*

Throughout Ethiopia, when farmers grow sorghum or maize, they use the *marasha* to create contour furrows within the standing crop during the (second) weeding operation (Gebreyesus et al., 2006). The technique has also been documented in Yemen (Bédoucha, 1986; Varisco, 2004). Besides weeding and plant thinning, the aim of the practice (*miguemas* in Tigrinya, *shilshalo* in Amharic and south Tigray; Table 1) is to enhance runoff capture, particularly in semi-arid areas. In our target area, and despite the existence of very heavy rains in August when *shilshalo* is practised (Nyssen et al., 2005), the furrows are made along the contour and are slightly curved upwards at both ends of the farmland to enhance the water harvesting effect. In addition, plant physiologists have pointed to the sorghum crop root pruning that takes place and which enhances root growth (Blum et al., 1977; Rajaram et al., 1991; Blum, 2004).

Table 2 Runoff and soil loss (July–September) from farmland on Vertisols treated with *derdero*+ and *terwah* ploughing (both without herbicide) as compared with traditional management

			Runoff											Soil loss								
			Conventional			*Derdero*+				*Terwah*				Conventional		*Derdero*+			*Terwah*			
Site	Year	*n*	$m^3\ ha^{-1}$	s.d.	RC	$m^3\ ha^{-1}$	s.d.	RC	%	$m^3\ ha^{-1}$	s.d.	RC	%	$t\ ha^{-1}$	s.d.	$t\ ha^{-1}$	s.d.	%	$t\ ha^{-1}$	s.d.	%	Source
Gum Selasa	2005	2	647		0.15	255		0.06	40	377		0.09	60	19.5		4.6		23	7.6		39	(Tewodros et al., 2009)
Gum Selasa	2006	2	928	152	0.27	467	104	0.13	50	782	60	0.22	81	9.7	2.3	1.4	0.3	14	4.6	1.6	47	(Tigist et al., 2010)
Gum Selasa	2007	2	1158	224	0.27	650	444	0.15	56	1366	229	0.32	118		–	–	–	–	–	–	–	(Tesfay et al., 2010)
May Zegzeg	2005	3	1620		0.16	1496		0.14	92	1592		0.15	98	10.4		8.2		78	11.0		107	(Fekadu 2006)
May Zegzeg	2007	3	1311	149	0.35	920	126	0.24	70	1129	326	0.30	86		–	–	–	–	–	–	–	(Own data)
Average			1133		0.24	758		0.14	62	1049		0.22	89	13.2		4.7		38	7.7		64	

RC = runoff coefficient; s.d. = standard deviation; % = results of *terwah* and *derdero*+ treatment expressed in percent of the conventional tillage.

Fig. 5 *Shilshalo* ploughing as practised on sorghum, some weeks after emergence in the May Zegzeg catchment. In one tillage operation, weeding, thinning and root pruning is done; the furrows also decrease runoff and enhance infiltration

Fig. 6 The indigenous *derdero* technique applied on a farmer's field with fenugreek near Densa (Lasta), August 2007. Book for scale. Tillage after sowing has created the typical bed-and-furrow topography and brought most of the seeds to the beds

In May Zegzeg, except in three farm fields, *shilshalo* was practised by all farmers who planted sorghum (Fig. 5). A comparison of crop yields sampled on 4-m^2 plots in these three farmers' fields yielded no significant difference with 14 randomly selected farm plots where *shilshalo* was applied: 0.86 (±0.34) t ha^{-1} grain on plots with *shilshalo* against 0.90 (±0.56) t ha^{-1} without *shilshalo*. Yet, all farmers in that area claim that without implementing *shilshalo* one can hardly grow sorghum.

Gebreyesus et al. (2006) obtained on an experimental plot in Avergelle, 100 km away, but lower, warmer and less rainy, mean sorghum yields of 1.78 t ha^{-1} for *shilshalo* and 1.48 t ha^{-1} for crops where no furrows were created after plant emergence (results of two years on an experimental plot with three replicates). This tends to indicate that the *shilshalo* practice is especially essential for sorghum production in rain-deficit areas. McHugh et al. (2007) also mention the indigenous practice of *shilshalo* which is practised by many farmers in Wello on their sorghum crops for the sake of moisture conservation. Tilahun et al. (2002) further recommended sowing an intercropped legume at the moment of *shilshalo*. Some authors, however, merely recognise *shilshalo* as a weeding technique (Rezene et al., 1992; Hussein et al., 2000). While identifying the *shilshalo* technique, Sasakawa Global 2000 abandoned it on its CA experimental plots with maize, replacing it by glyphosate spraying (Ito et al., 2007).

4.3 Derdero

In Lasta, the *derdero* system is applied by many farmers, especially for fenugreek (*Trigonella foenum-graecum*), wheat and tef. At the last tillage operation, the farmer broadcasts the seeds over the surface, and then prepares beds and furrows along the contour using the *marasha*, in order to conserve water (Table 1). Soil and seeds that are on the position of the furrows are moved to an upper position on the beds in the ploughing process. Most of the plants will then germinate on the beds (Fig. 6). The *derdero* technique is especially used on Vertisols: in rain-rich locations or rain-rich years, it allows one to have the plants grouped in a slightly higher position on the ridges, i.e. protected from waterlogging, while the excess water is drained towards the furrows where it ponds and slowly infiltrates.

Whereas *shilshalo* is applied after emergence (particularly of sorghum and maize), *derdero* tillage is done at sowing time, so that surface depressions exist from the date of sowing onwards.

This tillage system shows many similarities to the "corrugaciones" tillage system in wheat-growing areas in North America. Farmers sow the field, incorporate the seed with a last ploughing operation and subsequently make furrows. In recent years, the "corrugaciones" are frequently used in rainfed systems, where the furrows either serve to drain excess water of heavy rain showers, or to avoid runoff, and as such enhance the water harvest capacity of the field in semi-arid areas (Medina et al., 2003). A similar tillage system has been reported in Yemen (Behnstedt, 1987; Varisco, 2004).

5 Bed-and-furrows for the Northern Ethiopian Highlands

Having noted the appropriateness of the *marasha* for surface shaping for conservation, such as *terwah*, *shilshalo* and *derdero*, the next logical step was then to link the tool up with the wide international body of knowledge on CA (Wall, 2007). Through discussion, empirical design, formal experiments and promotion of on-farm use, our current concept of CA and CA-based resource-conserving technologies for smallholder farmers in the north Ethiopian highlands came to include the following:

- limited tillage activity, to enhance soil structure and to decrease the cost of maintaining oxen;
- use of *marasha* as a tool for CA-based resource-conserving technologies;
- leaving half the straw length as standing stubble after harvest;
- crop rotation;
- critical use of non-selective herbicide just before sowing; and
- abandonment of stubble grazing (to improve soil fertility, to decrease compaction and to avoid browsing of standing stubble as well as roaming livestock just after spraying of herbicide).

This newly developed tillage system is called *derdero*+, where the "plus" stands for the improvements made. The basic system that has been developed consists of permanent beds with standing stubble (5.1 in Table 1). Here, parallel furrows are made on the contour at intervals of approx. 60 cm, using half the width of the yoke as a measurement (Fig. 7). In the first year of implementation, the furrows are refreshed a few times during the period of land preparation. At sowing, crops are broadcast over the land, and the furrows reshaped, whereby the soil is moved to the beds, thereby covering the seed. In subsequent years, one single tillage pass at the moment of broadcast sowing allows a refreshment of the furrow, reshaping of the bed and covering of seeds at the same time (Fig. 7).

The *derdero*+ land preparation system (Figs. 7 and 8), in which the farmer needs to adjust his *marasha* in such a way that the sediment uplifted from the furrows is well spread over the beds, has proven to work with wheat (Tesfay et al., 2010), barley, chickpea, maize and grasspea. For tef, the implementation is slightly different: on the day of sowing, the furrows are first refreshed by *marasha*, whereafter the seed is sown over the surface, including beds and furrows. The conventional incorporation of the seeds into the top 1 cm is done using thorny *Acacia* sp. branches.

Fig. 7 The *derdero*+ system involves shaping of beds and furrows by a pair of draught animals with attached *marasha* (on a farmer's field in May Zegzeg, July 2009). Only a single, broad-spaced tillage operation is needed. The farmer replaced one of the oxen in the span by a (weak) cow, as only the sediment accumulated in the previous year's furrows needed to be reworked and less power was needed. Note how the left ox walks in the furrow, which provides guidance for the position of the plough tine in the immediately upslope furrow

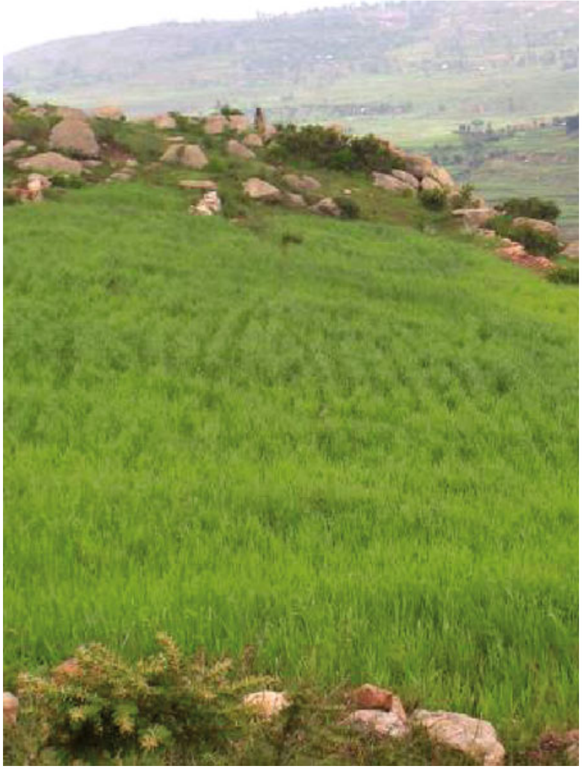

Fig. 8 Wheat on permanent beds (*derdero*+ system) in a farmer's field in May Zegzeg, August 2007. Beds were reshaped in a single tillage operation immediately after broadcast sowing

Results from our experimental sites show that this system leads to an average decrease in runoff of 38% and in soil loss of 62% (Table 2).

6 Future of the *Derdero*+ System

One particular problem that had to be overcome was weed infestation, as frequent tillage no longer takes place: at the beginning of our experimentation, there were large invasions of *Cynodon dactylon*, which could not be controlled by hand weeding and which particularly affected tef growth (Tigist et al., 2010) (Fig. 9).

We have recently introduced the use of glyphosate herbicide on the permanent beds, a few days before sowing, when maximum emergence of the seed bank has taken place. There is a good rationale for the use of herbicides, since tillage operations are carried out only by men and weeding is done mostly by women and children. Hence the introduction of *derdero*+ without use of herbicides would have led to significant shifts in workload from men to women and children. However, follow-up of appropriate use and dosing of the herbicide is of utmost importance (Christoffoleti et al., 2008). The constraints related to herbicide weed control are related to the fact that spraying is a knowledge-intensive technology. Hence, the extension of CA-based resource-conserving technologies needs to be combined with innovation systems that support training and knowledge transfers. As used to be the case in Ethiopia for fertiliser not so long ago, there is no structure/system in place at the moment for such inputs. Our working example is deemed to catalyse the innovation network and make the needed change to input and output markets.

Two intermediate systems were also introduced, which can be very useful for farmers who hesitate to use herbicide or who believe that "a good farmer ploughs his land many times" (Melesse et al., 2008). Firstly, to manage semi-permanent beds we apply the *derdero* technique, whereby in addition we keep standing stubble on the land (5.2 in Table 1). After several normal tillage operations, without herbicide application, the land will be shaped into contour beds and furrows immediately after broadcast sowing and beds and furrows remain throughout the crop-growing period. The second intermediate system consists of *terwah* ploughing for all crops, as promoted by the Bureau of Agriculture (5.3 in Table 1); traditionally it used only to be done for tef (Fig. 10).

At this initial stage of implementation, our agronomic results for permanent beds do not show a significant difference in crop and straw yield with traditional tillage. We measured a slight increase in wheat yield (Tewodros et al., 2009; Tesfay et al., 2010) and a decrease in tef yield (Tigist et al., 2010) (Fig. 9), which was most probably related to the earlier mentioned weed problems in the absence of herbicide use. Further increase is expected to occur in the future, when the weed seed bank becomes exhausted and soil physical and chemical properties improve. Obviously, for the same crop yield, labour investment and draught force requirement are strongly reduced with *derdero*+, with only one tillage pass that consists of simply refreshing furrows (Fig. 7). The use of cows and donkeys for tillage becomes possible. Similar to our case, Wall (2007) found that in many developing countries, one of the most important benefits of CA for

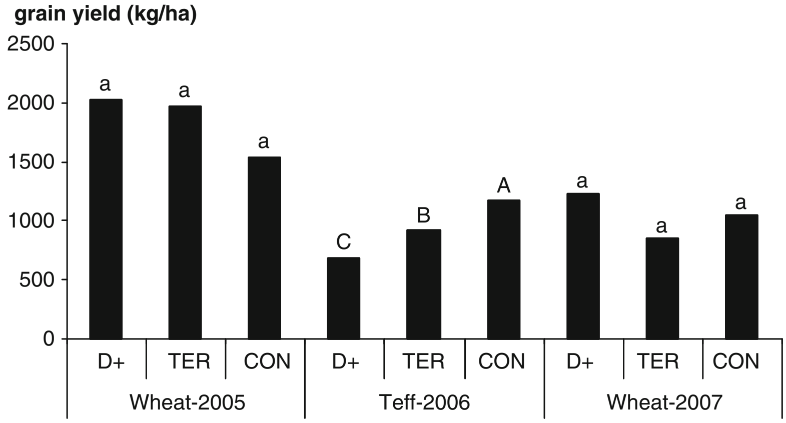

Fig. 9 Average grain yield (kg/ha) trend in conservation and conventional agriculture in Gum Selasa. Different letters stand for significant difference ($P < 0.05$) between treatments per year. D+ = *derdero*+ (without use of herbicide), TER = *terwah*, CON = conventional tillage (after Tesfay et al., 2010)

Fig. 10 The stripped pattern of this wheat farm at the ripening stage in May Mereb (5 km west of Hagere Selam; October 2005) is due to enhanced soil moisture in the area around *terwah* furrows (see detail in Fig. 2). Traditionally, *terwah* ploughing is only used with tef, but in recent years the local Bureau of Agriculture has promoted the establishment of such broad-spaced deeper furrows at sowing time of other crops as well

smallholder farmers is the reduction in labour requirements, both by oxen and humans, for tillage. However, if only manual weeding is practised, then labour requirements may increase, making the system unattractive to farmers (Muliokela et al., 2001). Hence the use of glyphosate in our *derdero*+ CA system.

The abandonment of stubble grazing, which has definite advantages in terms of soil conservation (Nyssen et al., 2009), is a prerequisite for implementation of resource-conserving technologies. The issue of leaving standing stubble fits in the same paradigm as the establishment of exclosures (Descheemaeker et al., 2006; Wolde et al., 2007), where previously overgrazed rangeland has been left to regrow and fodder is taken through cut and carry. The only way to get out of the vicious circle of land degradation caused by overgrazing is to increase total biomass production per land unit, so that one can cut and carry part of the straw and leave part for system sustainability. The aimed-for increase in productivity would allow both harvesting the upper part of the straw and leaving tall standing stubble. Zero grazing is promoted in the target area but meets with resistance as there is a strong tradition of off-season grazing. In May Zegzeg, where free grazing has been abolished, the developed *derdero*+technology has been implemented on ten farmers' fields (Fig. 9) with a total area of around 2 ha, with good results in terms of crop stand and crop yield, and strongly reduced draught requirement both in number of tillage operations and in required energy (Fig. 7). Farmers in that area also claim that in the absence of compaction by livestock hoofs and with reduced tillage, the topsoil has become particularly soft.

To enhance area-wide adoption, we agree with Ekboir (2002), who stated that although the development of no-till packages and their adoption by small-scale farmers followed different paths than for large-scale farmers, the paths shared one important common feature: all successful programmes resulted from networks that worked with participatory research approaches. Wall (2007) further stresses the need for interested agents to catalyse innovation systems and, based on the activities of innovative farmers, to help overcome problems observed in farmer experiments. These highly participatory innovation systems do not develop without a catalyst: an individual or organisation with a specific interest in advancing the technology and its adoption (Wall, 2007).

7 Conclusions

Research on CA and CA-based resource-conserving technologies in Ethiopia generally fits into two major paradigms: (1) flat seedbed with use of herbicide and one surficial tillage operation (Ito et al., 2007); or (2) surface shaping using various (often heavy and complex) tools (Hussein et al., 2000; Abiye et al., 2002; Gebreyesus et al., 2006; McHugh et al., 2007; and many others). In both cases, indigenous in situ soil and water conservation tillage technology, if recognised, is merely mentioned anecdotally (Gebreyesus et al., 2006; McHugh et al., 2007), and even considered as an interference with the experiment (Hussein et al., 2000; Ito et al., 2007).

Yet, in the northern Ethiopian highlands farmers traditionally use the *marasha* ard plough for in situ conservation by creating surface depressions, either at the moment of sowing (*terwah*, *derdero*) or after crop emergence (*shilshalo*). On Vertisols, the indigenous conservation technologies *terwah* and *derdero* aim to locally drain out the crops while curbing runoff and favouring infiltration.

Building upon this indigenous knowledge, we further developed CA-based resource-conserving technologies into *derdero*+ whereby the traditional ard plough was found suitable for a ‘bed-and-furrow’ system. The concept further integrates the retention of stubble, improving the soil structure by decreasing the number of tillage operations, whereby tillage becomes limited to transferring sediment from the furrows to the beds at the moment of sowing.

From the socio-economic point of view, implementation of permanent beds leads to decreased oxen (and hence straw) requirements, but also to increased need for weeding in the first years unless herbicide is used. These modified labour requirements need to be studied. The commonly practised stubble grazing system is incompatible with stubble management that is part of *derdero*+. It is expected that benefits from *derdero*+ will be an additional incentive for free grazing abandonment.

Furthermore, the decreased runoff (–51%) and soil loss (–81%) allow protection of the downslope areas from flooding, but soil nutrient build-up and soil structure improvement are slow processes, and hence the full benefit of the permanent bed system can only be expected after some years.

Overall, *derdero*+ can be part of the ongoing intensification process which includes physical soil and water conservation, slope reforestation and irrigation development, and will have to be implemented through a step-by-step process of adoption of resource-conserving technologies that lead to CA. It has its own niche: the cropped land sensu stricto, i.e. the most important part of the land, both for the farmer and for a nation that is striving for food security. Hence, it is suggested that the permanent bed *derdero*+ system could be widely implemented on farmers’ fields after several years of experimentation, when stable improved crop yields can be shown. The *terwah* system is a very good first step, resulting in decreased runoff (–11%) and soil loss (–36%), and we strongly recommend implementing it on all farmland in northern Ethiopia. However, the long-term goal is to achieve a permanent bed planting system.

In a country like Ethiopia, with an enabling environment for smallholder agriculture (Mitiku et al., 2006) and with the *marasha* as a tool that is almost ready for use, there are good prospects for CA and bed-and-furrow CA-based resource-conserving technologies.

Acknowledgements This study was carried out in the framework of Mekelle University Institutional University Cooperation, funded by VLIR, Belgium. We particularly thank the ten farmers who agreed to implement *derdero*+ on their lands. Numerous other Ethiopian farmers agreed to share their knowledge with us, as well as one Australian farmer (Leigh Vial), and Solomon Gebregziabher, Tigist Oicha, Fekadu Getnet and Tewodros Gebregziabher (Mekelle University, Ethiopia), Mulugeta Gebreselassie (Bureau of Agriculture, Mekelle, Ethiopia), Wagnew Ayalneh (International Livestock Research Institute, Addis Ababa, Ethiopia), and Dirk Raes and Josse Debaerdemaeker (K.U. Leuven, Belgium). The authorities of the villages and districts concerned facilitated the research. While carrying out fieldwork, J.N. was employed by K.U. Leuven, Belgium. Soil scientist Neil Munro (Dirleton, East Lothian, UK) revised and corrected the English. The editor and two anonymous reviewers are sincerely thanked for useful comments on an earlier version of this paper.

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Biological Nitrogen Fixation and Socioeconomic Factors for Legume Production in Sub-Saharan Africa

Jonas N. Chianu, E.M. Nkonya, F.S. Mairura, Justina N. Chianu, and F.K. Akinnifesi

Contents

Abstract Low crop productivity is a general problem facing most farming systems in sub-Saharan Africa (SSA). These low yields are pronounced in grain legumes and are often associated with declining soil fertility and reduced N_2-fixation due to biological and environmental factors. Unfortunately, the majority of African small farmers are now unable to afford the high mineral fertilizer prices. More than 75% of the fertilizers used in Africa are imported, putting pressure on foreign exchange. Low cost and sustainable technical solutions compatible with the socioeconomic conditions of small farmers are needed to solve soil fertility problems. Biological nitrogen fixation (BNF), a key source of N for farmers using little or no fertilizer, constitutes one of the potential solutions and plays a key role in sustainable grain legumes (e.g., soybean) production. Given the high cost of fertilizer in Africa and the limited market infrastructure for farm inputs, current research and extension efforts have been directed to integrated nutrient management, in which legumes play a crucial role. Inoculation with compatible and appropriate rhizobia may be necessary where a low population of native rhizobial strains predominates and is one of the solutions which grain legume farmers can use to optimize yields. It is critical for sustained yield in farmlands deficient in native rhizobia and where N supply limits production. Research on use of *Rhizobium* inoculants for production of grain legumes showed it is a cheaper and usually more effective agronomic practice for ensuring adequate N nutrition of legumes, compared with the application of N fertilizer. Here, we review past and on-going interventions in *Rhizobium* inoculation (with special reference to soybean) in the farming systems of SSA with a view to understanding the best way to effectively

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_17,

from Chianu et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010004

advise on future investments to enhance production and adoption of BNF and inoculant technologies in SSA. The major findings are: (1) complete absence of or very weak institutions, policy and budgetary support for biotechnology research and lack of its integration into wider agricultural and overall development objectives in SSA, (2) limited knowledge of inoculation responses of both promiscuous and specifically nodulating soybean varieties as well as the other factors that inhibit BNF, hence a weak basis for decision–making on biotechnology issues in SSA, (3) limited capacity and lack of sustainable investment, (4) poorly developed marketing channels and infrastructure, and limited involvement of the private sector in the distribution of inoculants, and (5) limited farmer awareness about and access to (much more than price) inoculants. The lessons learned include the need: (1) to increase investment in *Rhizobium* inoculation technology development, and strengthen policy and institutional support, (2) for public private partnership in the development, deployment and dissemination of BNF technologies, (3) to develop effective BNF dissemination strategies (including participatory approach) to reach farmers, (4) for greater emphasis on capacity building along the BNF value chain, and (5) for partnership between universities in SSA and those in the North on BNF research.

Keywords Low soil fertility • N_2 fixation • Inoculants • Soybean • Adoption drivers • Sub-Saharan Africa

Abbreviations

ASK	Agricultural Society of Kenya
BCR	Benefit Cost Ratio
BNF	Biological Nitrogen Fixation
CAN	Calcium Ammonium Nitrate
CIAT	Centro Internacional Agricultura de Tropical
DAP	Di-Ammonium Phosphate
DR-Congo	Democratic Republic of Congo
FAO	Food and Agriculture Organization of the United Nation
FYM	Farmyard manure
IAEA	International Atomic Energy Agency
IITA	International Institute of Tropical Agriculture
IFDC	International Fertilizer Development Center
INM	Integrated Nutrient Management
ISAR	Institut des Sciences Agronomique du Rwanda
KARI	Kenya Agricultural Research Institute
KIOF	Kenya Institute of Organic Farming
KShs	Kenya Shillings
LSD	Least Significant Difference
MIRCEN	Microbiological Resources Center
MRR	Marginal Rate of Returns
N	Nitrogen
NGO	Non-governmental organization
NPK	Nitrogen Phosphorus Potassium
OMMN	Organic Matter Management Network
P	Phosphorus
R&D	Research and Development
SPRL	Soil Productivity Research Laboratory
SSA	Sub-Saharan Africa
SUA	Sokoine University of Agriculture
TGx	Tropical Glycine crosses
UNEP	United Nations Environment Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
US$	United States of America Dollar

1 Introduction

Smallholder farming systems in sub-Saharan Africa (SSA) are constrained by low crop productivity. Grain legumes are being seen as the “meat for the poor” due to their rich protein content and the low prices of pulses compared with meat. High productivity of pulses becomes vital as most poor people in SSA depend on pulses for protein supply to meet their food, nutritional and health needs. The average yields of grain legumes have remained very low (e.g., about 622 kg/ha for common bean) under farmers’ conditions compared with research station yields (e.g., over 1500 kg/ha for common beans) (Mushi, 1997). Low yields are associated with declining soil fertility due to continuous cropping without soil replenishment and reduced N_2-fixation due to various biological and environmental factors (Dakora and Keya, 1997). This becomes more severe as farmers expand into marginal lands in response to population pressure. Arid and semiarid marginal lands are generally deficient in nitrogen (N), required to raise crop production (Mugabe, 1994).

Methods to enhance cost-effective soil nutrient supplies to small farmers have remained a challenge to scientists working to improve agricultural productivity in SSA. In the highland agricultural ecozones of SSA, N supply is also a key limiting factor in crop production for 35–45% of the farmers (Odame, 1997). Soybean is estimated to fix 80% of its N needs (Smaling et al., 2008). Many SSA countries have a growing need for mineral fertilizers to enhance crop yields (Mugabe, 1994; Morris et al., 2007; World Bank, 2008). However, the majority (about 60%) of African smallholder farmers are unable to afford the high prices of mineral fertilizers (Yanggen et al., 1998). A farmer has to sell about 10 kg of maize or 5 kg of common bean to buy 1 kg of N or P in the form of mineral fertilizers (Odame, 1997). This high conversion (crop to sell to purchase a unit of mineral fertilizer) ratio implies a low incentive to use fertilizers, and is largely explained by the high average farm gate prices of fertilizer in SSA. More than 75% of the mineral fertilizers used in Africa are imported (Mugabe, 1994), leading to reduced foreign exchange. For example, in the 1990s, Kenya spent over 30% of its foreign exchange annually on fertilizer importation (Mugabe, 1994).

Although grain legume yields can be improved by use of moderate levels of mineral fertilizers, legumes (unlike cereals) are rarely fertilized by farmers, probably due to the high cost of fertilizers and low awareness of the associated economic returns, or both (Ndakidemi et al., 2006). Supplementing legumes with soil nutrients has been shown to double yields (Dakora, 1984; da Silva et al., 1993), and increase plant growth and N_2-fixation compared with the unfertilized control (Ndakidemi et al., 2006). *Rhizobium* inoculation also helps to boost the yield of grain legumes, leading to land savings, and has been described as a cheap insurance for higher yields (Ndakidemi et al., 2006). However, despite its potential to address low N and its cost effectiveness, the demand for inoculants by farmers in SSA remains low (Kannaiyan, 1993). The reasons included poor quality, and inadequate and inefficient marketing outlets, as well as inadequate extension services covering inoculant use (Kannaiyan, 1993; Odame, 1997).

Given the high cost of fertilizer in Africa and the limited market infrastructure for agro-inputs, current research and extension efforts have been directed to integrated nutrient management (INM), in which leguminous crops, shrubs and trees play a key role (Chianu and Tsujii, 2005; Mafongoya et al., 2007). BNF is a key source of N for farmers who use little or no fertilizer, especially for legumes such as soybean (Smaling et al., 2008). Rinnofner et al. (2008) described BNF, in the context of legume catch crops, as an additional benefit. Based on the studies of Zotarelli et al. (1998), Alves et al. (1999, 2005), Hungria et al. (2006) and Araujo et al. (2006), it has been widely shown that up to 80% of the above-ground N accumulation in soybean is due to N fixation by rhizobia. A study carried out in Uganda shows that BNF contributes 22% of nitrogen inflows for perennial crops and 44% for annual crops (Nkonya et al., 2008). It is estimated that about 11.1 million metric tons of nitrogen is fixed annually through BNF in developing countries (Hardarson et al., 2003). If supplied through mineral fertilizers, about twice this amount will be required to achieve the same crop yield level (Hardarson et al., 2003). Most African countries could reduce expenditures on fertilizer imports through a full exploitation of BNF (Mugabe, 1994). It is estimated that *Rhizobium* alone could provide more than 50% of the fertilizer required for crop production in most of the marginal lands of Kenya, Zimbabwe and Tanzania (Mugabe, 1994). This underscores the importance of BNF in ensuring sustainable and low cost production by smallholder farmers in SSA. However, several constraints (socioeconomic, environmental, production, etc.) need to be addressed to tap the full benefits of BNF (Bohlool et al., 1992; Amijee and Giller, 1998; Date, 1999). The most serious problems affecting nodulation and N fixation in tropical cropping systems are limited skills, low quality inoculants, acid soils and high soil temperatures, among others.

The overall BNF history, especially in SSA, indicates that holistic studies addressing the challenges facing the use of inoculants by farmers have been rare. This contributes to limited knowledge and approaches on how to stimulate widespread use of BNF. Most studies on rhizobial inoculants have tended to address the biophysical components and the science of *Rhizobium* production (Amijee and Giller, 1998). Some research has also been carried out on the economic productivity of inoculants under experimental studies (Ndakidemi et al., 2006). Only a few studies have examined the socioeconomic and policy constraints affecting household adoption and utilization of inoculants by farmers. Meanwhile, lack of knowledge by farmers is a key factor explaining the low adoption, use and retention of inoculant technologies (Date, 1999).

Here, we review past and on-going interventions in *Rhizobium* inoculation (with special reference to soybean) in SSA in order to understand how best to effectively advise on future investments to popularize soybean enterprises and enhance production and adoption of BNF and inoculant technologies to improve the farming systems, household nutrition, income and overall welfare in SSA. We hypothesize that soybean inoculation significantly enhances the sustainability and productivity of farming systems in SSA and as a result, leads to reduction in poverty, and improvements in farm income and the overall welfare of farm families.

2 Production and Marketing of Inoculants in Sub-Saharan Africa

BNF and N-inoculants have had a long history in Africa, starting with the colonial agricultural research efforts to develop N-inoculants for pasture legumes aimed at increasing the productivity of exotic cattle (Odame, 1997). To promote BNF in developing countries, UNESCO established a number of Microbiological Resource Centers (MIRCENs) across five continents with support from the UNEP and the FAO (Odame, 1997). The broad responsibilities of MIRCENs in Africa (located in Dakar, Cairo and Nairobi) include the collection, identification, maintenance and testing of strains as well as preparing inoculants and distributing cultures compatible with local crops. Others are the deployment of local *rhizobia* inoculant technology, promotion of research, and the provision of advice, training and guidance to individuals and institutions engaged in rhizobiology research. For instance, the Nairobi MIRCEN project promoted and transferred BNF technologies (including inoculants of pulses, pasture legumes and trees) to researchers and other agricultural stakeholders in Kenya and all of east Africa. The Nairobi MIRCEN also diversified into screening *Rhizobium* strains for tolerance to abiotic stress (e.g., high temperatures, soil acidity, drought, etc.), especially given the numerous environmental stresses that affect the success of BNF and since two-thirds of Kenya's agricultural land suffers from these. The idea is to gradually intensify screening trials for rhizobia that are adapted to such ecological stresses. The Nairobi MIRCEN project also explored the potential of *mycorrhiza*, a fungal strain, on plant roots that assists the plant to extract P and water from the soil. The Nairobi MIRCEN also developed a marketable bio-fertilizer called *Biofix* (Odame, 1997). Two non-governmental organizations (NGOs) [Kenya Institute of Organic Farming (KIOF) and the Organic Matter Management Network (OMMN)] played a significant role in distributing Biofix to farmers. Over time, KIOF's active involvement in Biofix promotion over-stretched its financial and human resources and waned.

In the 1990s, the FAO supported a project to select better strains of *Rhizobia* in Tanzania (Mugabe, 1994). Through this, Sokoine University of Agriculture (SUA), Morogoro, developed a bio-fertilizer (*Nitrosua*) for use in soybean production. In collaboration with the Ministry of Agriculture and some NGOs, SUA also established extension activities to disseminate *Nitrosua* to local farmers. These activities also waned over time (Bala, 2008). Inoculants were produced in Uganda by at least two plants (Madhavani Ltd. and the BNF of Makerere University, established in 1990 with the help of USAID). These two plants functioned up to 1997 and, for the contract order by the FAO produced 14.2 tons of soybean inoculants between 1995 and 1997. In Rwanda, inoculant production started at the Institut des Sciences Agronomiques du Rwanda (ISAR) in 1984 and had by 1990 reached an annual production level of 2.4 tons (Cassien and Woomer, 1998). Activities were, however, disrupted by the civil war of the 1990s. Following the end of the war and the renovation of the laboratory, BNF activities resumed. However, pre-civil war levels are yet to be reached (Giller, 2001).

Commercially manufactured South African inoculants first appeared in the market in 1952 but were of doubtful quality until an independent quality control system was introduced in the early 1970s (Strijdom, 1998). Since 1976, all inoculants must be manufactured with sterilized peat and must contain at least 5×10^8 rhizobial cells g^{-1} of peat (Strijdom, 1998). The quality control measures ensured that South

African inoculants compared well with the best quality inoculants produced outside Africa (Strijdom and van Rensburg, 1981). A range of inoculants are produced for a number of crops including soybean, groundnut and cowpea (Deneyschen et al., 1998). In Malawi, commercially available inoculants for crops such as soybean and cowpea, produced by Chitedze Agricultural Research Station, Lilongwe, were available and sold in 50-g packets (Khonje, 1989), starting from the 1970s. Sales rose dramatically from 450 packets in 1976 to about 1800 in 1987/88. The presence of a large and well-established commercial soybean sector in Zimbabwe suggests widespread use of inoculants in the country (Mpepereki et al., 2000). The Soil Productivity Research Laboratory (SPRL) spearheaded a project in the 1990s for enhancing BNF technology in Zimbabwe, supported by the IAEA (IAEA, 1998 cited by Bala, 2008). The project developed the capacity to mass produce inoculants (120 000 packets per year) which were distributed to small farmers through the Government's extension system. The University of Zimbabwe also undertook *mycorrhizal* inoculation research in some regions (Mugabe, 1994).

3 Inoculation and Grain Legume Nodulation

Due to the importance of legumes in N_2-fixation, soil fertility improvement, and human and livestock nutrition in SSA, the agricultural extension services in SSA have traditionally promoted and encouraged their inclusion in the farming systems (e.g., through promotion of cereal-legume intercropping). The cereal-legume intercrop has been shown to increase the total value of cereal and legume production, land equivalent ratio and returns to labor and other inputs (Maingi et al., 2001; Dapaah et al., 2003). This suggests the suitability of the cereal-legume intercropping system for smallholder farmers with limited land and resources to buy inorganic fertilizer and other external inputs. Hence promotion of the cereal-legume intercropping system by the extension services in SSA will also increase the adoption of *Rhizobium* inoculation technology.

Studies have shown that *Rhizobium* inoculation is needed in all agricultural lands deficient in N and where N supply is a key limiting factor in crop production (Odame, 1997). Examples of such lands abound in SSA (e.g., marginal lands, arid and semiarid lands, some highland areas that have lost their fertility) (Mugabe, 1994). Inoculating legumes with species-specific *Rhizobium* increases the success of their establishment, root nodulation, biomass and biomass N yields (Java et al., 1995; Zhu et al., 1998). Inoculation helps to increase the number of effective rhizobia (Boahen, 2008). It may also be necessary if a legume, newly introduced into an area, is to form effective symbiosis. Kaizzi (2002) and Giller et al. (1994) reported that Velvet bean (*Mucuna pruriens)* accumulated 68–220 kg N ha^{-1} in eastern Uganda, 50% of which was derived from the atmosphere through BNF. Indigenous *Bradyrhizobium* spp. also does not meet the demand for N by soybean all the time. Poor yields may occur whenever effective bradyrhizobia are inadequate or even when available in adequate numbers (Ken Giller, pers. commun.). The overuse of classical N fertilizers substantially contributes to environmental degradation through groundwater pollution by nitrates (Paynel et al., 2008). This leaching of N into waters is an economic flaw since only a part of the fertilizer is used by the plants. *Rhizobium* inoculants have been used to address the problems of soil fertility and inadequate fertilizer application in grain legume production and have been found to be a cheaper and usually more effective agronomic practice for ensuring adequate N nutrition of legumes, compared with the application of N fertilizer (Tran, 2004; Paynel et al., 2008). Studies have also shown that under the natural BNF inhibiting conditions, inoculation is much cheaper than mineral N fertilizer (Odame, 1997). A 100-g packet of Biofix (a form of *Rhizobium* inoculant), sufficient to inoculate 15 kg of common bean seed, enough to plant 1 acre, costs KShs 75 (or US$1.20). In contrast, 100 kg of inorganic CAN (Calcium Ammonium Nitrate) fertilizer, required for 1 acre, is sold for KShs 2040 (or US$34). Apart from being cheaper (28 times) than commercially produced N fertilizers, a 100-g packet of Biofix is also lighter to transport.

Worldwide, the use of *Bradyrhizobium* in soybean has been a major success in bio-fertilizers and constitutes an example of traditional biotechnology application. However, it is important to note that unlike soybean, cowpea hardly responds to inoculation in most parts of SSA because of the presence of suitable native rhizobia in the soil.

3.1 Inoculation Response of Commercial vs. Promiscuous Soybean Varieties

Commercial soybean varieties are specific and require repeated inoculation due to non-persistence of the rhizobia. Corby (1965) was the first to describe the nodulation of soybean by rhizobia indigenous to African soils. He observed that a variety, 'Hernon 147', nodulated effectively with indigenous rhizobia and did not respond to inoculation in five out of six sites in Zambia and Zimbabwe. Field studies at the IITA (International Institute of Tropical Agriculture) in Nigeria showed that seed inoculation with commercial rhizobial inoculants significantly improved neither nodulation nor yield of three Asian soybean varieties, 'Malayan', 'Orba' and 'TGm 686', while the American varieties 'TGm 294-4-2371', 'Bossier' and 'Jupiter' formed very few nodules without inoculation and showed yield increases of 40 to 97% (Nangju, 1980). The Asian varieties rarely responded to inoculation even when inoculation more than doubled the yields of the American varieties (Pulver et al., 1982). Weaver and Frederick (1974) suggested that inoculant rhizobial numbers of up to 1000 cells g^{-1} soil need to be applied to soybean seed to obtain greater nodule occupancy by inoculant rhizobia than the indigenous strains.

In some cases, however, the introduced legume such as soybean could form effective symbiosis with sufficient numbers of local rhizobia, thus obviating the need for inoculation. Chowdhury (1977) reported that soybean had been grown successfully without inoculation in certain parts of Nigeria, Tanzania and DR-Congo and attributed this to the selection for and increase in efficient *Rhizobium* strains due to cultivation over the years. All the varieties tested by Chowdhury (1977) formed nodules but only those bred locally from 'Hernon' varieties or 'Malayan' (Asian variety) formed many nodules. However, Sanginga et al. (2000) noted that promiscuous soybean is incapable of nodulating effectively with indigenous rhizobia in all locations in the moist savanna zone of Nigeria. Similarly, Bala (2008) observed that it is also not clear whether promiscuous soybean cultivars are effectively nodulated by indigenous rhizobial populations in all soils and under all conditions. A study by Eaglesham (1989) showed that the promiscuous soybean varieties showed inconsistent response to inoculation. This author, therefore, concluded that it may be safer to rely on effective inoculant strains rather than breed for the ability to nodulate with indigenous rhizobial strains of unknown potential. Early studies in South Africa showed local soybean cultivars to specifically form symbiosis with *B. japonicum* (Van Rensburg and Strijdom, 1969). However, it is important to note that even promiscuously nodulating soybeans (that often do not require inoculation), developed and cultivated in some parts of Africa, sometimes respond to inoculation. A study carried out by Osunde et al. (2003) at five sites in the moist savanna region of Nigeria showed that promiscuous soybean varieties (Tropical Glycine cross TGx 1456-2E and TGx 1660-19F) responded to inoculation (see Table 1). However, 'Magoye', an exceptionally promiscuous line released in Zambia in 1981, nodulates readily in all soils of southern Africa where it has been tested and rarely responds to inoculation in Zambia and Zimbabwe (Mpepereki et al., 2000).

Promiscuity was used as a major selection criterion in the IITA for more than 10 years without in-depth

Table 1 Nitrogen fixation and N accumulation by two promiscuous soybean cultivars [with(out) inoculation] across five sites in the southern Guinea savanna of Nigeria

Treatment	Stover N (kg ha^{-1})	Grain N (kg ha^{-1})	Total N[a] (kg ha^{-1})	N_2 fixed (%)	N_2 fixed (kg ha^{-1})
N source:					
Inoculated	36	131	166	50	83
Uninoculated	32	121	153	46	70
60 kg N ha^{-1}	26	98	124	26	32
LSD (5%)[b]	4	21	25	10	11
Cultivar:					
TGx 1456-2E	30	135	165	43	70
TGx 1660-19F	33	98	131	39	50
LSD (5%)[b]	NS[b]	27	31	NS	15

[a] Total N of above-ground dry matter; [b] Least Significant Difference; NS = not significant. Source: adapted from Osunde et al., 2003.

microbiological studies (Sanginga et al., 2001). Recent studies on symbiotic effectiveness of indigenous rhizobia nodulating promiscuous soybean varieties in 92 Zimbabwean soils led to the identification of three isolates with superior N_2-fixing potential in the 'Magoye' variety than the commercial strain MAR 1491 (Musiyiwa et al., 2005a), although the M3 isolate was later identified as superior to the commercial strains MAR 1491 and 1495 (Zengeni and Giller, 2007). Thus, local soybean cultivars in South Africa may have had some evolutionary promiscuity, which might have been overlooked by the exclusive use of the specifically nodulating commercial varieties. Using four promiscuous soybean varieties (TGx 1485-1D, TGx 1456-2E, TGx 1448-2E and TGx 1660-19F), Okogun and Sanginga (2003) observed no significant difference between the yield of inoculated and uninoculated crops at three sites in the savanna of Nigeria even though the number of native rhizobia in soils at the three sites differed by orders of magnitude. On the basis of these results, the IITA initiated a soybean breeding program in 1978 to develop 'promiscuous' soybean varieties that nodulate with indigenous soil bradyrhizobia, thus eliminating the need for inoculation (Kueneman et al., 1984). The aim was to confer the ability to nodulate with indigenous rhizobia on the American soybean varieties, which had greater yield potential and better tolerance to diseases than the Asian varieties. This effort led to the development of over 60 TGx soybean lines. Studies aimed at establishing the degree of promiscuity of soybeans with indigenous rhizobia need to be further investigated to confirm these results (Musiyiwa et al., 2005b). According to Mpepereki et al. (2000), the symbiotic interaction between soybean genotypes and rhizobial isolates varies widely (e.g., in terms of ability to nodulate, and effectiveness in N_2-fixation). All plant genotypes tested by Mpepereki et al. (2000), including varieties considered to be highly specific, nodulated with indigenous isolates in some soils.

3.2 Response to Inoculation: Variability and Determinants

Among others, inoculation response (e.g., soybean response to *Bradyrhizobium* inoculation) is regulated and influenced by the number and quality of indigenous rhizobia as well as abiotic and biotic factors (Abaidoo and Woomer, 2008). Figure 1 shows that the likelihood of response to inoculation by soybean is strongly influenced by the number of effective rhizobia in the soil. Soybean growing in a soil with a small number of effective rhizobia (20–50 cells g^{-1} soil) will likely respond to inoculation (Singleton and Tavares, 1986; Thies et al., 1991).

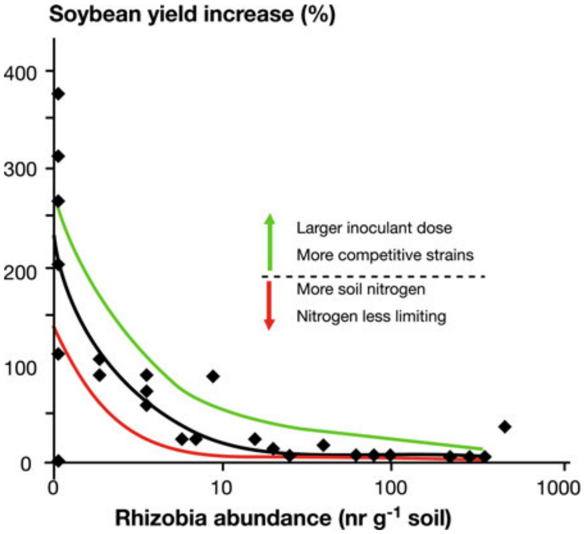

Fig. 1 Soybean response to inoculation as regulated by the number of indigenous rhizobia (after Thies et al., 1991)

Weaver and Frederick (1974) suggested that for inoculation to be beneficial, nodule occupancy by inoculant strains must be more than 50%, requiring an inoculum rate of at least 1000 times the soil population. In practice, however, the presence of a large indigenous population of compatible rhizobia does not necessarily preclude response to inoculation, provided the inoculant rhizobial strains are competitive and highly effective (Giller, 2001; Osunde et al., 2003). According to Danso (1990), by increasing the numbers of inoculant strains, it is possible to increase the number of nodules they occupy.

A number of biotic and abiotic factors affect N_2-fixation (see Table 2). Symbiotic N_2-fixation is highly sensitive to water stress, a major limiting factor to legume productivity in semiarid tropics (Serraj et al., 1999). Nitrogen fixation in soybean also declines under soil moisture deficit (Devries et al., 1989;

Table 2 Factors limiting biological nitrogen fixation in soybean and recommendations

Factor	Effect	Reference	Recommendations
High soil temperature	Reduces the survival of rhizobia in soil and inhibits nodulation and N_2-fixation	Munevar and Wollum, 1982; Michiels et al., 1994	Surface mulching; placement of inoculum in deeper soil layers; select heat-tolerant strains
Soil moisture	Reduces rhizobial numbers, limits migration of rhizobia, reduces nodulation and N_2-fixation	Hunt et al., 1981	Optimization of soil moisture; select moisture-stress-tolerant strains
Soil acidity	Reduces the survival of rhizobia in soil, inhibits nodulation and N_2-fixation and leads to P fixation. Increases aluminum toxicity and calcium deficiency	Graham et al., 1994; Zahran, 1999; Giller, 2001	Use of acid-tolerant legume cultivars and rhizobia; liming of soil to pH at which Al and Mn are no longer toxic
P deficiency	Inhibits nodulation, N_2-fixation and rhizobial growth	Gates and Muller, 1979; Cassman et al., 1981	Addition of P fertilizers, amelioration of soil acidity, inoculation with effective mycorrhiza, and selection of P-efficient cultivars
Salt stress	Reduces nodule formation, respiration and nitrogenous activity	Tu, 1981; Delgado et al., 1994	Select salt-tolerant strains
High soil N level	Inhibits root infection, nodule development & nitrogenous activity	Abdel-Wahab et al., 1996; Imsande, 1986; Arreseigor et al., 1997	Breed cultivars which are less sensitive to mineral N
Herbicides, fungicides and insecticides	Inhibits rhizobial growth; reduces nodulation and N_2-fixation; deforms root hairs and inhibits plant growth	Mallik and Tesfai, 1993; Isol and Yoshida, 1990	Test the particular rhizobial inoculum and its behavior in respect of the product used before application; separate placement of rhizobia and fungicides
Competition from native organisms	Suppression of inoculation by native rhizobia	Dowlig and Broughton, 1986	Targeted research

Kirda et al., 1989). Similarly, Sinclair et al. (1987) observed that N_2-fixation in soybean was sensitive to drought, a situation that underscores the shortcomings of N_2-fixation in the drylands, where 38% of the rural poor in SSA live (Ryan and Spencer, 2001). High temperature, soil acidity and salinity also inhibit growth of *Rhizobium*. Nutrient deficiencies, especially P, and the lack of efficient strains of rhizobia are also among the major factors limiting symbiotic N_2-fixation (Zaman-Allah et al., 2007).

These and other factors have led to efforts to inoculate legumes. Therefore, trials for establishing the need for inoculation should include tests for the limitation of BNF by other nutrients (Bohlool et al., 1992). For example, El-Hamdaoui et al. (2003) observed that application of boron and calcium increased N_2-fixation in salt-stressed soils.

3.3 Nitrogen Fixation by Promiscuous Soybean Varieties

A major advantage of promiscuous soybean varieties is their indeterminate growth, resulting in the production of large biomass (Mpepereki et al., 2000). Results from measurements based on the ^{15}N isotope dilution method showed that the amount of N_2 fixed by five promiscuous soybean varieties planted for 2 years in the southern Guinea savanna of Nigeria averaged

Table 3 Nitrogen fixation (kg/ha) by selected breeding lines grown for two seasons in three sites with poor N in the Guinea savanna of Nigeria

	Site			
Breeding line	Gidanwaya	Mokwa	Zaria	Means
High fixers:				
1485-ID	63	71	55	64
1830-20E	67	74	42	61
1526-5E	60	64	57	60
1798-7F	54	75	43	58
1799-8F	44	72	53	57
Intermediate fixers:				
1838-10E	47	50	39	46
1831-28E	39	54	38	44
1837-6E	40	52	36	43
1833-20E	36	60	33	43
Samsoy-2	34	54	38	42
Low fixers:				
1838-5E	45	34	21	33
1805-33F	37	27	36	33
1740-3F	32	37	27	32
1837-2E	29	35	32	32
1814-2E	23	32	37	31
Means	41	55	39	45
LSD[a]	26	25	19	13

[a] LSD = least significant difference.
Source: Sanginga et al., 2000.

91 kg N ha^{-1}, representing 46% of the total plant N. In general, however, different lines of promiscuous soybean varieties growing on the same soil vary considerably in their ability to fix nitrogen (see Table 3, further summarized in Table 4).

Differences in their efficiency for N_2-fixation among the genotypes, reproducible across sites, have also been a basis for selection in current soybean breeding work of the IITA, aimed at enhancing N_2-fixation in the absence of inoculation (Sanginga et al., 2000).

4 Success in the Use of Inoculants to Increase Yields: Trials

Early inoculation studies indicate yield advantage in soybean grain yield in tropical Africa (e.g., Sivestre, 1970; Nangju, 1980; Bromfield and Ayanaba, 1980). Sivestre (1970) noted yields of 1440 kg ha^{-1} in inoculated soybean compared with 240 kg ha^{-1} for the uninoculated. Bonnie (1957, cited by Bala, 2008) had reported yield increases of 80–300% with inoculation in DR-Congo. In Nigeria, a series of field experiments conducted in 1978 to screen strains of *B. japonicum* that were efficient in N_2-fixation showed that grain yields of the American soybean cultivars Bossier and TGm 294-4 were increased by as much as 100%, while cultivars of Asian origin showed no significant response (Ranga-Rao et al., 1981). Inoculation also led to grain yield increases of 40–79% in American soybean cultivars when grown in the southern Guinea savanna of Nigeria (Nangju, 1980; Pulver et al., 1982; Ranga-Rao et al., 1984). Bromfield and Ayanaba (1980) reported that inoculation of soybean in acid sands of south-eastern Nigeria resulted in grain yield increases of 300–500% when lime was added and 270–970% in the absence of lime.

In an on-farm experiment in two districts (Moshi and Rombo) in northern Tanzania, using rhizobial inoculants (*Rhizobium tropici* strain CIAT 899 for common bean, and *Bradyrhizobium japonicum* strain USDA 110 for soybean), Ndakidemi et al. (2006) observed that at harvest, soybean and common bean growth was significantly ($P \leqslant 0.05$) greater with (brady)rhizobial inoculation compared with N and P supply or uninoculated control. Relative to uninoculated unfertilized plots, grain yields of common bean increased from inoculation alone (60–78%) and from inoculation +26 kg P/ha (82–95%); with soybean,

Table 4 Nitrogen fixation by class of soybean breeding lines in three sites with poor N in the Guinea savanna of Nigeria

Class of breeding line	Lower end N fixed (kg/ha)	Upper end N fixed (kg/ha)	Mean N fixed (kg/ha) across sites	Lower end breeding & site	Upper end breeding & site
Low N-fixers	21	45	32.3	1838-5E (Zaria)	1838-5E (Gidanwaya)
Intermediate N-fixers	33	60	43.3	1833-20E (Zaria)	1833-20E (Gidanwaya)
High N-fixers	44	75	59.6	1799-8F (Gidanwaya)	1798-7F (Mokwa)

Source: derived from Sanginga et al., 2000.

there was 127–139% increase in grain yield from inoculation alone, and 207–231% from inoculation +26 kg P/ha. Thus, the combined application of bacterial inoculants and P fertilizer to soybean and common bean increased biomass production and grain yield compared with the singular use of N and P or (brady)rhizobial strains (Ndakidemi et al., 2006). Economic analysis shows that the increase in grain yield with inoculation translated into higher marginal rate of return (MRR) and profitability for soybean and common bean small farms. With common bean, relative to the control, there was 66% increase in profit with inoculation in the Moshi district and 92% in the Rombo district; with provision of supplemental P (26 kg P/ha), these profit margins rose to 84% (Moshi) and 102% (Rombo). With soybean, however, the increase in profit with inoculation was much larger, about 140% (Rombo) and 153% (Moshi). With P supplementation, these rose to 224% (Rombo) and 250% (Moshi). In Zimbabwe, studies have shown that, depending on soil fertility and rainfall, inoculant use can be more cost-effective than mineral fertilizer application (Brenner, 1996). However, Kipkoech et al. (2007, p. 18) compared the efficacy of *Rhizobium* (in groundnut cropping systems) with other soil fertility-enhancing technologies (Diammonium phosphate, DAP, NPK and Farm yard manure, FYM) and the control in western Kenya. Results show that groundnut yield under *Rhizobium* inoculation (1362.9 kg/ha) ranked third after yield under DAP (1800 kg/ha) and NPK (1646 kg/ha). It was, however, better than the yield under FYM (1218.5 kg/ha) and the control (1208.7 kg/ha). The benefit cost ratio (BCR) follows the same trend, with *Rhizobium* inoculation coming third (with a BCR of 2.5:1) after DAP (with a BCR of 3.0:1) and NPK (with a BCR of 2.8:1). With a BCR of 2.2:1, FYM even trailed behind the control (BCR of 2.4:1) (Kipkoech et al., 2007, p. 19). These results show the importance of promoting inoculant use in African agriculture, especially among resource-poor farmers who cannot afford expensive mineral fertilizers (Ndakidemi et al., 2006). Besides, economic analysis of another four trials showed that farmers gained 19% more benefits from inoculation compared with urea application (Tran, 2004). In virtually all situations, there was economic benefit of inoculation, both for the legume itself and for subsequent crops. Soybean N_2-fixation has an economic value in terms of the N that it supplies to the plant from the air which otherwise would need to come from soil and/or fertilizer sources. There is also an economic value in the residual benefits for soil N fertility and increased productivity of subsequent crops (Tran, 2004). In most soils, the savings were equivalent to 40–60 kg N/ha. In a report by Duong et al. (1984), 240 kg N/ha would have been required to produce an equivalent grain yield to the inoculated treatment.

The key lesson learned with the successes of *Rhizobium* inoculation based on on-station experiments or experiments simulating farmers' conditions is that it does not really guarantee widespread uptake by the smallholder farmers, no matter how attractive the results are.

5 Success in the Use of Inoculants to Increase Yields: Farmers' Fields

Based on the obvious benefits of inoculation of legumes with suitable *Rhizobium* strains, a lot of trial research has been directed at BNF in SSA, with significant advances made. This section reviews the farm-level adoption of inoculants and the growing of promiscuous soybean varieties in Africa. Smallholder farmers in many parts of the moist savanna of Nigeria have widely adopted promiscuous soybean varieties, especially the high-yielding TGx 1448-2E (Manyong et al., 1998; Sanginga et al., 2001). In southern Africa, the exceptionally promiscuous nature of 'Magoye' led to its widespread promotion and adoption (Mpepereki et al., 2000). However, inoculating soybean with effective rhizobia is a key strategic research intervention that has contributed to significant improvement in the productivity of soybean in SSA. The development of local production of inoculants in Rwanda led to rapid expansion of the area under soybean production (Saint Macary et al., 1986). In Zimbabwe, a soybean promotion program including the use of inoculants saw to its widespread adoption by smallholder farmers, with farmer education on inoculation being a major driving force for the success of the program (Marufu et al., 1995). Presently, over 55 000 smallholder farmers in Zimbabwe inoculate and grow soybean as a result of this promotion (Mpepereki, pers. commun.). Initially designed to promote the cultivation of 'Magoye', farmers' improved access to seeds and inoculants encouraged them to adopt both 'Magoye' and specifically nodulating cultivars. Farmers were keen to plant the latter for their high yields and income generation, and 'Magoye' for its non-requirement for inoculants as well as its large biomass used as fodder and for soil fertility maintenance and improvement. Overall, the trial discoveries have led to very few opportunities for enhancing grain legume (e.g., soybean) production among smallholder farmers in Africa due to limited farm-level availability and adoption of inoculants (Bala, 2008). There is, therefore, relatively little evidence (across SSA) to show any substantial inoculation practice among smallholder farmers.

Key lessons learned on the drivers of success at farmers' field level indicate that these include: (i) widespread demonstration of the inoculants with particular attention to the needs of small farmers; (ii) within-country collaboration and involvement of media agencies; (iii) well-coordinated collaborative research-for-development efforts; (iv) involvement of top government officers and decision-makers; (v) joint efforts of a number of national, international and private-sector organizations over many years; (vi) involvement of the private sector in production and dissemination of the inoculants, and (vii) farmer education on inoculation. These strategies have worked well in pilot areas and should be scaled up and out to reach more smallholder farmers that produce the bulk of the food (especially grain legumes) eaten in SSA. This scaling could be attained through an appropriate innovation platform involving all stakeholders. Appropriate incentives are also needed to support the private sector and industries for an effective role and delivery in the scaling up of the inoculant technology.

6 Limitations to the Use of Inoculants to Increase Yields

There have been several failures in the effort to use inoculants to increase the yields of grain legumes in different parts of the world, especially SSA. Adoption and use of bio-fertilizers is very limited among small farmers in many African countries such as Kenya (Odame, 1997). This author also noted that many of the modern biotechnology products (Bt. Maize, transgenic sweet potato, genetically-engineered livestock vaccines, etc.) have yet to be used by farmers. Private technology markets are undeveloped in many SSA countries (Brenner, 1996). Therefore, specific measures, such as tax incentives and exemptions, will be needed to stimulate the development of BNF technology markets and the creation of local firms (Brenner, 1996). Kueneman et al. (1984) described inoculation of soybean with specific strains of *Bradyrhizobium japonicum* as an investment that most farmers cannot afford. With very few exceptions, countries in SSA do not have industries to produce viable inoculants at prices that smallholder farmers can afford (Kueneman et al., 1984). In West Africa, other than experiments that are limited to research farms, there is hardly any country where rhizobial inoculation is commonly undertaken by farmers. The Dakar (Senegal) MIRCEN, which has a mandate for inoculant production, does not appear to be actively involved in either inoculation trials or the production of inoculants. The most visible BNF endeavors appear to come from East and Southern Africa. In Zimbabwe, the cultivation of specifically nodulating soybean with inoculants was promoted among smallholder farmers in the 1980s. However, when the project ended, soybean cultivation failed to make any appreciable increase, largely because of farmers' difficulty in accessing seed and inoculants (Mpepereki et al., 2000). In Kenya, in line with the obvious potential of *Biofix* to replace the often unavailable and expensive mineral fertilizers, together with the Agricultural Society of Kenya (ASK), the Nairobi (Kenya) MIRCEN sells inoculants to farmers. Between 1992 and 1993, average sales were estimated at 1350 kg per year (Mugabe, 1994). However, cutbacks in government expenditure as a part of the structural adjustment policies resulted in a 40% reduction in financial support to extension programs, affecting their agricultural programs (Mugabe, 1994). This demonstrates the unsustainable dependence on government as a major consumer of the inoculants and the need to commercialize inoculant marketing and consumption. There is also the lack of specific policy incentives to stimulate the involvement of the private sector at all stages of the innovation process to induce adoption (Brenner, 1996).

Experiences from Zambia show that the technical feasibility of *Rhizobium* inoculants for common beans remains doubtful (Sakala, 1990; Mvula et al., 1996). Elsewhere, it has been noted that, except for soybean, responses to inoculation are sporadic (Silver, 1976), mainly due to the presence of an adequate and aggressive native rhizobial population (Woomer et al., 1997), ineffective strains (Parker, 1977) or competition from indigenous rhizobial flora (Silver, 1976; Miller and May, 1991; Woomer et al., 1997). In Malawi, inoculant use has not been widely adopted by smallholder farmers, largely because they are mostly not well informed of the technology. Another problem is the fact that inoculants require refrigeration, an investment that is obviously beyond an average smallholder farmer (Khonje, 1989).

6.1 Lessons from the Reasons for Failure in the Use of Inoculants to Increase Yields

Some key lessons have been learned on the causes of failures in the use of inoculants to increase the yield of grain legumes. These include: (i) a disconnect between farmers' problems and biotechnological emphasis, (ii) the path from research to development and to dissemination of a biotechnology product is fraught with uncertainty (Brenner, 1996), (iii) policy and institutional arrangements are critical for widespread dissemination and adoption of *Rhizobium* inoculants, (iv) increase in the involvement of other local actors (e.g., private entrepreneurs, NGOs, etc.) may be more sustainable interventions for BNF distribution than total reliance on government agencies (Odame, 1997), (v) the problems of Biofix were caused by a variety of technical factors that include: complexity of tropical soils, selectivity of *Rhizobium* strains in infecting specific legume species, residual effects of inoculation, and the need for phosphate fertilizers to stimulate nodulation and nitrogen fixation. The other lessons are: (vi) farmers' concerns on the efficacy of *Rhizobium* inoculants (e.g., Biofix) in increasing crop yields as well as their operational feasibility, (vii) inadequate participatory and interactive approach among key stakeholders (including farmers) in the development of *Rhizobium* inoculation technology, (viii) production and marketing of inoculants (e.g., Biofix) was centralized, affecting effective distribution, (ix) weak linkages with private-sector manufacturers, local stockists, NGOs and farmers, and (x) inadequate capacity building and technical assistance for African Universities in *Rhizobium* inoculation technology development.

7 Constraints and Challenges to Production and Farmers' Use of Inoculants in SSA

This section reviews the social, economic, cultural and policy constraints and challenges to the production and farmers' use of *Rhizobium* inoculants in SSA. These, including their effects and possible recommendations, are summarized in Table 5. Due to limited capacity many national agricultural research systems in SSA lack the skills to set priorities in the application of biotechnology. This situation hampers the development of BNF technologies. Current research and development programs in Africa are often isolated, with little coordination, coupled with inadequate funding. Most of the time, these organizations are not need-driven and lack the ability to develop specific products. Besides, there is weak protection of intellectual property rights in many parts of Africa, hampering innovations, inventions, investments, and development of new technologies including biotechnologies.

7.1 Socioeconomic Constraints and Challenges

A successful transfer of *Rhizobium* inoculation technology from the laboratory to farmers' field depends on some crucial interactions among many players (research, policy, etc.). Farmers' access to inoculants remains the most controversial phase in the evolution of *Rhizobium* inoculation technology (Woomer et al., 1997) and explains why despite its acclaimed attributes the use of inoculants among smallholder farmers has been limited, making it a technology with a low rate of adoption.

Most farmers in SSA are not aware of inoculants or that legumes fix N in their nodules, yet traditional and modern farming systems almost invariably include grain legumes. Contrary to the thinking of Kueneman et al. (1984), the cost of inoculants is probably not usually a constraint to farmers who normally set aside some funds for seeds that are clearly more costly than *Rhizobium* inoculants. However, for farmers that use non-commercial seeds, there may be little incentive to purchase inoculants. Poshiwa et al. (2006) found that inoculant awareness in Zimbabwe was extremely

Table 5 Socioeconomic and policy constraints to the use of *Rhizobium* inoculation technology in sub-Saharan Africa and possible intervention measures

Socioeconomic and policy constraint	Effect	Reference	Recommendations
Limited farmer awareness of and access to inoculants	Low adoption and use of inoculants in farming systems	Odame, 1997; Woomer et al., 1997; Kipkoech et al., 2007	Private sector involvement
Poor quality control of inoculants	Low viability of *Rhizobium* inoculants and uncertain performance	Johnson et al., 1994; Odame, 1997	Cold storage, use of modern technologies, and more research
Lack of trained personnel	Limited awareness by farmers of the existence of BNF (including inoculants)	Kannaiyan, 1993; Odame, 1997; Woomer et al., 1997	Raise farmer awareness about legume root nodules; familiarize farmers with *Rhizobium* inoculants
Fear over possible human and livestock health risks of inoculants by farmers	Limited adoption and use of *Rhizobium* inoculants to increase legume productivity	Odame, 1997	Involvement of farmers in the process of development of inoculants; participatory approach
Absence of policy or weak policy support and insufficient biotechnological framework	Forestalls widespread adoption; weak development of the production and marketing of inoculants	Mugabe, 1994; Odame, 1997; Silver and Nkwiine, 2007	Include the issue of bio-fertilizers in governments' effort towards addressing the problems of low and declining soil fertility
Limited scientific expertise, applied BNF brain drain, and poor research funding	Limited production of inoculants and low quality inoculants	Brenner, 1996; Ndakidemi et al., 2006	Linkages between Universities in SSA with those in the North with expertise in *Rhizobium* science; government policy support

low (2%). In central Kenya, among farmers who cultivated grain legumes, less than 15% used inoculants (Woomer et al., 1999). Although Poshiwa et al. (2006) and Woomer et al. (1999) collected data on social characteristics (e.g., age, gender, education) of the heads of the households surveyed, none of them linked these characteristics with the adoption of BNF, critical information required to explain better the low use being widely observed. The BNF adoption parameters examined by Woomer et al. (1999) includes: legume cultivation, nodule awareness, inoculation benefit awareness and inoculant use. There is the need to expand the knowledge base on BNF utilization among farmers in SSA beyond binary measures (e.g., awareness or use) to include more qualitative aspects of farmers' knowledge, willingness to pay and the long-term relevance of inoculants in farm objectives. Generally, it is necessary to understand the structure and function of knowledge held by farmers, especially the adopters of inoculants, so as to bridge the gaps between farmers' and scientific knowledge.

Brain drain, lack of trained personnel and inadequate resources have also been noted to be a major concern (Odame, 1997), just like the lack of capacity by scientists to understand the full potential for BNF application in local agro-ecosystems. The capacity building efforts on BNF have also been challenged by the mass exodus of plant biologists from applied BNF to other fields (Bohlool et al., 1992). It is clear that lack of complete understanding of legume BNF interactions under diverse farming systems contributes to the low understanding among experts in particular settings. Besides, a vast majority of smallholder farmers in SSA are not aware of the existence of BNF (including inoculants) due to lack of resources to create awareness in BNF and improve its distribution (Odame, 1997). This explains why researchers at the Nairobi MIRCEN project attributed the limited use of Biofix to "technological ignorance of smallholders", poor communication, and limited understanding of *Rhizobium* and plant biology (Kannaiyan, 1993; Woomer et al., 1997). They emphasized the need to raise farmer awareness about legume root nodules (in which *Rhizobium* bacteria live) as part of the familiarization with *Rhizobium* inoculants (Woomer et al., 1997).

There are problems related to inoculant packaging size, influencing uptake potential by farmers. The minimum quantity of inoculants that a farmer has to buy is a key issue. A 100-g packet of inoculants (e.g., Biofix) is needed for 15–16 kg of common bean seed (for instance), sufficient for planting one acre of land. However, few smallholders can plant such a relatively large area with common bean. On average, farmers require only 25 g of Biofix – sufficient for 2–3 kg of common bean seed, to be planted on 0.25 acres of land, which is the approximate farm size usually allocated to legumes in SSA. Thus, if the packet size is not reduced, each packet needs to be opened and shared among several farmers, raising the issue of high administrative and other costs. Lastly, there were fears over possible health risks to young children and domestic animals. Participants at a focus group discussion described Biofix as 'poison', the leftover of which they were unsure of what to do with (Odame, 1997).

7.2 Quality Constraints and Challenges

The uncertain performance of *Rhizobium* inoculants may explain the limited farmer adoption despite their potential to reduce the mineral fertilizer requirement, and reduce cost of production, and the fact they are lighter to transport and are more environmentally friendly than mineral N fertilizers (Odame, 1997; Kipkoech et al., 2007). Poor quality control in inoculant production processes as well as transportation and storage problems negatively affect the viability of inoculants (Odame, 1997). Based on a report for the World Bank, difficulty in obtaining and keeping inoculants was one of the factors that constrained soybean production in Zimbabwe (Johnson et al., 1994). Cold storage which might improve its viability is beyond the reach of smallholder farmers. Another constraint has to do with the limited production of inoculants due partly to limited scientific expertise, inadequately funded agricultural research and extension, and poor infrastructure (Ndakidemi et al., 2006). Limited investment of public funds in R&D and the diffusion of new technology through the national extension systems add to this problem (Brenner, 1996).

7.3 Policy Constraints and Challenges

Rhizobium inoculation has the potential to offer more environmentally friendly agricultural production than the mineral fertilizer-intensive model (Brenner, 1996). However, this will only be realized if certain conditions that involve difficult policy choices and trade-offs are met (Brenner, 1996). From a policy point of view, the constraints and challenges range from a complete absence of to very weak policy support for BNF technologies. Research into the basic mechanisms of BNF processes is an important goal for improving N_2-fixation. Much knowledge on BNF has been gathered with several successes documented, especially in developed countries. However, bio-technology research in SSA has generally been science-driven, ad hoc (and non-holistic), and not integrated into the wider agricultural and overall development objectives (Brenner, 1996). It is concentrated in universities which have little tradition of interaction with farmers and the private sector. The focus of scientists has been on the supply side of biotechnology (Brenner, 1996). Even in that area, there are still issues widely unattended to, including bio-safety and intellectual property rights.

Lack of policy and political support at the national level has been blamed for forestalling widespread adoption of *Rhizobium* inoculation and other suitable forms of biotechnology (e.g., germplasm) in SSA (Odame, 1997; Silver and Nkwiine, 2007). Efforts of many governments to address soil fertility problems in SSA have mostly been directed to mineral fertilizer subsidies (Jayne and Boughton, 2006). The limited commitment to non-fertilizer soil fertility management options has contributed to the weak development of the production and marketing of inoculants in SSA. For instance, *Nitrosua*, developed by SUA, could not be effectively disseminated to local farmers in Tanzania due to poor government budgets for agricultural extension (Mugabe, 1994).

In many SSA countries, bio-fertilizers are not fully addressed in national fertilizer recommendations (Odame, 1997). Only a few SSA countries (e.g., Zimbabwe) have set up national biotechnology institutions and implemented bio-safety procedures (Brenner, 1996). Besides, the question of IPRs (Intellectual Property Rights) related to biotechnology is still unresolved in most countries (Brenner, 1996).

The current research agenda on BNF has evolved due mostly to individual efforts with limited government policy support (Mugabe, 1994). African governments must reconsider their policy on agricultural biotechnology research so as to provide a strong institutional basis for BNF (Mugabe, 1994). In the same vein, although genetically modified plants could result in increased productivity, serious concerns (e.g., health and environmental safety, etc.) have been raised over their R&D policy process in SSA (Odame, 1997). To efficiently develop, produce and disseminate new technologies, an elaborate value chain [to enhance scaling up and involving key stakeholders (researchers, farmers, extension agents, agro-input dealers, etc.)] adequately backed up with relevant policies is required. There is limited linkage among inoculant stakeholders presently. Although such linkages are poor in most other agricultural technologies in SSA, the inoculants' value chain is probably among the most poorly developed value chains, limiting its trade and dissemination.

7.4 Other Constraints and Challenges

These do not strictly fall into either socioeconomic, quality or policy categories. The most important has to do with decision-making about the level of biotechnology required in different SSA countries. These challenges fall into: (i) development of the knowledge base for appropriate decision-making on use of biotechnology approaches, (ii) lack of sufficient studies that have clearly analyzed biotechnology-related data to assist policy-makers on *Rhizobium*-related policies, (iii) lack of priority setting for biotechnology aimed at solving problems of national importance, (iv) capacity development, and (v) the establishment of linkage and cooperative mechanisms for biotechnology development, transfer, and sustainable applications. The emphasis given to *Rhizobium* in bio-fertilizer research shows its high specificity to only legumes (common bean 47%; lucerne 23%; soybean 14%; desmodium (a leguminous pasture species) 9%; and other minor legumes 7%; in that order) unlike mycorrhiza, that works in 80% of all plants (Odame, 1997). A wider use of *Rhizobium* inoculants in marginal areas depends on the ability to develop strains which are tolerant to high temperatures, soil acidity, drought and salinity (Odame, 1997).

8 Opportunities for Production and Use of Inoculants in Africa

Given that widespread decline in soil fertility and agricultural productivity has largely been blamed for widespread poverty in Africa and that biotechnology has great potential to lead to increases in agricultural production, as has been demonstrated in some of the instances in this review article, we see a great opportunity for increases in the production and use of *Rhizobium* inoculants in the farming systems of SSA. The recent (2008) increases in the price of mineral fertilizers (even over and above the oil prices), driven by growing demand for mineral fertilizers resulting from commercial responses to increasing food prices (IFDC, 2008; Nehring et al., 2008) have also reinforced the need to develop alternative soil fertility management strategies. The price of nitrogen-based fertilizers rose from US$277 per ton in January 2007 to over US$450 per ton in August 2008 (IFDC, 2008). The dramatic increase and soaring prices of food reveal the large potential for developing and disseminating BNF and inoculation technologies to poor farmers who cannot afford the high fertilizer prices. The increasing concern about environmental pollution also presents an opportunity for increasing efforts to develop and promote BNF and inoculation. It is estimated that only 30–40% of fertilizer applied worldwide is used by plants. The remainder is lost, especially through leaching and volatilization, etc. (Hardarson et al., 2003).

However, these opportunities can be exploited if SSA countries develop long-term policies on BNF and biotechnology in general. Such policies should: (i) promote national biotechnology need assessment and implementation, (ii) target biotechnology research and execution to needs, (iii) provide incentives and environment for commercialization of biotechnology research and enterprises, (iv) promote partnerships among local public R&D and foreign industries in biotechnology, (v) improve scientific capacities and technological infrastructure for optimal biotechnology execution, and (vi) integrate biotechnology risk management into existing environmental, health and agricultural regimes. Otherwise, the potential benefits of biotechnology (e.g., *Rhizobium* inoculation) may not be tapped for the improvement of human welfare in SSA. Besides, policy related to biotechnology (e.g., BNF, *Rhizobium* inoculation) should address the need to: (i) strengthen institutions (e.g., agricultural extension, NGOs) that serve the interests of smallholder farmers as they adopt biotechnology, (ii) enhance their capacity, and (iii) improve their participation in adapting and testing BNF and *Rhizobium* inoculation technologies. Instead of ad hoc approaches, SSA countries must opt for an integrated approach for biotechnology, which also requires policy intervention (Brenner, 1996). The integrated approach will ensure that biotechnology research is at the service of problems confronting smallholder agriculture.

9 Highlights and Key Messages

Given the unsettled state of knowledge on the response of different varieties of soybean to inoculation and the fragmentary evidence of its economic benefits at the farm level, there is the need to initiate further studies on inoculation response of promiscuous soybean varieties as well as the commercial or specifically nodulating soybean varieties. Holistic studies addressing the challenges facing the use of inoculants by farmers have been rare in SSA. For instance, unlike experimental data, only a few studies have examined the socioeconomic and policy constraints affecting household adoption and utilization of inoculants by farmers. Trials for establishing the need for inoculation should include tests for the limitation of BNF by other nutrients (e,g., boron, calcium, etc.).

Secondly, there is also a pressing need for more in-depth analysis of short- and long-term economic and social costs and benefits of *Rhizobium* inoculation and the need to expand the knowledge base on BNF utilization among farmers in SSA beyond binary measures (e.g., awareness or use) to include more qualitative aspects of farmers' knowledge, willingness to pay and the long-term relevance of inoculants in farm objectives.

Thirdly, policies and institutions promoting the development of soybean inoculants and widespread farmer adoption for increased production of both promiscuous and commercial soybean varieties are needed. This must be accompanied by targeted research to effectively address the specific needs of specific soybean growing areas in SSA. This is because, despite its potential to address low N and its "assumed" cost effectiveness and importance in ensuring sustainable and low cost production by smallholder farmers in SSA, the demand for inoculants remains low for reasons that include poor quality, and inadequate and inefficient marketing channels and outlets, as well as inadequate extension services covering inoculant use. In virtually all situations, there was economic benefit of inoculation, both for the legume itself and for subsequent crops. The lesson learned with the successes of *Rhizobium* inoculation based on on-station experiments or experiments simulating farmers' conditions is that it does not really guarantee widespread uptake by the smallholder farmers, no matter how attractive the results are. Like *Rhizobium* inoculants, many of the modern biotechnology products (Bt. Maize, transgenic sweet potato, genetically-engineered livestock vaccines, etc.) have yet to be used by SSA farmers, especially given that private technology markets are still highly undeveloped in many SSA countries. Specific measures, such as tax incentives and exemptions, will be needed to stimulate the development of BNF technology markets and the creation of local firms. There is the need for specific policy incentives to stimulate the involvement of the private sector at all stages of the innovation process to induce adoption. The trial discoveries have led to very few opportunities for enhancing grain legumes (e.g., soybean) production among smallholder farmers in Africa due to limited farm-level availability and adoption of inoculants (Bala, 2008). There is, therefore, relatively little evidence (across SSA) to show any substantial inoculation practice among smallholder farmers. There is the need for SSA countries to develop long-term policies on BNF and biotechnology in general.

Fourthly, the review article indicates that *Rhizobium* inoculation is needed in all agricultural lands deficient in N and where N supply is a key limiting factor in crop production, especially since it is not clear whether promiscuous soybean cultivars are effectively nodulated by indigenous rhizobial populations in all soils and under all conditions (e.g., soil fertility and rainfall status). Although smallholder farmers in selected countries in SSA (e.g., Nigeria, Zimbabwe, Zambia, etc.) have widely adopted promiscuous soybean varieties (e.g., TGx 1448-2E, Magoye, etc.), inoculating soybean with effective rhizobia is still a key strategic research intervention that has the potential to significantly contribute to noticeable improvements in the productivity of soybean in SSA. However, some exceptionally promiscuous soybean varieties (e.g., Magoye) nodulate readily in most soils and rarely respond to inoculation. Results also confirmed the importance of promoting inoculant use in African agriculture, especially among resource-poor farmers who cannot afford expensive mineral fertilizers (e.g., DAP, NPK, etc.).

Fifthly, experiences from some SSA countries (e.g., Zambia) show that the technical feasibility of *Rhizobium* inoculants for some grain legumes (e.g., common beans) remains doubtful. It has also been noted that, except for soybean, responses to inoculation are sporadic, mainly due to the presence of adequate and aggressive native rhizobial population, ineffective strains or competition from indigenous rhizobial flora. Finally, there is the need to involve other local actors (e.g., NGOs, agro-input dealers, etc.) to ensure more sustainable interventions for BNF distribution than total reliance on government agencies. There is also widespread lack of skills to set priorities in the application of biotechnology in SSA, hampering the development of BNF technologies and often leading only to isolated cases with little coordination and inadequate funding. The increasing concern about environmental pollution also presents an opportunity for increasing efforts to develop and promote BNF and inoculation.

10 Conclusion

Several conclusions could be drawn from the outcomes of this review article. Firstly, while in principle, privatization of public sector institutions and activities is being encouraged, biotechnology R&D in SSA is presently focused on improving agriculture with over 85–90% of the biotechnology R&D in the region still within the public sector. Little or no incentives have been provided to encourage private sector interest in the development and application of biotechnology products. Except for South Africa, development, production and dissemination of biotechnology are limited to public institutions. Secondly, most issues around *Rhizobium* inoculation in parts of SSA still revolve around research with little or no effort to disseminate the results and products. Virtually no attention has been given to the demand side and to the ways in which biotechnology could best contribute to solving farmers' production problems. Most research activities and information generated started and ended with scientists who are yet to finalize even on issues of quality control. There were unsustainable interventions in the *Rhizobium* inoculant distribution, due to too much reliance on government agencies and little or no serious involvement of local actors (e.g., private entrepreneurs, non-governmental organizations, farmers' cooperatives, local stockists, trade associations, etc.). A coordinated program is needed to develop a capacity to produce and supply sufficient quantities of high quality inoculants to farmers and to educate extension workers and farmers about the benefits of inoculation. Thirdly, although bio-fertilizers could potentially benefit smallholder farming systems in SSA, policy support necessary to create a suitable environment for the active participation of non-government agency players is critical to ensure widespread production, distribution and application of bio-fertilizers. Unfortunately, such policies are nearly absent in most SSA countries. Fourthly, bio-fertilizer research in SSA has been science-driven, *ad hoc*, uncoordinated and unintegrated. Attention has essentially been focused on the supply side (e.g., training of scientists in molecular biology, biochemistry, microbiology, etc.). Little effort has been made to set clear priorities and to integrate research efforts with the broader objectives set for agricultural research. There is little interaction among the multiple actors and institutions (e.g., biotechnologists and plant breeders; the public and private sectors; scientists and farmers; and among the scientific community and policy-makers) most closely involved with biotechnology R&D and diffusion. Indeed, public research institutions at times tend to compete (rather than collaborate) for scarce resources. More importantly, there is little interaction and/or collaboration between the public and private sectors. Fifthly, efforts to develop rhizobial inoculants also need to be accompanied by research that facilitates their efficient use that is relevant to resource-poor farmers. Breeding programs can also help to develop crop varieties that nodulate under abiotic stresses. Finally, there is a strong need to train policy-makers and scientists to increase their awareness of the need to reshape biotechnology towards the needs of smallholders, and for farmer participation in prioritizing areas to be addressed by biotechnology research and policy.

Acknowledgements The authors wish to thank Dr. Abdullahi Bala of the Federal University of Technology, Minna, Nigeria and Professor Ken Giller of Wageningen University for their critical review of and constructive comments on the earlier versions of this review article.

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Conventionalisation of Organic Farming Practices: From Structural Criteria Towards an Assessment Based on Organic Principles

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Contents

Abstract Organic farming is widely perceived as being more environmentally friendly than conventional farming. As a form of sustainable agriculture, it receives substantial support from policy for its contribution to environmental protection as well as the provision of amenities such as biodiversity and cultural landscapes. Consumers are attracted to organic foods as they are produced without synthetic chemicals and comply with higher animal welfare standards. Although organic farming certainly has the potential to fulfil these expectations, studies have shown that some certified organic farms do not. Their practices comply with the regulations, but not with the principles of organic farming. This trend has been called ‘conventionalisation’ of organic farming. In this paper we review the studies that discuss the conventionalisation of organic farming, focusing on the farm level and on evidence from Europe. We argue that to strengthen organic farming’s transformative potential, the debate must move beyond its focus on the bifurcation between artisanal and conventionalised organic farms, so as to capture the full range of empirical heterogeneity. Our core argument is that to adequately understand the dynamics within organic farming and their potential impact on the ability of organic farming to fulfil the expectations of consumers and policy-makers, it is not sufficient to focus on structural changes. Instead, we

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_18,

from Darnhofer et al., Agron. Sustain. Dev. 30 (2010) 67–81. DOI: 10.1051/agro:2009011

need to assess whether or not the observed changes comply with the principles and values that are the fundament of organic farming.

Keywords Organic agriculture • Conventionalisation debate • Europe • Plant production • Animal husbandry

1 Introduction

Organic farming was developed in the 1940s in Switzerland by Hans Müller, Maria Biegler and Hans Peter Rusch, as well as in the United Kingdom by Lady Eve Balfour and Sir Albert Howard. Their developments were based in part on the concepts of biodynamic farming initiated in the 1920s by Rudolf Steiner in Germany. However, it was only in the 1980s, when the negative impact of intensive conventional agricultural methods became apparent, that interest in organic farming reached a broader public. In the European Union (EU) organic farming has received policy support since the early 1990s for its potential to contribute to environmental protection, rural development and animal welfare (EC, 2004; Häring et al., 2004; Nieberg et al., 2007). Organic farming is also linked to the production of quality food, and policies have been implemented to ensure transparency and fair competition on the market. Support by consumers and by policy-makers has resulted in a sizeable uptake of organic farming methods. In 2005, around 4% of the Utilised Agricultural Area of the 25 Member States of the EU was certified organic, representing over 6 million ha and nearly 158 000 organic producers (Eurostat, 2007). In the last decade the market for organic products has grown steadily, both in Europe and elsewhere, and is expected to keep increasing (Michelsen et al., 1999; Willer et al., 2008).

Research on agronomic aspects of organic farming has shown that crop yields tend to be lower in organic farming (Mäder et al., 2002; Kaut et al., 2008), albeit less variable and less susceptible to drought (Lotter, 2003). Organic farming methods tend to increase soil organic matter and thus enhance soil fertility (Langmeier et al., 2002; Mäder et al., 2002), making it less dependent on external inputs. Organic farming may also provide ecosystem services, increase biodiversity and have a positive impact on the landscape (Letourneau and Bothwell, 2008; Norton et al., 2009). However, studies have pointed out that the potential benefits of organic farming are not always realised in all places and under all management systems (e.g., Trewavas, 2001; Rigby and Cáceres, 2001; Degré et al., 2007; Letourneau and Bothwell, 2008).

In the social sciences, there have been numerous studies on reasons for farmers to convert to organic farming (e.g., Fairweather, 1999; Padel, 2008) and for consumers to purchase organic foods (e.g., Brand, 2006; Holt, 2006). Research has also addressed the potential of organic farming to contribute to endogenous rural development, through fostering direct producer-consumer relationships (e.g., Moore, 2008; Renting et al., 2008; DuPuis and Gillon, 2009) or through service provision (e.g., Darnhofer, 2005; Schermer, 2006; Frederiksen and Langer, 2008; Lobley et al., 2009). Finally, a number of researchers have analysed the influence of legal regulations as well as of the entry of agribusiness and supermarkets into the organic food chain (e.g., Allen and Kovach, 2000; Alrøe and Noe, 2008; Tomlinson, 2008). However, as Lamine and Bellon (2009) have shown, there has been little dialogue between the agricultural and social sciences as well as a general lack of emphasis on the trajectories of organic farms and the understanding of transitions.

Transitions in organic agriculture and the trajectories of organic farms play a central role in the debate surrounding the conventionalisation hypothesis. The hypothesis was first put forward by Buck, Getz and Guthman in 1997 and has led to an on-going, intense debate on how the developments within organic farming can be understood and which patterns can be discerned. The debate essentially hinges on two aspects: whether the developments observed in the organic vegetable sector in California are universal and whether they are inevitable.

According to the conventionalisation hypothesis, organic farming is becoming a slightly modified version of modern conventional agriculture, replicating the same history, resulting in many of the same social, technical and economic characteristics (Buck et al., 1997; Hall and Mogyorody, 2001; Guthman, 2004a). Organic farming could thus be subjected to 'industrialisation', i.e. the implementation of economies of scale at the farm level (larger farms), increased reliance on purchased non-farm inputs (machinery, fertilisers, feed, agrichemicals), resource substitution (capital for land and labour), implementation of organisational features associated with the concept of the 'firm', and mechanisation of the production process (Bowler, 1992). These mechanisms have been observed both at the farm level as well as in processing and marketing of organic food (Guthman, 2004a).

Conventionalisation is widely seen as problematic since organic farming has received public support for its potential to contribute to environmental protection and rural development (CEC, 2004). However, if organic farming increasingly comes to resemble conventional farming, this potential contribution is jeopardised, and organic farming might lose the support it currently receives from both consumers and policy-makers.

In this paper we present a brief review of the debate that has surrounded the conventionalisation hypothesis. We also discuss the empirical evidence that has been gathered to support and modify this hypothesis. We focus on the contributions from the EU[1] as the Common Agricultural Policy and the multifunctional model of agriculture are likely to offer different opportunities and constraints to organic farms than liberal agricultural policies. We will argue that a number of the 'symptoms', that have been used to identify conventionalisation at the farm level, may not be reliable indicators and that they are thus not well suited to achieve a comprehensive assessment of the type and direction of changes in organic farming. To overcome these weaknesses, we suggest that it may be necessary to design an assessment framework that is explicitly based on the ethical principles and values that are the foundation of organic farming. Such an assessment framework could be used both to assess the 'level of conventionalisation' on individual organic farms and to guide their development in accordance with the principles.

In this paper we will focus on conventionalisation at the farm level rather than taking an agrarian political economy approach. This in no way denies the importance of sophisticated, comprehensive studies of food networks (e.g. Goodman, 1999; Green and Foster, 2005; Brand, 2006; Lockie et al., 2006; Follett, 2009), nor does it indicate that we are not aware of the fact that all farmers operate within a larger political and economic framework that affects their agronomical practices (e.g., Allen and Kovach, 2000; DeLind, 2000; Guthman, 2004b; Thomas and Groß, 2005; Obach, 2007; Alrøe and Noe, 2008; Tomlinson, 2008). We also fully acknowledge the importance of consumer motivations (Lockie et al., 2002; Bähr et al., 2004; Padel and Foster, 2005; Holt, 2006) and the role supermarkets can play (Burch and Lawrence, 2005; Konefal et al., 2005) in the development of organic farming. However, we argue that if the imperative of agricultural intensification undermines the potential of organic farming to contribute to agricultural sustainability, then there should be clear evidence at the farm level. Also, as organic quality is mostly based on the production process, it is in large part defined by what happens at the farm level. Arguably, conventionalisation should lead to tangible changes in farm practices, which would threaten the core identity of organic farming. The farm is thus probably the most important level mediating the various influences exerted by the different scales in the hierarchies of agri-food networks.

2 An Overview of the Conventionalisation Debate

2.1 Core Issues of the Debate

Trends towards conventionalisation were first reported from California (Buck et al., 1997; Guthman, 2004a), where high-value crops within the organic vegetable commodity chain were being appropriated by conventionally-based agribusiness. Many of these commercial farms were abandoning the more sustainable agronomic and marketing practices associated with organic agriculture (Guthman, 2000, 2004a). A first thread of the conventionalisation debate thus tries to assess whether such developments can also be identified in other locations and if so, how widespread such developments are. The relevant evidence from international studies has been reviewed by Lockie et al. (2006). They conclude that case studies from around the world, e.g. by Hall and Mogyorody, 2001; Lockie and Halpin, 2005; Jordan et al., 2006, show that concentration, de-localisation, institutionalisation and input substitution certainly are occurring to a significant extent (Lockie et al., 2006).

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[1] Especially in the 15 Member States of the former EU-15, as the Member States that joined the EU since 2004 tend to have a different agricultural structure and heritage, stemming from past socialist policies.

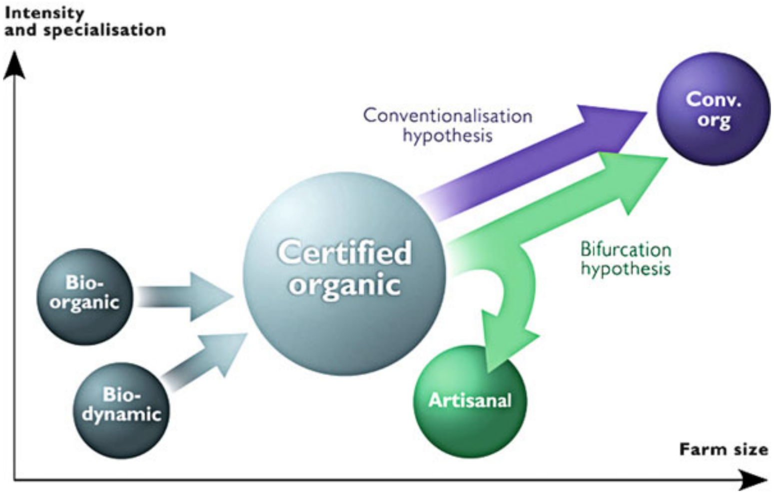


Fig. 1 Schematic development of organic farming from the pioneers in bio-dynamic and bio-organic farming in the 1940s towards certified organic farming in the 1990s and expected further development according to the conventionalisation and the bifurcation hypotheses. (Note: the graphic is indicative of trends and is not based on empirical data.)

A second thread of the conventionalisation debate hinges on whether conventionalisation affects all farms equally, or whether there might be a 'bifurcation' of the organic sector (see Fig. 1). A bifurcation would result if conventionalisation primarily affects large operations that specialise in mass-producing a few high-growth, high-profit crops (Coombes and Campbell, 1998). The smaller, 'artisanal' farms continue to implement diversification strategies, using artisanal methods to grow a variety of marketable crops (Buck et al., 1997; Coombes and Campbell, 1998). This thread of the debate focuses on the ability of these 'artisanal' organic farms to resist the economic pressures exerted by the large operations, i.e. their ability to survive in the long term and ensuring that conventionalisation does not spread to all organic farms.

Guthman (2004b) has argued that agribusiness involvement unleashes the logic of intensification and therefore alters the conditions under which all organic growers operate. Through their control over processing and marketing, and through their introduction of industrial inputs, agribusinesses make the smaller operations less profitable, as they compete directly with the larger producers on the same markets. This puts pressure on the artisanal organic farmers to adopt conventional cropping, labour and marketing practices if they are to survive. The growing constraints in decision-making, coupled with increases in the economic pressures farmers face, could thus cause an erosion of the ethical attitudes and behaviours of farmers (Hendrickson and James, 2005).

Other authors, although acknowledging these pressures, have emphasised the smallholder's ability to resist concentration and specialisation and have pointed out that a number of factors countervail conventionalisation (Coombes and Campbell, 1998; Lockie et al., 2006; Guptill, 2009). These include the technology barriers faced by larger and more monocultural operations; the biological limits to input substitution; the ability of household-based enterprises to cope with unfavourable returns through self-exploitation and reduced consumption; the competitive advantage of small enterprises in a range of markets and under a range of policy conditions; their ability to target market niches; as well as consumer demands for what are perceived to be local, speciality products. Coombes and Campbell (1998), in their analysis of the development of organic farming in New Zealand, not only emphasise that smallholders are expected to survive, but also show that the relationship between small and large growers may well be complementary. Indeed, larger actors may initiate research and market development, and/or focus on different products or markets.

The various contributions to the conventionalisation debate have thus shown that although symptoms of conventionalisation can be identified fairly easily, a widespread conventionalisation of organic farming is (still) constrained. This conclusion needs to be understood as being based on case studies in countries with a liberalised agricultural policy, e.g. California (USA), Ontario (Canada), New Zealand and Australia. In such a context, farmers receive few or no subsidies and are thus more likely to be affected by the vagaries of market forces. However, as Guthman (2004b) points out, the type and degree of state support along with the agrarian structures on which organic farming was built are likely to have a strong influence on the extent, severity and pervasiveness of conventionalisation.

2.2 Conventionalisation in the European Context

In the EU, agriculture is characterised by a vast majority of family farms and a minority of corporate farms. The Common Agricultural Policy has embraced the concept of multifunctional agriculture, thus recognising that farms not only produce food, but also provide services such as landscape amenities, recreational space, environmental protection and preservation of cultural heritage. Whereas large-scale agriculture may be efficient in producing food and fibre, small-scale farms tend to be more efficient in supplying services that are valued at a local level. Farms thus receive direct payments for the provision of public goods. This supportive policy environment creates a different set of opportunities for organic farmers.

Still, case studies assessing the changes in organic farming in Europe report on trends and practices that could indicate conventionalisation processes. For example, the size of organic farms is increasing, e.g. in Denmark (Langer and Frederiksen, 2005) and in Germany (Best, 2008). An analysis of dairy herds in Norway shows that later entrants tend to have a higher level of registered disease treatments per cow, and an intensification of milk production based on a higher use of concentrates (Flaten et al., 2006). Smith and Marsden (2004) point out that the over-supply of some organic products (e.g., milk) has led to a 'farm-based cost-price squeeze' in the UK, which might be forcing farmers into progressively more intensive production strategies. Best (2008) finds that later entrants seem to be somewhat less concerned about the environment. He also reports fewer mixed farms and a drop in direct marketing. De Wit and Verhoog (2007) report conventionalisation trends in organic pig and poultry production in the Netherlands. Conventionalisation has also been reported from arable farming, where permitted fertilisers of conventional origin are increasingly used (e.g. vinasse, a byproduct of the sugar beet industry). The intensive use of fertiliser has resulted in mineral surpluses in the soil and higher nitrate levels, e.g. in organic carrots (De Wit and Verhoog, 2007; Padel et al., 2007). Despite these symptoms of conventionalisation, a Europe-wide study concludes that conventionalisation does not (yet) seem to be a dominant phenomenon in organic farming as a whole (De Wit and Verhoog, 2007).

Thus, in Europe as elsewhere, some organic farms are implementing practices that may not be sustainable but that are not explicitly prohibited by the standards (Padel et al., 2007). Certified organic farming by itself is thus no guarantee for its alternativeness, as has been pointed out by Guthman (2004b). The question thus arises whether the symptoms that have been identified indicate that conventionalisation is progressing, thus undermining the potential contribution of organic farming to quality food and to environmental protection. To answer this question it is not only necessary to assess whether the identified practices are spreading, it is also necessary to assess whether the practices and symptoms reported in the studies are valid and reliably indicate the conventionalisation of organic farming.

2.3 Shortcomings of the Debate Surrounding the Conventionalisation Hypothesis

When critically assessing the methods used in the various studies, a range of weaknesses can be identified. Most of these are due to the fact that the available data does not allow to sufficiently capture the heterogeneity and complexity of the on-going processes within organic farming. Indeed, there has been a focus on analysing aggregated statistical data, and there has been a lack of distinction between farm types, e.g. part-time vs. full-time farmers or corporate vs. family farms. Furthermore, there is a lack of statistical time-series data to ascertain long-term trends. In this section we review some of the approaches leading to conclusions that are based on circumstantial evidence rather than on valid variables and rigorous analysis of comprehensive data sets.

One of the methodological weaknesses lies in comparing early converters with later converters and deriving conclusions on the change in attitudes, values and practices of organic farmers. In this approach, a small group of pioneers who have been organic for an extended period of time are compared with farmers who have limited experience with organic farming as they converted recently. This comparison is fundamentally problematic, as it tends to ignore the learning processes leading to change in knowledge and attitudes that farmers undergo after conversion (Padel, 2008). To reliably assess changes in attitudes, a longitudinal study of both early and later converters would be required, but none has been reported so far.

A similar weakness can be found in studies that analyse aggregated statistical data and assess changes in the ‘average’ organic farm at two points in time. These studies often conclude that organic farms are now larger than they used to be, or that they are more specialised than they used to be. These changes are then assumed to derive from farmers’ preferences, indicating conventionalisation of on-farm practices. The problem with this approach is that the influence of changes in the wider environment (markets, policies, technologies) tends to be under-theorised. The identified differences may thus be the result of processes that are not linked to conventionalisation. For example, a reduction in the share of organic farms involved in animal keeping might be due to the spread of organic farming in a new agro-ecological environment, i.e. a new cohort of organic farms, rather than different practices by the same cohort. For instance, in Austria most organic farms in the period 1995–2000 were grassland-based dairy farms. After the year 2000 arable farms started converting to organic farming, but these had few, if any, animals even prior to conversion. Similar shifts in the national composition of organic farms were also reported from Denmark (Langer, 2002). Thus, data indicating changes in the ‘average’ organic farm must be interpreted carefully, so as not to infer unwarranted causal links.

Unwarranted conclusions can also be the result of a lack of distinction between farm types, e.g. because the survey sample is not large enough or because the official statistics do not contain the necessary variables. However, capturing the heterogeneity of organic farms is important as it is likely that different farm types, e.g. full-time vs. part-time farmers, family farms vs. corporate farms, mixed farms vs. grassland-based dairy farms (which are often found in less favoured areas), have different options to face market pressures and price squeezes. Indeed, as part-time farmers derive most of their household income from off-farm work, they are not affected by market pressures to the same extent as corporate farms (Best, 2008). This type of distinction is important, as in many European countries, a large share of farms are managed part-time: in the sample surveyed by Best (2008) 68% were part-time farmers; in Austria 61% of all farmers are part-time farmers (BMLFUW, 2007). To be able to accurately capture conventionalisation would require an analysis differentiating between, e.g., farm types, commodities and marketing channels. Only then will it be possible to do justice to the complexity of organic production (Sylvander et al., 2006; Rosin and Campbell, 2009; Lamine and Bellon, 2009).

The lack of an adequate, nuanced analysis has also been raised by Lockie and Halpin (2005, p. 287), who point out that the binary opposition between ‘artisanal’ and ‘industrial’, between ‘lifestyle’ and ‘agribusiness’ producers, can be problematic, as it “conflates differences in economic scale with differences in production practices, market relationships and grower motivations that have not been empirically verified”. They thus point out the need to “unpack the concept of conventionalisation and avoid the uncritical aggregation of multiple dualisms between small and large, artisanal and industrial, radical and regulatory, local and international, regenerative and substitutionist, and so on through the related concept of bifurcation” (Lockie and Halpin 2005, p. 304).

At a more general level, the conventionalisation debate also suffers from resting on implicit and poorly justified stereotypes. As Langer and Frederiksen (2005) have pointed out, conventionalisation builds on the notion that until a decade or two ago organic farms were smaller, less specialised and less intensive than conventional farms, and that this might now be changing. This assertion regarding ‘early’ organic farms is often made, although there is little (if any) historical data on organic farms. We thus do not have sufficient data to ascertain whether they all were mixed farms or whether there has always been a certain level of heterogeneity. The stereotype of the 'small' organic farm is also surprising, as in most European countries organic farms are larger than conventional farms: the average size of organic holdings in the EU-25 in 2005 was 38.7 ha, compared with 16.0 ha for all holdings (Eurostat, 2007). In this context, an increase in farm size is hardly an indication of a convergence between organic and conventional farms. Thus, whereas conventionalisation has been linked to increased farm sizes, the converse need not be true: not every instance of scale increase necessarily indicates conventionalisation.

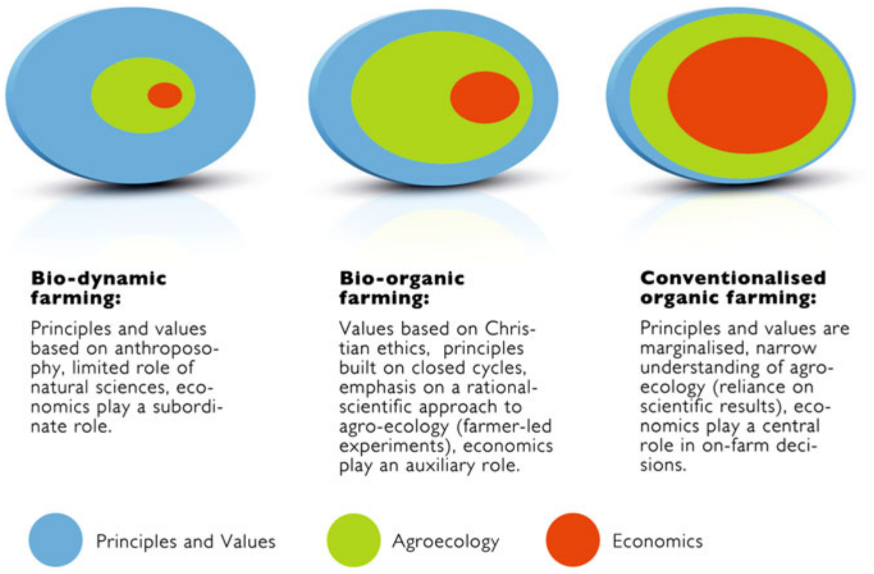
The conventionalisation debate may also have suffered from being mostly conducted by social scientists. Due to the dearth of interdisciplinary studies (Watson et al., 2008; Lamine and Bellon, 2009), the links between the changes noted by social scientists and the agronomic practices of farmers have not been established. In other words, the implied effect of the changes within organic farming on variables indicating environmental protection, sustainable plant production, animal welfare and soil fertility is rarely based on empirical evidence. For example, a reduction of mixed farms and of animal keeping is hypothesised to indicate a stronger reliance on off-farm fertiliser and thus input substitution. However, before reaching this conclusion, it would be important to assess whether the number of animals kept in the first place was sufficient to cover the nutrient needs of the field crops. Indeed, from an agronomic point of view it would be hard to argue that 100 growing-fattening pigs make a sizeable contribution to nutrient cycling on a 50-ha crop farm. Thus, if the conventionalisation debate is to be relevant to practitioners and inform them about the development options within organic farming, then the debate will need to integrate other disciplines into the discourse, e.g., crop and livestock production scientists as well as soil scientists.

Overall, we conclude that despite the case studies reporting symptoms that have been linked to it, the available data is inadequate to confirm or to refute the conventionalisation hypothesis in the European context. This is not least due to the fact that the variables used to identify the changes do not reliably indicate conventionalisation and that the available data does not allow for the nuanced analysis required to adequately capture the heterogeneity of organic farms or the complexity of the change dynamics.

3 A More Discerning Approach to Studying Change in Organic Farming

The review of the debate surrounding the conventionalisation hypothesis has shown that there are changes within organic farming. These transition dynamics are sometimes – if implicitly – seen as problematic in and of themselves. In this view, the pioneers are understood as the original, truest proponents of organic farming. The goal of organic farming is thus to replicate their practices, now and in the future. The original practices should not be changed or modified as it would undermine the nature of organic farming. This view presents conventionalisation as problematic, primarily because it implies a departure from the practices of the pioneers. This justification for opposing conventionalisation is questionable, if there is a lack of awareness that the converse is not necessarily the case. Indeed, although conventionalisation is a departure from the practices of the pioneers, not every departure from the practices of the pioneers is an indication of conventionalisation. We thus should no longer focus on whether organic farming is changing or not: there is ample evidence that it is. It would be more fruitful to focus on how organic farming is changing, taking care to capture the whole range of changes.

Indeed, organic farming is not a Luddite movement that seeks to turn back the clock. Organic farming should not be limited to the practices and methods of the pioneers. Not least because some of their practices, especially regarding animal welfare, were questionable (Sundrum, 2005). Rather, organic farming needs to be understood as dynamic, i.e. a system that responds to internal and external demands and conditions (IFOAM, 2005). Organic farming is thus involved in an on-going process of reviewing existing methods, assessing new technological developments, e.g. plant breeding, soil management and tillage, mechanical weed management, and implementing relevant insights from agro-ecological research (Niggli, 2007). Looking at the 1940s, it is clear that the pioneers also understood organic farming as dynamic and did not have qualms adjusting those aspects that they found unsuitable. For example, the bio-organic farming as developed by Müller and Rush is no longer based on anthroposophy, as was (and still is) the case of biodynamic farming (Aeberhard and Rist, 2009). Instead



Bio-dynamic farming:

Principles and values based on anthroposophy, limited role of natural sciences, economics play a subordinate role.

Bio-organic farming:

Values based on Christian ethics, principles built on closed cycles, emphasis on a rational-scientific approach to agro-ecology (farmer-led experiments), economics play an auxiliary role.

Conventionalised organic farming:

Principles and values are marginalised, narrow understanding of agro-ecology (reliance on scientific results), economics play a central role in on-farm decisions.

Fig. 2 Schematic representation of changes in the relative importance of values, natural sciences and economics for production decisions on organic farms

of using antrophosophy as their guideline, Müller and Rush have given agro-ecology and scientific thinking a much larger role (Fig. 2).

Changes are thus not problematic in and of themselves and organic farming has benefited from them in the past. Moreover, change is necessary for the survival of organic farming. Indeed, farmers now face an environment that is very different from the one faced by the pioneers: they have more regulatory constraints, but access to better developed markets; they face higher competition, but have more scientific and practical advice available to them; they face rapid changes, but have better communication possibilities. Both the constraints and the opportunities open to organic farmers are different, and as the farmers tackle these demands, it is bound to reflect on their on-farm practices.

What needs more attention is the diversity and type of changes taking place. Darnhofer (2006) has proposed to distinguish between first-order changes, i.e. changes that do not undermine the principles of organic farming (what may be termed 'professionalisation') and second-order changes. Second-order changes entail a discontinuity, they involve a shift in the rules that govern on-farm decisions. Conventionalisation can be labeled a second-order change, as the organic principles are sidelined in favour of economic profitability. Although organic farmers have always had to ensure that their farm is economically sustainable, within conventionalisation, economic considerations tend to marginalise both the principles and agroecology (Fig. 2).

Moving towards a more discerning analysis of the changes taking place on certified organic farms, we propose to define 'conventionalisation' as the introduction of farming practices that undermine the principles of organic farming, i.e. a second-order change. The assessment of a move towards conventionalisation should thus be based on criteria and indicators showing that the principles of organic farming are being undermined, not on structural changes (that may or may not indicate an undermining of principles). This approach implies moving away from focusing on the two extremes of 'true organic' vs. 'conventionalised'. Instead of focusing on the extremes, it might be more fruitful for research to focus on the field in-between, on the range of possibilities within organic farming. The goal is to leave organic farming room to change while providing farmers with guidance on the development paths that are in line with the principles of organic farming. This seems more constructive than debating whether organic farming is being conventionalised or not.

4 Towards Principle-Based Indicators of Conventionalisation

In this section we present a potential approach towards a principle-based indicator that would allow to distinguish between changes that follow the principles of organic farming and those that do not. We present an outline that will require substantial further development to be applicable empirically. Our goal is not to present a polished toolbox, but to indicate a way to overcome the weaknesses of previous approaches to assessing change in organic farming. These have proven too rough and thus could not capture the developments within organic farming with the required discernment.

4.1 *Organic Farming as Value-Based Agriculture*

Organic farming has set out to be an alternative to conventional agriculture and food chains. It is based on principles and values (Luttikholt, 2007; Besson, 2008). The International Federation of Organic Agriculture Movement (IFOAM), after a concerted and participatory process, has formulated four principles to inspire action (IFOAM, 2005):

- the principle of health: “Organic agriculture should sustain and enhance the health of soil, plant, animal and human as one and indivisible”;
- the principle of ecology: “Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them”;
- the principle of fairness: “Organic agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities”; and
- the principle of care: “Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment”.

These principles have a strong ethical component and display a much wider view of agriculture compared with the Good Agricultural Practice which may serve as a guide to conventional farming (e.g. DARD, 2008). However, these values are only partially codified in rules and regulations, thereby allowing the compromising of a more holistic vision of organic farming (Milestad et al., 2008). Indeed, the organic standards tend to focus on values and practices that are easy to codify and audit through the inspection and certification process, such as what inputs are permitted or excluded (Lockie et al., 2006; Padel, 2007). It has been argued that the dominant regulatory focus on inputs is much more likely to encourage entrants who can substitute allowed materials for disallowed materials (Rosset and Altieri, 1997; Guthman, 1998; Michelsen, 2001). Thus, the necessity of transparency in the interest of trade has made possible a rationalisation and simplification of organic meanings (Tovey, 1997; Allen and Kovach, 2000). The new European Regulation for organic production (EC, 2007) does include principles for organic production. However, not all are translated into production rules that can be part of inspection and certification (Padel et al., 2007). This mostly affects agro-ecological system values such as bio-diversity and nutrient recycling, as well as the lack of social considerations (Padel, 2007; Lockie et al., 2006). However, as Padel (2007) points out, the fact that some core values are not part of the standards does not mean that they are less important to organic stakeholders.

The organic farming associations may thus be called upon to ensure that their members do not focus exclusively on implementing the minimum requirements necessary for certification. They may also need to counter the attractiveness of exploiting legal loopholes. The goal would be for the associations to uphold the impetus to keep developing on-farm practices that implement the principles in ever more comprehensive ways. To achieve this, an assessment framework whose indicators are based on the principles of organic farming would seem a useful tool. Although developing this framework in detail is beyond the scope of this paper, we would like to propose some preliminary concepts to concretise our proposal.

4.2 *Exploring Options for Building an Assessment Framework*

The goal of developing an assessment framework is for it to serve as a tool to assess and guide future

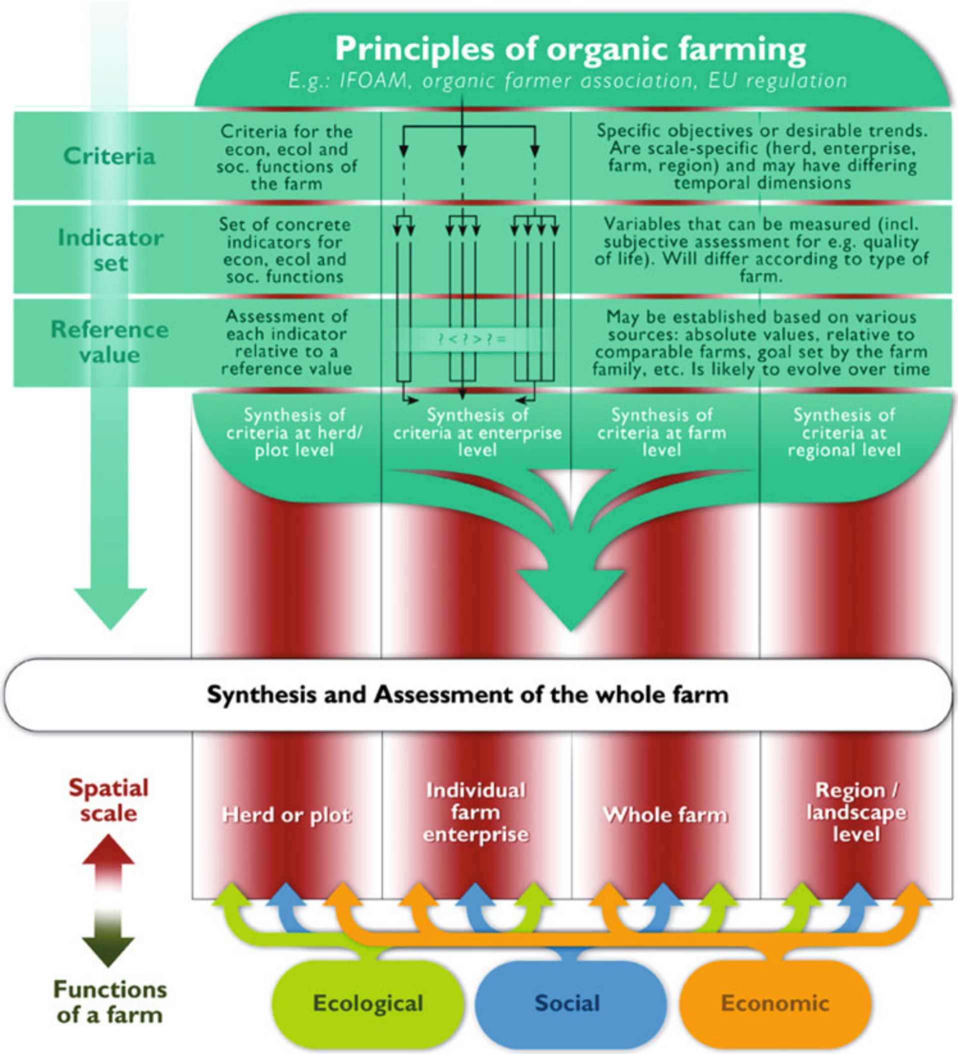


Fig. 3 Schematic structure of a tool to assess the extent to which an organic farm complies with the principles of organic farming

developments of organic farming methods and practices. We are aware of the practical and conceptual challenges involved in developing such an indicator set. Various authors have discussed the issues related to the necessity to cope with different units of measurement, as well as the trade-offs between complex index measurements and simplified approaches that can be understood by practitioners who do not have a background in statistics (e.g., Andreoli and Tellarini, 2000; Malkina-Pykh and Pykh, 2008; Meul et al., 2008). Despite these hurdles, a number of models for constructing an indicator-based framework to assess sustainability of farms have already been built (e.g., van Cauwenbergh et al., 2007; Knickel, 2008).

As the goal is to derive indicators from the principles, we would suggest a hierarchical framework (see van Cauwenbergh et al., 2007). Figure 3 presents a concept of such a framework linking principles, criteria, indicators and reference values which are measured on different spatial scales (plot, farm, landscape). The first level of the hierarchy is made of the principles of organic farming, i.e., the general objectives to be achieved, which clearly go beyond the organic standards.

The second hierarchical level is the criterion, i.e. the resulting state or aspect of the farming system when its related principle is respected. Criteria are thus specific objectives or desirable trends. Criteria would need to encompass the environmental, economic and social functions of an organic farm. Criteria challenging the positivistic view of traditional science, such as subjective experiences, may also be considered (Lund and Röcklingsberg, 2001; Meul et al., 2008).

The third hierarchical level is the indicator. Indicators are variables of any type that are used to measure compliance with a criterion. A set of indicator values should provide a representative picture of the implementation level of the organic farming principles on a farm. The goal would thus be to ensure the selection of a core, coherent and consistent list of indicators. A number of the indicators should be site- and scale-specific as some criteria and indicators may not be relevant for all regions or all farm types. Some indicators could also be based on existing measurements, e.g. the animal needs index; the indexes used for selecting breeding animals for a number of different traits at the same time (Hazel, 1943); or indicators developed to assess the environmental performance at the farm level (Braband et al., 2003; Meul et al., 2008; Niemeijer and de Groot, 2008).

The fourth and lowest level of the framework is made up of the reference values which describe the desired level for each indicator. The reference values can be either an absolute value or a comparative value, e.g. based on similar organic farms in the same agro-ecological region. The absolute reference values might be either target values, i.e. desirable conditions, or they could be threshold values in the form of minimum or maximum levels, or a range of acceptable values.

We are aware of the contested and negotiated nature of indicators and reference values. It will be a challenge to legitimise indicators when faced with divergent claims made by different groups (Slee, 2007). The selection of the indicators and the reference values should thus be part of a participative process which includes a wide range of stakeholders (Knickel, 2008; van Ittersum et al., 2008). The discussions could be used as a tool to make the range of positions and reasoning transparent.

Such a principle-based assessment framework needs to ensure a holistic approach to organic farming, by at least being based on a whole-farm assessment. The framework needs to be based on an understanding of the farm as a complex organism, rather than the sum of more or less independent activities. The latter is a reductionistic approach that does not do justice to the systemic underpinning of organic farming. The goal would be to show the extent to which a farm implements the integrity approach, rather than the no-chemicals or the agro-ecology approach (Verhoog et al., 2003; Baars and Baars, 2007).

The framework itself would be expected to change over time, as the principles of organic farming are refined (Luttikholt, 2007), scientific knowledge increases or societal values and concerns evolve. The goal would be to ensure a coevolutionary process, between organic farming principles, practices of organic farmers, and technical and economic feasibility as well as societal expectations.

As the assessment is based at the field, at the farm and at the landscape level, it allows the links between management by the farmer and impacts and effects on the agro-ecosystem to be addressed explicitly. This would allow the assessments derived from the framework to be used by a variety of actors: organic farmers, organic farming associations, researchers and regional policy decisionmakers. Within the framework, farms could be compared within a regional best-practice assessment. Such a regional ranking would take into account what is feasible in the region and provide each farm with a nuanced and detailed profile of the areas in which it is performing well, and those criteria where improvements seem desirable and possible. The assessment would also be dynamic since it takes into account developments and improvements in best practices.

4.3 Examples of Potential Indicators for Conventionalisation

To illustrate the types of indicators that can be considered within the assessment framework, we have compiled some indicators for crop production (see Table 1) and animal husbandry (see Table 2). These indicators were selected focusing on those areas where practices threaten to undermine the principles. As stated in the previous section, we want to emphasise that any individual indicator is only meaningful when seen within the total constellation of a set of indicators (Niemeijer and de Groot, 2008). Indeed, an individual indicator cannot effectively capture a causal network, i.e., the whole range of causes and effects and their

Table 1 Examples of indicators that could be used to identify conventionalisation in crop production, and related principles of organic farming (IFOAM, 2005)

Indicators of conventionalisation	Justification and comments	Principle of organic farming[1]
Low share of legumes in the crop rotation.	Legumes are necessary for humus build-up and a key element of the nitrogen supply. As an indicative reference value, there should be at least 20% legumes, but this could be higher, depending on the agro-ecological specificities of a site.	Ecology – Production is to be based on ecological processes. Nourishment is achieved through the ecology of the specific production environment. In the case of crops this is based on soil fertility and diverse crop rotation (to build up resilience of the agro-ecological system).
High share of cereals in the crop rotation.	A high share of cereals tends to lead to plant diseases and pests, deficiencies in the nutrient supply and poor humus build-up. As an indicative reference value, cereals should not use more than 70% in the crop rotation.	Ecology – Organic agriculture should attain ecological balance and resilience (see above) through the design of cropping/farming systems.
Inadequate crop rotation or unbalanced crop sequence.	The incidence of (soil-borne) pests, diseases and weeds increases. Also, crop rotations are needed to balance the soil nutrient demands of various crops and to avoid soil depletion.	Ecology – Organic agriculture should be based on living agro-ecological systems and cycles, work with them, emulate them and help sustain them.
Reliance on easily soluble (nitrogen) fertilisers (e.g. vinasse).	High levels of easily soluble nutrients (esp. nitrogen) threaten plant health, lower product quality and lead to problematic NO_3 and N_2O emissions.	Ecology – Production is to be based on ecological processes and recycling.
Prolonged and intensive use of plant protection products that are known to be problematic (e.g. sulphur, copper, pyrethrum).	These products are known to be toxic and to accumulate in the soil. They threaten beneficial insects and soil fertility.	Ecology and Health – Organic agriculture should avoid the use of fertilisers, pesticides, etc. that may have negative ecological and health effects.
Widespread use of practices that require a high level of external inputs (energy, fertilisers, feedstuffs, materials).	A farm (or a region) should aim at a balanced energy and nutrient budget and not be excessively dependent on external inputs. External inputs tend to be linked to high levels of CO_2 emissions and energy use.	Ecology – Inputs should be reduced by reuse, recycling and efficient management of materials and energy in order to maintain and improve environmental quality and conserve resources.
High share of varieties or cultivars which are not adapted to organic farming.	As these varieties are not adapted to the local agro-ecosystem and/or organic management they do not lead to stable yields appropriate to the local conditions. They usually require higher levels of external inputs.	Ecology – Organic management must be adapted to local conditions, ecology, culture and scale, in order to build up agro-ecological resilience.
Low level of biodiversity on the cropland.	The agro-ecological stability and the biodiversity are reduced. Cropland biodiversity includes the types of crops planted each year, and the number of cultivars used for a crop as well as the varieties used as green manure.	Ecology – Organic cropping is based on resilient agro-ecological systems, where biodiversity plays an important role. Organic agriculture should contribute to the maintenance of genetic and agricultural diversity.
Low level of biodiversity around the cropland (e.g., hedges, field margins).	Reduced agro-ecological stability (e.g. of beneficial organisms), poor protection of natural resources, lack of habitats and lack of contribution towards the cultural landscape.	Ecology – Organic farming should protect and benefit biodiversity (e.g. rare species), air and water. Role of biodiversity in resilience.
Few measures to actively protect and care for ecologically sensitive areas on the farm (e.g., ponds, marshes).	Reduced agro-ecological stability; lack of habitats. These areas are often of high ecological value, but of low economic/productive value.	Ecology – Those who produce organic products should protect and benefit the common environment including landscapes [and] habitats.

[1] These are examples, as most conventionalisation indicators are related to several principles and several aspects of the same principle.

Table 2 Examples of indicators that could be used to identify conventionalisation in animal husbandry and grassland management, and related principles of organic farming (IFOAM, 2005)

Indicators of conventionalisation	Justification and comments	Principle of organic farming[1]
High incidence rate of metabolic disorders/Frequent veterinary treatments required.	Metabolic disorders (e.g. acidosis or ketosis in ruminants, which are often referred to as 'production diseases') can be an indicator of high production intensity, inadequate animal housing conditions, or feeding.	Health – Organic agriculture should sustain and enhance the health of (. . .) animal[s]. Health is the wholeness and integrity of living systems. It is not simply the absence of illness.
High incidence rate of abnormal animal behaviour.	Atypical behaviour (e.g. feather pecking and cannibalism in laying hens) tends to indicate a high animal population density, inadequate housing system, a lack of appropriate stimuli, or lack of space to allow natural behaviour.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.
Average life time of the animals is substantially below the average productive life span of the species.	In organic farming animals should not be conceived of as 'throw-away' animals. The focus should be on the potential productive life span of the species.	Fairness – Respect, justice and stewardship of the shared world (...) in their [the people's] relations to other living beings.
Use of breeds that are not appropriate for organic farming systems (e.g. poultry hybrids, breeds with extreme muscle conformation, dairy breeds with a predisposition for metabolic disorders).	Increases the incidence of diseases due to low robustness of the animals.	Ecology – Organic management must be adapted to local conditions, ecology, culture and scale.
Number of rare breeds kept on-farm.	The use of rare breeds is a valuable contribution to the maintenance of biodiversity. However, they are often linked to lower economic productivity and thus neglected.	Ecology – Organic agriculture should contribute to the maintenance of genetic and agricultural diversity.
High share of reproduction through artificial insemination. The sires used stem from the use of embryo transfer (ET bulls).	It would be preferable to keep male animals on the farm (or on a neighbouring farm) and allow the animals to mate naturally.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.
High share of concentrates in the ration of herbivores (e.g. dairy cows).	This is more useful as an indicator than e.g. the milk production per cow, which can vary strongly depending on the genetic potential and on the agro-ecological conditions.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.
High share of feed that is purchased (industrially produced) rather than produced on the farm (or by neighbouring farms).	The number of animals kept on-farm needs to be in relation to the feed that can be produced on the farm's own fields.	Ecology – Nourishment and well-being are achieved through the ecology of the specific production environment.
Restricted access to pastures (for e.g. cows).	Each species should be allowed to display typical behaviour (e.g. grazing for cows). Sufficient exercise also reduces the risk of lameness.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.

(continued)

Table 2 (continued)

Indicators of conventionalisation	Justification and comments	Principle of organic farming[1]
Few structuring elements in the animal housing, including the outdoor run.	Structural elements are necessary for orientation and motivation for species-specific behaviour, e.g. separate resting and activity areas, perches for poultry.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.
Type of storage, treatment and use of animal urine and manure (i.e. prevalence of slurry).	Whether urine and manure are collected and stored separately or as slurry has implications for their use (i.e. effect on the species composition of the grassland).	Ecology – Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.
Animal housing makes extensive use of non-renewable materials, such as plastic sheets, rubber mats, etc.	Use of ecologically sound design and of renewable materials would be preferable.	Ecology – Inputs should be reduced by reuse, recycling and efficient management of materials (. . .) to (. . .) conserve resources.
Low amounts of litter provided (i.e., animal housing that is geared towards litter minimised system).	The amount of litter should be adequate to fulfil the needs and natural behaviour of the animals.	Fairness – Animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.
Low time budget for the care of the individual animal and for herd management.	Increasing herd sizes reduces the time budget per animal of the stockpersons. If this time budget decreases below a threshold (which needs to be defined separately for each livestock category), animal welfare suffers.	Care – Organic agriculture should be managed in a precautionary (. . .) manner.
Only a few animal types are kept on-farm.	It would be desirable for farmers to keep ruminants as well as monogastric livestock to integrate animal husbandry better with plant production and thereby balance demand and supply of feed resources within a farm.	Ecology – Inputs should be reduced by reuse, recycling and efficient management of materials and energy. Production is to be based on ecological processes, and recycling.
The choice of mowing dates of the grassland is not based on ecological criteria.	Grassland management needs to take into account ecological criteria to ensure the upkeep of the biodiversity in the grassland (flora and fauna), and the ecological stability of the grassland.	Ecology – Organic pastoral systems should fit the cycles and ecological balances in nature (role of biodiversity, s. a. and enhancement of rare species).
High incidence rate of broad-leaved dock on grassland.	Broad-leaved dock tends to be linked to a level of slurry application that is not adequate for the local conditions, as well as a high frequency and poor timing of mowing. This threatens the yield, fodder quality and the ecological stability of grassland.	Ecology – Organic management must be adapted to local conditions [and] ecology.

[1] These are examples, as most conventionalisation indicators are related to several aspects of the same principle and several principles.

inter-relation. A set of indicators, however, should be able to identify the production logic underlying farm management.

Most of the indicators in Tables 1 and 2 are linked to finding "conventional" solutions to challenges faced in production and management decisions. They are mostly linked to a production logic that seeks to maximise production, i.e. aiming for yields above the level adapted to the local agro-ecological conditions, and that lacks an understanding of the interrelationships between different farm activities; and/or to short-term thinking that might be the result of economic pressures and the (perceived) need to 'cut corners', e.g., to reduce the share of legumes in the crop rotation, as legumes tend to have a low gross margin.

Indeed, a number of the indicators are linked to economic profitability, being a dominating decision criterion in conventionalised farming (Fig. 2). However, in organic farming short-term economic aspects need to be balanced with long-term ecological and social considerations to achieve sustainability. It reflects the approach at the core of organic farming of the ethical values of stewardship and moral care for the land and thus organic farmers "say no to using chemicals, not to (always) putting every acre under tillage, not to (always) striving for the largest yield" (Stock, 2007, p. 97).

A departure from the organic principles is also reflected in practices that do not seek systemic solutions to problems, but focus on the topical alleviation of a production problem. The farm is thus not understood as a whole where each part needs to be seen in the context of the other parts. Instead, each part of the farm is perceived as separate and problems appearing in that part are solved 'locally' (separately) without considering links to other activities on the farm.

For example, a high incidence of broad-leaved dock (Rumex obtusifolius) in permanent grassland can be an indicator of intensification. It tends to be found on farms where management is guided more by economic goals and technical objectives, sidelining ecological knowledge and locally adapted management. Problems with broad-leaved dock can be linked to the application of slurry, intensive grassland use (e.g. high frequency and poor timing of mowing), as well as poor grassland management, resulting in low plant cover and soil compaction (Dietl and Lehmann 2004; FiBL, 2006). To solve the problem, it is thus usually not sufficient to reduce the number of dock plants. The farm should be seen at the systems level. For example, it might be helpful to change animal housing on those farms using litter-minimised systems, to reduce the share of concentrate in the ration (and thus reduce the nitrogen available on-farm), seek an alternative to collecting animal manure as slurry, as well as seek a grassland management appropriate for the local ecological conditions (Dietl and Lehmann, 2004).

Another example is that nutrient cycles need to be considered when selecting the number and type of animals to be kept on a farm. A specialisation in monogastric livestock (pigs, poultry) frequently reduces the share of forage legumes in the crop rotation, because this type of livestock does not use plant material rich in fibre well. Besides other challenges, the long-term consequences of such a reduction may involve severe imbalances in the farm nutrient cycles and weed problems. Systems thinking and ensuring closed nutrient cycles is a foundation of organic farming and thus needs to be the basis of all farm decisions.

5 Conclusion

Reports both in scientific journals and in popular media show that some of the developments within organic farming can lead to conventionalisation. By undermining the principles of organic farming, practices will undermine its transformative potential and thus its contribution to sustainable agriculture. However, we have shown that a number of the methods used in the conventionalisation debate do not reliably assess conventionalisation as they tend to focus on structural characteristics.

To be able to capture the changes at the farm level and be able to assess whether or not they amount to conventionalisation, a comprehensive framework built on the principles of organic farming would be useful. Indicators will need to capture the whole range of causes and effects as well as their interrelations and will clearly go above and beyond the requirements for organic certification. The indicators will need to comprehensively cover all four principles of organic farming defined by IFOAM (2005) and do justice to the systemic nature of organic farming. Defining criteria, deriving indicators and setting reference values need to be done in a participative process.

The assessment framework could be used by, e.g., organic farmer associations to provide guidance and support extension activities. Farmer associations might implement a yearly assessment to encourage farmers to implement improved practices and thus to demonstrate their commitment to the organic principles. If the assessment includes a form of certificate, the farmers can use that certificate to convey their merits to customers.

Clearly, this would not prevent some farms from opting for conventionalised practices, but it would make their (lack of) commitment to organic principles transparent. This would allow addressing the increasing heterogeneity in organic farming practices. There are farmers practising ‘organic light’ and those that go ‘beyond organic’. If the latter are to be supported and strengthened in their resistance to conventionalisation, their contribution to agricultural sustainability must be made visible. An assessment framework explicitly based on the principles of organic farming could contribute to both the reflexivity of organic farmers and to the academic discourse regarding the changes in and further development of organic farming.

Acknowledgements We would like to thank all those who participated in the conventionalisation workshop in May 2007 in Vienna for their open and constructive contribution and for their assessment of the changes in organic farming in Austria. We would like to thank AERU at Lincoln University for providing the supportive environment that made this paper possible. We are grateful to the anonymous reviewers and to the editor for their constructive and helpful suggestions on an earlier version of this paper. Of course, the authors alone are responsible for the content of the paper.

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Conservation Tillage in Turkish Dryland Research

Muzaffer Avci

Contents

Abstract Central Anatolian soils have high risk of erosion, degradation and intensive cultivation. They are in danger of exhausting their agricultural use unless conservation agricultural practices are adopted. Conservation agriculture is a key tool in sustainable production systems throughout the world and is developed around soil management technology that minimizes soil disturbance, maximizes soil cover and promotes crop diversity to offer benefits to farmers and to environment. It has been particularly effective at sustaining crop production in semi-arid rain-fed regions such as the Central Anatolian soils, where potential evaporation exceeds precipitation during most months of the year, dry farming is extensively practiced, water and wind erosion is common, and proper application of water- and soil-conserving tillage technology is critical. The area under plow expanded its limits as the number of tractors in agriculture dramatically increased during the 1960s. This is the starting point for inappropriate use of the agricultural land. The conservation agricultural technologies, therefore, are of utmost importance for the region. Common farmers' practices of the current fallow-wheat system in the central plateau of Turkey are incompatible with the conservation agriculture concept. The objective of this review is to re-evaluate the performances of the partial and full conservation tillage practices previously tried in the region. The review reached the following conclusions: (1) agreeing with the conservation principles, fall tillage as a primary operation in the fallow areas was found to be useless compared with leaving the land without tillage; (2) therefore, much research has focused on spring tillage as a primary operation and employed conventional, semi-conservative and conservative methods. Results showed that the conventional

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_19,

from Avci, Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010022

system, in addition to being ecologically unfriendly, is unprofitable as compared with other conservation practices regarding the updated cost analysis; (3) similarly, tillage depth in primary spring tillage was determined to be shallower than the depths currently practiced by farmers, in agreement with the conservation principles; (4) fallow tillage operations in summer to create dust mulch for eliminating soil moisture loss did not increase the crop yields and soil moisture as compared with chemical fallow; (5) no-till fallow was similar to the conventional clean fallow system in terms of moisture and yield levels. However, no-tillage resulted in 50% reduction in the cost of tillage besides its ecologically-friendly effects; (6) the existing dryland agricultural systems in the plateau should be transformed into or changed toward sustainable systems, although further research is required on residue and stubble management, and integrated weed control methods, planting equipments and methods on the fields with high amounts of residue.

Keywords Dryland farming • Summer fallow • Erosion • Stubble • Straw • Tillage • No-tillage • Weed control

1 Introduction

Conservation tillage is variously defined around the globe. In the USA, the definition includes a minimum of 30% soil cover after planting to reduce soil erosion by water, or where soil erosion by wind is the primary concern, a minimum of 1120 kg ha^{-1} of flat, small grain residue equivalent on the soil surface throughout the critical wind erosion period (CTIC, 2004). However, precise definitions of conservation tillage can only be made in the context of the crop species and varieties, soil types and conditions, and climates (Carter, 1994). Conservation agriculture is developed around soil management technology that minimizes soil disturbance, maximizes the soil cover and promotes crop diversity to offer benefits to farmers and to the environment.

Common agricultural practices regarding the fallow-wheat system in Central Anatolia are incompatible with the conservation agriculture concept. Surface soil has no residue cover and loses its aggregation and becomes dustlike structure as a result of frequent tillage operations during the 16 months of the fallow period. In addition to fallow fields, the planted fields have no crop residue cover and are under high risk of wind and water erosion during the growing period of 10 months, but especially immediately before and after drilling. Measures to protect soils from wind and water losses are the prerequisite for the sustainable use of the croplands. In Turkey, as the importance of water erosion is well known, wind erosion has been regarded as unimportant and confined to special areas such as Karapınar-Konya and İncesu-Kayseri towns. For the last 5 years, we have observed severe wind erosion (especially during the Lodos – south wind) in Central Anatolia during the winter months. Roadsides and sheltered fields are covered with the sediments eroded from open fields (Fig. 1). However, evidence of erosion is removed by rain or snowfall and soils then bear a resemblance to those affected by successive freezing and thawing events (Fig. 2). Soykan (1962) reported that the soils blown by wind accumulated in sheltered places and sometimes heaped up to the windows of the houses in Central Anatolia. He also emphasized the fact that soil erosion was started even by the gentle winds in the case of soils with no or little residue on them and low organic matter. Mutaf (1970) indicated that abrasion and eradication of young plants by wind and covering of planted fields with the eroded soil were regarded as ordinary events in Central Anatolia. Christiansen Veniger (1973) provided some photos emphasizing the wind erosion problems in the central plateau. In order to reduce the hazard of severe wind erosion, many researchers recommended reconsideration of the summer (clean) fallow practices which make the soil more vulnerable to wind erosion by successive summer tillage operations and construction of wind breaks (Beşkök, 1957; Beşkök, 1966; Hans Johaim Spath, 1975; and Aydemir, 1975). Soil

Fig. 1 Field covered by sediments eroded by south winds (Lodos) in Central Anatolia

Fig. 2 Post-winter scene of soils eroded by south winds (Lodos) in Central Anatolia, 2006

surface cover of living vegetation or crop residue is the simplest and surest way of reducing erosion. Small-stemmed residues such as wheat or barley create more friction with the wind than large-stemmed ones such as corn or sunflower stalks. Standing residue is more effective than lying residue to control wind erosion.

The crop residue cover reduces evaporation, and the standing stubble traps snow for extra soil moisture. At the same time, the crop residue reduces wind speed on the soil surface, and standing stubble anchors the soil, and traps the snow (MWPS-45, 1992).

Most soils require 30 percent ground cover to prevent wind erosion. For cereal crop residues, this is equivalent to about 900 to 1100 kg ha^{-1} of residue. The best way to reduce water and wind erosion is to leave the crop residue on the soil surface. The amount of residue mulch reduces erosion to a different extent. Residue mulches of 1.24 and 2.47 t ha^{-1} minimized the loss of runoff water and soils on slopes of different lengths and steepness (Kramer and Meyer, 1969). While 2.0 t ha^{-1} reduced the loss by 80%, a mulch rate of only 0.5 t ha^{-1} decreased the loss by 40% (Lattanzi et al., 1974). In another study, erosion of 0.56 t ha^{-1} and 1.12 t ha^{-1} mulch rates was less than 25% of erosion without mulch (Meyer et al., 1970).

In the central plateau, crop residue research was carried out on fallow fields by using treatments with and without stubble (burning). The effects of the treatments of stubble management on the fallow fields were evaluated on the next years' crop phase. Thus, wheat stubble left on the field caused 110 to 950 kg ha^{-1} less soil and 3.5 to 20 mm less soil water losses during the fallow phase, and 17 to 25% more subsequent wheat yields than stubble burning (Ayday, 1980; Sayın, 1983).

Besides soil erosion control, the soil organic matter content is increased by leaving stubble on the soil surface. There is consensus that carbon sequestration potential is higher in humid temperate areas (0.1 to 0.5 t C/ha/yr) than in semi-arid and tropical areas (0.05 to 0.20 t C/ha/yr). Palm et al. (2000) measured carbon stocks, losses and rates of accumulation in Brazil, Cameroon and Indonesia. They concluded that carbon accumulation rates are much higher in aboveground biomass (at least 2 t C/ha/yr) than in soils (0.2–0.6 t C/ha/yr), and also indicate that tree-based agro-ecosystems, either plantation crops (e.g. oil palm, cacao and rubber agro-forests) or on smallholder farms, bring the greatest dividend, accumulating 3.0–9.3 t C/ha/yr (Sanchez et al., 1999; Sanchez and Jama, 2000). Rapid decomposition occurred when the stubbles were buried to 12 cm depth and almost all the stubbles were mineralized in 18 months of the fallow period. On the other hand, only 33% of stubbles were decomposed when they were kept on the soil surface for the same period (Özbek et al., 1976; Cogle et al., 1987). Thus, a substantial part (60–65%) of the stubbles accumulates on the soil each year. Our observations also showed that no debris from the stubbles of the previous harvest can be seen on the field at the planting time of wheat. This clearly indicates that all the residue which was buried by the moldboard plow is decomposed during the fallow season.

The purpose of this review is to re-evaluate the performances of the conservation tillage practices previously studied in dryland conditions in view of the current socio-economic circumstances; and thereby shed light on the current and future research agenda by presenting the pros and cons of the practices.

2 Fall Tillage in Fallow

The earliest studies on soil tillage in the fallow period were performed by Kıraç (1937) during the 1930s. In his studies, tillage practices were evaluated in terms of soil moisture conservation and nitrate accumulation as well as grain yield of wheat. Comparing his findings with those found in dryland areas of the USA, Kıraç discussed the results of fall tillage practices more prudently than his successors and tested various implements (moldboard plow, disc harrow and non-tillage) for the following expectations: (1) controlling the weeds and volunteers during the fall; (2) burying weed seeds and eliminating them after germination; (3) increasing the rainfall penetration into the soil; and (4) enrichment of soil organic matter. Unfortunately, the results were in contrast to his expectations and one to 15% yield increase was obtained with non-tillage in the fall. Further investigating fall tillage, Gerek (1968) compared tillage and non-tillage on wet (good tilth) and dry soils with the control (moldboard plow in March) treatment under slope and bottom soils. The 18 years of results showed that while there was not any yield difference among the treatments on dry soil, significant differences were detected on bottom soils in favor of tillage. The advantage of tillage on bottom soil can partly be attributed to successful weed control by tillage treatment because the weed problem is expected to be more severe on bottom soils than slope soils. Volunteer and weed seeds germinate easily in the wet soil until the winter months and then are eliminated by the fall tillage. Thus, the fall tillage operation can contribute much to the weed control in wet soils. In another study, tillage treatments consisting of different reduced tillage methods and the moldboard plow were compared in 16 locations in Central Anatolia. Fall, spring and summer tillage practices were evaluated separately. The results of the fall tillage, although the results of statistical analysis was not given, showed that the moldboard plow provided more average yield than a chisel, subsoiler and no-tillage. The moldboard plow was superior to the other methods in only single year of the research. Therefore, by referencing the previous research results, the authors concluded no difference among the tillage methods, and recommended non-tillage in the fall for the central plateau (Pehlivantürk et al., 1977). Doğan et al. (1977) tested various fall tillage methods in the plateau for three years. The results indicated that chisel plowing was superior to non-tillage and the subsoiler in terms of grain yield and moisture conservation. However, the yield difference was not found to be statistically significant. Therefore, they decided in favor of non-tillage. As a summary of fall tillage, it was concluded by all the research results that tillage practice in the fall did not make a difference. This conclusion supports the idea of conservation agriculture. Leaving standing stubble on the field by non-tillage keeps the snow in the fields, increases the infiltration capacity of the soil and reduces erosion. These facts make it unnecessary to till the field in the fall. If an area has soils which propagate weed infestation, or an impermeable soil layer, chisel plowing can be an alternative to non-tillage for those areas.

3 Spring Tillage as Primary Operation

Because the fall tillage as a primary operation in the fallow phase was shown to be a useless operation as compared with non-tillage, tillage in spring became the main concern for many researchers. Kıraç (1937) conducted his experiments by using the moldboard plow as a primary tillage tool. He compared and wanted to replace this costly equipment with cheap disc harrowing in March and April. He found equal yields and equal soil moisture but different nitrate contents. The amount of nitrate was high in moldboard-plowed plots, because intensive cultivation encouraged microorganism activity which led to rapid and complete decomposition of the wheat stubble. In those years, the wooden plow was common in the Anatolian plateau. The demand to use the moldboard plow instead of the wooden plow (saban) was very high because of its extensive use and popularity in the developed countries. The people advocating the wooden plow were said to be "reactionary" to modern agriculture. Kıraç (1937) handled the issue in a scientific way and tested the moldboard plow, wooden plow and lister as primary tillage implements for three years (1932 to 1934). The lister was preferred because it does similar work to the wooden plow. The results indicated that the wooden plow, contrary to expectations, was not as "dangerous" as a primary tillage tool and produced grain yields as high as the moldboard plow. However, it was stated by the author that it should be converted into an efficient metal tool by agricultural engineers. Gerek (1968) continued Kıraç's experiments on sloping and bottom lands for 18 years and found 1.32, 0.94, 1.00 and 0.99 t ha^{-1} average grain yields with March moldboard plowing as a control, the wooden plow, lister and cultivator, respectively, on sloping land. Great yield differences between the implements in some years (1939, 40, 44, 46 and 48) were not discussed by the author. This makes it difficult to comment on those yield differences. However, Kıraç (1937) observed that the lister and the wooden plow were characterized by tilling the soils shallowly, capturing the severe rainfalls of spring months in the soil and preventing wind and water erosion. As a consequence, we can conclude that the wooden plow and lister can be considered as the right equipment to be used in conservation agriculture and can replace the moldboard plow. On the bottom land, significant differences were not detected between the control (moldboard plow) treatment (1.48 t ha^{-1}) and the wooden plow (1.41 t ha^{-1}). The lister and cultivator yielded 12 and 15% less than the control treatment, respectively. Doğan et al. (1977) followed the recommendation made by Kıraç (1937) and modified the wooden plow into a metal form (Anatolian saban) which had a steel frame with several shanks. They compared the moldboard plow, the reduced moldboard plow, the metallic saban, Russian-type plow and sweep plow for four years. The modified metallic (saban) plow provided the highest yield in two years out of four and the second highest in the remaining other two years. The sweep plow also provided satisfactory performances. They recommended the Anatolian saban or the reduced moldboard plow as the primary tillage equipment (Doğan and Küçükçakar, 1987). All the research results showed that equipment similar to the wooden plow will do a satisfactory job on the plateau. Mutaf (1970) tested the moldboard plow with a small cutting edge in front, moldboard plows in different forms, disc plow, field cultivator, and sweep (Graham) plow for three years at the Polatlı State Farm. The highest wheat yield was obtained with the moldboard plow with a small cutting edge in front. The lowest yields were obtained with cultivator and subsoiling implements. He suggested that the cutting edge plow buries the structureless topsoil deeply and brings well-structured deeper soil to the surface; weeds, pests and organic residue on the surface are also buried deep in the soil, and humus is formed. Contrary to Mutaf's (1970) perception, much of the literature agrees that inverted soils lose their organic matter and structure rapidly (Mannering et al., 1975; Hill, 1990; Lal et al., 1998; Reicosky et al., 1995; Allmaras et al., 2000; Wilhelm et al., 2004). Inverting of soil one in two years in fallow/wheat sequences only postpones the degradation process and does not eliminate or reverse it. As was stated by Reicosky et al. (1995): "it is practically impossible to increase soil organic matter where moldboard plowing is taking place". The present situation on the plateau also shows that our soils lose aggregation and become tiny particles due to low organic matter content (Eyüpoğlu, 1999) and primarily excessive moldboard plow tillage. Moldboard plow tillage and different reduced tillage methods were compared in 16 locations in Central Anatolia in the 1971–1977 period (Pehlivantürk et al., 1977). The moldboard plow was recommended for soils with slopes up to 8%. It was suggested that areas in the slope ranges of 3 to 8% should be plowed perpendicular to the slope direction, and areas with more than 8% of slope be allocating as grazing land. Those recommendations deserve some criticism. First of all, this perception did not consider wind erosion, that is not directly related to slope. Secondly, tilling the fields in the central plateau perpendicular to the slope direction is not a practical issue because most of the fields have a long border parallel to the slope direction. The last criticism is that a considerable amount of arable land on the plateau has slopes of more than 8% and there are no rules or laws acting to restrict the current use of those areas. Thus, the recommended method (moldboard plowing) and current extensive use of it by the farmers is not the right solution and involves none of the conservation measures that would prevent increased water and wind erosion.

The depth of tillage is very important in terms of water and soil conservation. The tilled layer retains more rain water than the untilled layer because of increased soil porosity. On the other hand, it accelerates the mineralization process of soil organic matter through enhanced oxidation. Surface (shallow) tillage results in lower soil disturbance and lesser amount of residue incorporation than deep tillage; consequently it leaves more residues on the soil surface (Sprague, 1986). Because the main aim of conservation agriculture is to leave a sufficient amount of plant residue on the seedbed, whatever technology that achieves this can be welcomed. Thus, the moldboard plow operated shallowly during tillage can leave more residues on the soil surface as compared with deep and complete inversion of the soil. Tillage depths were evaluated by several researchers in primary tillage operations in the central plateau. Deep tillage (20 to 25 cm), control (15 cm) and shallow (10 cm) were compared in terms of grain yields of wheat (Kıraç, 1937). The results showed that there was no difference between the deep and the control treatments and only a slight (2.5%) difference between the shallow and the control. Gerek (1968) tested the same treatments on different dates and in different soil conditions (on bottom and sloping soils) for 18 years. He concluded that shallow (10 cm) tillage operation in April was superior to the control and the deep treatments on both the slope and the bottom lands. He reported that shallow tillage did not completely invert the soil, resulting in partly buried stubble. He suggested that this greatly prevents wind and water erosion. He also emphasized the fuel economy of shallow tillage and prevention of loss of fertility caused by mixing topsoil with infertile parent material brought up by deep operations in the shallow profile soils common in the central plateau. Berkmen (1961) tested 10, 20 and 30 cm of primary tillage depths for 7 years and was in favor of depths less than 20 cm. The difference between 10 and 20 cm was 2.6% of grain yield on average. Ünver (1978) investigated time, implement and depth of primary tillage operations on soil moisture. Shallow tillage (11 to 13 cm) with the reduced moldboard plow and the sweep plow resulted in higher moisture than the moldboard plow. However, in cases of deep tillage (18–20 cm) the reverse was true. All of the research results we have discussed clearly demonstrate that, contrary to the current common farmers' practices of deep moldboard plow tillage, shallow primary tillage with the sweep plow and reduced moldboard plow, or even with the normal moldboard plow, tends to conserve more moisture than deep moldboard plow tillage, thereby emphasizing the importance of conservation agriculture for the region (Kıraç, 1937; Gerek, 1961; Berkmen, 1961; Ünver, 1978).

4 Summer Tillage

The hot and dry summer period follows intensive rainy spring months on the plateau. Reducing soil moisture loss to a minimum during this period is the most important objective of the summer tillage. Moisture conservation can be achieved by reducing evapo-transpiration during this period. For this purpose, dust mulch and weed control are considered as two important tools. The significance of a surface dust layer (dust mulch) in terms of moisture conservation has always been emphasized when dry farming is the concern. The value of dust mulch was evaluated for moisture conservation. Four years of results indicated that weeds were the only reason for the moisture loss in fallow areas. Dust mulch caused slightly more (1%) moisture in the upper 30-cm layer, which can play a significant role in nitrate accumulation and crop emergence. Unfortunately, this extra moisture was not coupled with yield increases (Kıraç, 1937). Similar results were found when dust mulch and chemical weed control (no-tillage) were compared in terms of wheat yield and soil moisture (Karaca et al., 1981). The effects and importance of summer tillage operations in terms of conservation agriculture depend on the previous primary tillage operation on the fallow fields with standing and/or lying stubble. Inversion of soil as a primary tillage on those fields buries all the stubble into the soil. The subsequent tillage (summer tillage) operations will be of little or no value due to the unavailability of the crop residue on the field. Summer tillage research focusing on clean fallow is obviously beyond the scope of this paper (Doğan et al., 1977; Ünver, 1978; Pala, 1982; Doğan and Küçükçakar, 1987; and Güler et al., 1989). However, it is remarkable to see that following the same primary tillage methods, chemical weed control outperformed the summer tillage alternatives in terms of yield and net benefits (Table 2).

5 Economic Considerations

Much of the research on tillage systems was carried out during the 1970s. The price of petroleum was much cheaper, but herbicide prices were very high. Hence, the economic analysis carried out for the tillage systems should be updated. Different tillage systems comprise equipment for primary tillage and a sweep harrow combination and chemical spray for summer tillage; these were tested in five provinces of the Anatolian plateau over five years (Annual Reports of Soils and Crop Managements of Wheat and Training Project, 1972 to 1977). According to the economic analysis (partial budget) on the updated costs and prices, yield losses with sweep tillage and offset disc harrows as primary tillage and chemical sprays as follow-up operations were compensated for by their reduced costs and reached identical profitability with moldboard plow systems (Table 2). An interesting result was the difference between tillage and chemical treatments the moldboard plow methods. The greater economic benefit of chemical sprays was due to their lower cost (Table 2). The economic analysis of the trials comparing different conservation tillage methods with the conventional method at a state farm (Altınova, Konya) for three years (Annual Reports of National Wheat Project (Agronomy) for 1971, 1972 and 1973) also showed similar results. Chemical fallow provided 130 to 260 USD ha^{-1} more profit than the method of conventional fallow. It should be noted that the herbicides used in chemical fallow were contact herbicides and could not control all weeds. Today, we have non-selective, systemic herbicides which control almost all types of weeds. Although we spend more money with the current moldboard plowing than the conservation fallow system, there is no guarantee of obtaining more return with the conventional fallow technology due to crop hazards, e.g., cold, frost and drought, which are becoming more frequent due to climate change. Therefore, Kıraç (1937) formulated a principle for the farming system on the plateau; which may be valid for all dryland production systems. "We need the low cost technologies which may be subject to partial yield loss more than the costly technologies which may have potential yield increase".

6 No-Tillage

No-till is defined as planting crops in previous crop residue and unprepared soil by opening a hole, narrow slot, trench or band of the smallest width and depth needed to obtain proper coverage of the seed. This is a single technology to achieve different goals simultaneously; it sustains agricultural production, and protects the environment. It is the most developed conservation agriculture technology, which has many agro-ecological advantages: controlling water and wind erosion by leaving crop residue on the field surface, increasing organic matter, preventing stubble burning, common on the plateau, and reducing the cost of production and greenhouse gas release to the atmosphere by eliminating tillage. Central Anatolian soils oxidize to two meters, and are friable due to high calcium content and frequent freezing and thawing. Thereby, the physical properties (tilth) expected from tillage occur by themselves under natural processes (Kıraç, 1937). So, we do not see substantial differences among the tillage methods affecting soil properties and crop yields. This implies that tillage can be easily avoided if stubble and weed problems can be overcome in our soils. No-till fallow (hand weed control) and till fallow plots plowed at various depths as primary tillage and follow-up monthly cultivations were compared for soil moisture contents at wheat planting time for three years (Kıraç, 1937). No considerable differences were found between primary tillage methods including different tillage depths and no-tillage in terms of soil moisture. Stubble mulch on till or no-till plots increased the amount of moisture stored and surface moisture. In dry years, no-till provided higher wheat yields than tilled plots, and vice versa in wet years. Soil moisture at 30 cm is less in no-till than tilled plots (Kıraç, 1937). This research clearly shows that if the weeds are controlled, no-tillage can replace the conventional fallow system. If straw was spread on the no-till plots (straw mulch), more soil moisture would be accumulated than in the conventional system. The results of only one season (1935/36) in which the soil was not frozen and allowed rainwater penetration into deeper zones show the effect of surface conditions (Table 1). In a three-year experiment, Kıraç (1937) observed deeper penetration of precipitation into the soil profile with no-till and residue-covered plots than tilled plots. The soils of no-till plots froze later and to a shallower depth and kept water longer than tilled plots. They were more effective in terms of moisture storage than hand-weeded control plots and plowed stubble cover and showed the benefit of conservation agriculture. The no-till system will play a vital role in preventing common stubble burning, reducing tillage costs by 30% and reducing soil and water loss. The research at the Altınova State Farm (annual reports for 1971, 1972 and 1973) showed that chemical fallow was more profitable, with the net benefit amounting to 260 USD ha^{-1} than tillage systems. The research conducted by the CRIFC produced infor-

Table 1 Impact of various tillage and surface management techniques on stored available soil water (in 180-cm profile) at the end of the rainy period (mid-June, 1932) (Kıraç, 1937)

Types of management	Soil moisture (mm)*
Stubble cover	159
Sand cover	153
No-tillage + hand weed control	146
Plow in March at 15 cm depth	139
No-tillage + no weed control	132
Dry plowing at 50 cm depth	127
Soil dust (road dust) cover	95

*Total precipitation during the experimental period was 226.2 mm.

Table 2 Effects of different fallow tillage methods (primary + summer tillage operations) on average yields, and economic parameters

Yield, cost and benefits	Tillage methods Sweep + 2 passes of sweep + harrow combination	Sweep + 2 times herbicide sprays	Offset disc + 2 passes of sweep + harrow combination	Offset disc + 2 times herbicide sprays	M. Plow + 2 passes of sweep + harrow combination (Control)	M. Plow + 2 times herbicide sprays
Average yields, kg ha^{-1}	2118.0	2065.0	2245.0	2101.0	2335.0	2281.0
Yield differences, kg ha^{-1}	217.0	270.0	90.0	234.0	0.0	54.0
Costs, USD ha^{-1}	250.0	233.3	291.7	275.0	333.3	283.3
Benefit, USD ha^{-1}	794.3	774.4	841.9	787.9	875.6	855.4
Net Benefits (NB), USD ha^{-1}	544.3	541.0	550.2	512.9	542.3	572.0
NB diff. from Control	–2.0	1.3	–7.9	29.4	0.0	–29.7

* Average of 16 experiments carried out in different provinces of the plateau.

** One pass of m. plow, sweep and offset disc and chemical spray costs 166.6, 83.3, 125.0 and 75.0 USD ha^{-1}, respectively.

mative results on what the type and the structure of planting machines should be in terms of suitability for the Anatolian region for the success of the no-till system. As a result, two types of drills were developed. Studies on conservation agriculture and no-till started in 1998 and are ongoing. The seven years of research showed that the grain yields of wheat were similar in no-till and till systems; however, the cost of no-till was 50% less (134.5 USD ha^{-1}) than the cost of a tillage system in fallow/wheat rotation (Avcı, 2005 and unpublished data). Similar results were also attained with a legume/wheat system. The Dutch Ankara Embassy supported no-till activities for the transfer of this technology to the Central Anatolian farmers during the 2003–2007 period. We conducted on-farm field trials and field demonstrations and farmer training during this period. In a village (Çeltikli-Haymana), the benefit from no-till (chemical fallowing) was 37% more than the farmers' system (clean fallow). This went up to 53% with the use of the improved wheat cultivar Ikizce96 instead of an existing variety, Bezostaya1. In another test area (Temelli-Ankara), no-till vetch hay was 1240 kg ha^{-1} and 23% more than tillage vetch hay production. At the Polatlı State farm vetch (hay) and lentil yields were 0.5 t ha^{-1} and 0.45 t ha^{-1} more than the yields obtained from tillage systems. Two farmers in Bala and in Yerköy with large areas of land were in favor of the no-till system when they saw the benefits in the extremely dry season of 2006/2007 (project reports of 2003, 2005 and 2007). They would like to extend the cooperation with research people and planned to plant a considerable amount of land with no-till systems. Similar climates to that of the plateau where conservation agriculture is well documented are in southern Australia and in the Pacific Northwest (PNW) of the USA. It will be instructive to use the experience of those regions in conservation agriculture. In southern Australia, the main elements in the conservation agriculture systems of direct drilling and stubble retention have been widely promoted. The benefits of conservation agriculture systems, which are reductions in erosion, increase in soil fertility, improvement in soil surface physical characteristics, and reduction in soil loss and bulk density, are well recognized (Poole, 1987; Steed et al., 1994). Despite these benefits the adoption process has been considered slow although about 36% of farmers in southern Australia use no-till and 80% retain stubble to some extent. The main reason for slow adoption is the expectation that the yield of crops in these systems will not increase relative to conventional systems; however, results from field experiments show that yields of wheat with conservation agriculture may be either higher or lower than with conventional tillage, and that there is considerable year-to-year variation both within and between sites (Kirkegaard, 1995). Conservation tillage in the PNW, like southern Australia, has been greatly influenced by soil degradation (Sojka and Carter, 1994), but particularly by the high rates of erosion and the need for water retention in the drier areas. Freeze-thaw events and the associated problems with soil water storage and erosion have also been drivers in the adoption of conservation agriculture practices in the PNW. Like southern Australia, the rates of adoption have been low. In 2002, just 27% of the cropland in the states of the PNW was managed with conservation agriculture, and only 7.5% was managed with no-till systems (CTIC, 2004). The constraints to adoption are similar to those mentioned for southern Australia, with the exception of concerns over inadequate seed zone water for crop establishment under conservation agriculture. In the PNW, early seeding, often ahead of the first substantial winter rains, is vital for early establishment of the winter wheat to ensure adequate winter survival. The experience of the PNW and southern Australia in conservation agriculture shows that the main driving force in the adoption of conservation and no-tillage technologies was to obtain more yield or more returns with conservation than with conventional technologies. We are at the beginning in the adoption of conservation agriculture for the Anatolian plateau. Farmers do not have sufficient information on the concept of conservation agriculture. Environmental indicators (erosion, flood) are not taken seriously by farmers. The growing concern regarding erosion problems amid intellectual circles and some NGOs such as the TEMA Foundation is focused mainly on the non-agricultural sites (rangeland and forest).

7 Challenges and Implications

Efficient and sustainable agricultural production requires that we continue to strive for systems that are efficient in their sustainability. In semi-arid regions of our country, conservation agriculture should be considered the primary standard by which systems and practices have been compared. Reduced productivity of our soils due to soil erosion, frequent tillage, intensive crop production and residue removal can be eliminated by reduced tillage systems. For example, previous research on tillage unanimously emphasizes non-tillage in the fall. For spring (primary) tillage, the Anatolian saban and the reduced moldboard and chemical fallow (no-tillage) outperformed in most of the experiments. However, there is not enough information about the residue levels and surface conditions in most of the studies. Therefore, studies on the amount and the state of crop residue (standing or lying of straw or stubble), and on the soil, water and crop yields are required for future research. On the plateau, demand for stubble grazing has been reducing because intensive animal production is replacing extensive systems. On the other hand, most of the straw is left in the fields besides the standing stubble in high-yielding years. Consequently, we have a chance to use the crop residue to improve and protect our soils. Some of the research (Karaca, 1987; Güler et al., 1989) highlighted the grassy weed population increase with reduced tillage and its control by the moldboard plow. Problems with grassy weeds can be overcome when we understand the weed biology. Seeds of downy brome buried five cm or more in the soil cannot emerge and only 1% survived after two years (Wicks et al., 1971; Durutan, 1982). Some of the chemical herbicides, metribuzin and diclofob, were found effective in control of downy brome (Durutan, 1982). New herbicides, Attribut (a.i. propoxycarbazoneNa) and Monitor (a.i. sulfosulfuron.), to control grassy weeds involving downy brome have been produced and they are effective when following the manufacturer's instructions. IPM (integrated pest management) research, which takes into consideration crop rotations, herbicide application and agronomic practices, is needed for grassy weed control in the dryland plateau. The unavailability of good drills which can perform well in high residue conditions was the main obstacle to obtaining high yields with reduced tillage methods in the past. In order to facilitate drilling with the available seed drills, chemical fallow fields were tilled prior to seeding to prepare a good seedbed. The performances of the available drills in no-tillage agriculture are very critical to the adoption of no-tillage agriculture by farmers on the plateau. Most producers will want to use machinery that is already owned. Some small modifications to the current drills by attachment of cutting disks should be tried in the fall and the spring plantings. Because the soil is very soft and swollen, the spring planting seems more feasible than the fall planting and also important for the implementation of no-tillage. Purchase of additional equipment adds significant costs unless it can be charged against production costs over enough hectares and years. The practical benefit of slightly more moisture in the upper zone of clean fallow than reduced tillage at planting time was not studied in detail. The interpretation was about the potential benefit on the germination and emergence and seedling failure “ala tav" after planting (Kıraç, 1937; Pala, 1982). However, the research on crop emergence and its pre-winter development is very limited in the region.

Acknowledgements I gratefully acknowledge all the people who conducted or participated in the previous research activities mentioned in this paper, and also respectfully remember those who have passed away.

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Part IV

Climate Change

Biofuels, Greenhouse Gases and Climate Change

Cécile Bessou, Fabien Ferchaud, Benoît Gabrielle, and Bruno Mary

Contents

Abstract Biofuels are fuels produced from biomass, mostly in liquid form, within a time frame sufficiently short to consider that their feedstock (biomass) can be renewed, contrarily to fossil fuels. This paper reviews the current and future biofuel technologies, and their development impacts (including on the climate) within given policy and economic frameworks. Current technologies make it possible to provide first generation biodiesel, ethanol or biogas to the transport sector to be blended with fossil fuels. Still under-development 2nd generation biofuels from lignocellulose should be available on the market by 2020. Research is active on the improvement of their conversion efficiency. A ten-fold increase compared with current cost-effective capacities would make them highly competitive. Within bioenergy policies, emphasis has been put on biofuels for transportation as this sector is fast-growing and represents a major source of anthropogenic greenhouse gas emissions. Compared with fossil fuels, biofuel combustion can emit less greenhouse gases throughout their life cycle, considering that part of the emitted CO_2 returns to the atmosphere where it was fixed from by photosynthesis in the first place. Life cycle assessment (LCA) is commonly used to assess the potential environmental impacts of biofuel chains, notably the impact on global warming. This tool, whose holistic nature is fundamental to avoid pollution trade-offs, is a standardised methodology that should make comparisons between biofuel and fossil fuel chains objective and thorough. However, it is a complex and time-consuming process, which requires lots of data, and whose methodology is still lacking harmonisation. Hence the life-cycle performances of biofuel chains vary widely in the literature. Furthermore,

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_20,

from Bessou et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2009039

LCA is a site- and time-independent tool that cannot take into account the spatial and temporal dimensions of emissions, and can hardly serve as a decision-making tool either at local or regional levels. Focusing on greenhouse gases, emission factors used in LCAs give a rough estimate of the potential average emissions on a national level. However, they do not take into account the types of crop, soil or management practices, for instance. Modelling the impact of local factors on the determinism of greenhouse gas emissions can provide better estimates for LCA on the local level, which would be the relevant scale and degree of reliability for decision-making purposes. Nevertheless, a deeper understanding of the processes involved, most notably N_2O emissions, is still needed to definitely improve the accuracy of LCA. Perennial crops are a promising option for biofuels, due to their rapid and efficient use of nitrogen, and their limited farming operations. However, the main overall limiting factor to biofuel development will ultimately be land availability. Given the available land areas, population growth rate and consumption behaviours, it would be possible to reach by 2030 a global 10% biofuel share in the transport sector, contributing to lower global greenhouse gas emissions by up to 1 $GtCO_{2\,eq}$ per year (IEA, 2006), provided that harmonised policies ensure that sustainability criteria for the production systems are respected worldwide. Furthermore, policies should also be more integrative across sectors, so that changes in energy efficiency, the automotive sector and global consumption patterns converge towards drastic reduction of the pressure on resources. Indeed, neither biofuels nor other energy source or carriers are likely to mitigate the impacts of anthropogenic pressure on resources in a range that would compensate for this pressure growth. Hence, the first step is to reduce this pressure by starting from the variable that drives it up, i.e. anthropic consumptions.

Keywords Biofuels • Energy crops • Perennials • LCA • Greenhouse gases • Climate change • Political and economic frameworks • Bioenergy potential • Land-use change • Nitrous oxide • Carbon dioxide • Agricultural practices

1 Introduction

Until the middle of the 19th century, American citizens lit their houses with whale-oil lamps. In 1892, the first Rudolf Diesel motor ran on peanut oil. Liquid fuels can be easily stored and transported and offer, for a given volume, a better exchange surface for combustion compared with solid fuels. Oils, in particular, can deliver a high energy amount by volume unit. No wonder then that biofuels were the first candidates to supply the newly developing automotive industry. However, they were almost immediately overtaken by petroleum products that appeared to be an energy godsend, remaining very cheap for more than a century. However, today the Black Gold Age is coming to an end.

In 2005, the world total primary energy supply approximated 11 430 Mtoe yr^{-1} (479 EJ yr^{-1} [1]), compared with 6 130 Mtoe yr^{-1} (257 EJ yr^{-1}) in 1973 (IEA, 2007a). According to the FAO, the world population will grow from around 6.5 billion people today to 8.3 in 2030 (UN, 2006). World energy demand is expected to rise by some 60% by 2030. More than two-thirds of the growth in world energy use will come from the developing countries, where economic and population growths are highest (CEC, 2006a). Fossil fuels will continue to dominate energy supplies, meeting more than 80% of the projected increase in primary energy demand. Global oil reserves today exceed the cumulative projected production between now and 2030, but reserves will need to be "proved up" in order to avoid a peak in production before the end of the projection period. Effective exploitation capacity today is almost fully used, so growing demand for refined products can only be met with additional capacity (IEA, 2005). The exact cost of finding and exploiting new resources over the coming decades is uncertain, but will certainly be substantial. Financing the required investments in non-OECD countries is one of the biggest challenges posed by our energy-supply projections

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[1] Mtoe yr^{-1}: million ton oil equivalent: IEA conversion factor used throughout the article: 1 Mtoe yr^{-1} = 4.1868×10^4 TJ. Selected units for the article are Joules; however, conversions are indicated in brackets when quoted figures are given in other units.

(IEA, 2005). As an example, Saudi Arabia, with 25% of the world's best proven reserves, is already investing US $ 50 billion to increase its production capacity by 2 million barrels per day (Mb/d); the global worldwide current production averaging 86 Mb/d (ASPO, 2008).

According to some experts the peak oil will occur in 20 years, whereas others argue that the world is already at peak production. Although one might argue on the exact moment, it is generally accepted now that it will happen soon and that an energy transition is unavoidable (Van der Drift and Boerrigter, 2006). ASPO, The Association for the Study of Peak Oil, states that overall oil and gas production will be at their peak by 2010, and the conventional oil peak would already be overcome in most regions (ASPO, 2008). According to Andris Piebalgs, European Union Energy Commissioner, the oil crisis of the 1970s presented a discrepancy between oil supply and demand of only 5%, but in a post-peak oil scenario, the gap between supply capacity and demand could reach 20% within five years (as quoted by ASPO, 2008). In 2030, the European Union energy dependency on imports could account for 70% of its global energy needs. Today, this dependency is already around 50% and the energy demand has kept increasing by 1 to 2% each year since 1986 (EU DG-TREN, 2005). Cheap reserves will not be sufficient to fulfil the world's growing energy demand and fossil fuels have been shown to be the main anthropogenic cause of global warming. These increasing supply and environmental costs make petroleum no longer the only candidate as the universal energy source; other sources may now be competitive. However, there is no alternative energy godsend and, as industries have been relying on petroleum for too long, clean technologies are late. There is no other solution than diversifying the energy mix with a growing contribution of cleaner energy sources.

Current global energy supplies are dominated by fossil fuels (81% in 2005), with much smaller contributions from nuclear power (6.3%) and hydropower (2.2%). Bioenergy provides about 10% of the total energy supplies, making it by far the most important renewable energy source used; solar, wind and other renewable energy sources accounting for the last 0.5% (IEA, 2007a). On average, in the industrialised countries biomass contributes less than 10% to the total energy supplies, but in developing countries the proportion is as high as 20–30%. In a number of countries biomass supplies 50−90% of the total energy demand. A considerable part of this biomass use is, however, non-commercial, and relates to cooking and space heating, generally by the poorer part of the world's population (IEA Bioenergy, 2007). The contribution of bioenergy to the global supply mix has scarcely evolved since 1973, whereas other renewable energy sources have been consequently fostered and nuclear power widely developed (IEA, 2007a). Bioenergy could play a bigger role, especially in the industrial countries, which consume a lot of fossil energy and are therefore the main contributors to atmospheric pollution and global warming. According to the intergovernmental panel on climate change (IPCC), greenhouse gas emissions have already made the world 0.6°C warmer during the last three decades. The EU-25 and the four other largest emitters, the United States, China, Russia and India, contribute all together approximately 61% of global emissions. Energy-related emissions represent 60% of global emissions in CO_2 equivalent (Baumert et al., 2005).

Transport is a major energy consumer [27.6% of total final energy consumption worldwide (IEA, 2007a), 31% in the EU-27 (EU DG-TREN, 2007); two-thirds of the projected increase in oil demand will come from transport (IEA world outlook 2005)] and a large greenhouse gas emitter. In 2004, the transport sector produced 6.3 $GtCO_2$, i.e. 23% of world energy-related CO_2 emissions (Ribeiro et al., 2007) or roughly 13.5% of global greenhouse gas emissions (Baumert et al., 2005). In the EU-27, this sector accounted for 22% of total greenhouse gas emissions in 2005 (EEA, 2008). Moreover, vehicle emissions are the single most rapidly growing source of CO_2 emissions. Achievement of a levelling off of vehicle emissions, given continuing growth in the number of vehicles on the road, requires both: (1) a substantial reduction in vehicle emissions during the next several years and (2) advances in technology in the longer term that fundamentally reduce CO_2 emissions, because energy will always be at a premium (Hansen, 2006). The automotive market is logically evolving towards electric motors, whose energy efficiency is roughly 7.5 times higher than that of internal combustion engines. The compacity and lightness of liquid fuels still enable fifty-fold higher energy storage than the best current batteries (Roby, 2006). Fuel cells may in the future replace these limited electro-chemical accumulators, but these are considered long-term technologies requiring significant research and development efforts.

Their deployment also hinges on changes in the market and consumption behaviours. Lastly, electricity or H_2 are secondary energy carriers that need to be produced from primary energy sources, involving possibly high CO_2 emissions.

Biofuels can contribute to reducing the dependency on fossil fuels and lower greenhouse gas emissions from transport, provided that the savings of greenhouse gases through the use of bioenergy is not counteracted by an increase in the same emissions during the production and transformation of the biomass. Agriculture and land-use change already account for some 15% and 13% of global greenhouse gas emissions, respectively (Baumert et al., 2005; Smith et al., 2007; Houghton, 2008). Can biofuels finally be considered as an advantageous clean energy source? Here, we address this question by first reviewing the various biofuels, the state of the art of the technologies, and the current production and consumption rates. We then present the political and economic frameworks that aim at promoting the development of biofuels but still fail at convincing all stakeholders about biofuel sustainability. We finally address the issue of biofuel quality in terms of environmental impacts, with a special focus on greenhouse gas emissions and the potential of biofuels to contribute to climate change mitigation.

2 Definitions

This section provides definitions of the following key concepts: bioenergy, renewable, biofuels and biorefinery.

Bioenergy is the chemical energy contained in organic materials that can be converted into direct, useful energy sources via biological, mechanical or thermochemical processes. The most common and ancestral bioenergy source is firewood, which nowadays still represents 15% of global energy consumption (ADEME, 2006), some 90% of the world's woodfuel being produced and consumed in the developing countries (Parikka, 2004). The prefix "bio", from the Greek "$\beta\acute{\iota}o\varsigma$" (meaning "life")[2], refers here to the origin of the energy converted through the metabolism of living organisms with, at the basis of the food chain, autotrophic organisms converting solar energy into chemical energy contained in the molecules they produce via photosynthesis[3]. The total sum of living organisms is called the biosphere in opposition to lithosphere, hydrosphere and atmosphere. It is also referred to as "biomass" or "biota" by biologists and ecologists. Biomass, in the energy sector, refers to biological material which can be used as fuel for transport, or an energy source to produce industrial or domestic heat and electricity (feedstock and conversion routes, Fig. 1). Bioenergy comes from biomass. In contrast, fossil energies are mineral resources, stocked in the lithosphere. The carbon fossil energy sources are the result of mineralisation transforming organic matter into mineral matter. This transformation takes millions of years, meaning that fossil resources are non-renewable on a human time scale.

"Renewable" does not mean "sustainable". Renewable resources consist of two main types of natural resources: flow resources and renewable stock resources. Flow resources, such as solar or wind energies, are non-limited resources despite intermittence. On the contrary, renewable stock resources, mainly biomass, are limited resources and their availability depends both on other primary natural resources (e.g. lands, water, ecosystems, etc.) and on natural regeneration/degeneration rates and/or anthropic production/consumption rates. The term "renewables" in the energy field encompasses all energies coming from renewable resources, e.g. photovoltaic energy, wind energy, bioenergy, etc. It is also referred to as RES, standing for renewable energy sources.

Considering biomass, "renewable" indicates that it will in theory stay available in an infinite time perspective as it can regenerate or be grown. However in practice, the renewal of biomass also depends on its management, which should ensure that primary resources are not overexploited or even depleted. If resource management is technically appropriate, environmentally non-degrading, socially favourable and economically viable, then the renewable resource will

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[2] Thus the prefix "bio" has nothing to do with the organic production label called "BIO" in France or Germany, for instance, which actually corresponds to specific management guidelines for agricultural production that aim at minimising the harmful impacts on the environment.

[3] Other autotrophic processes than photosynthesis exist when enlacing the whole biosphere, but they are less relevant in quantitative terms when focusing on bioenergy.

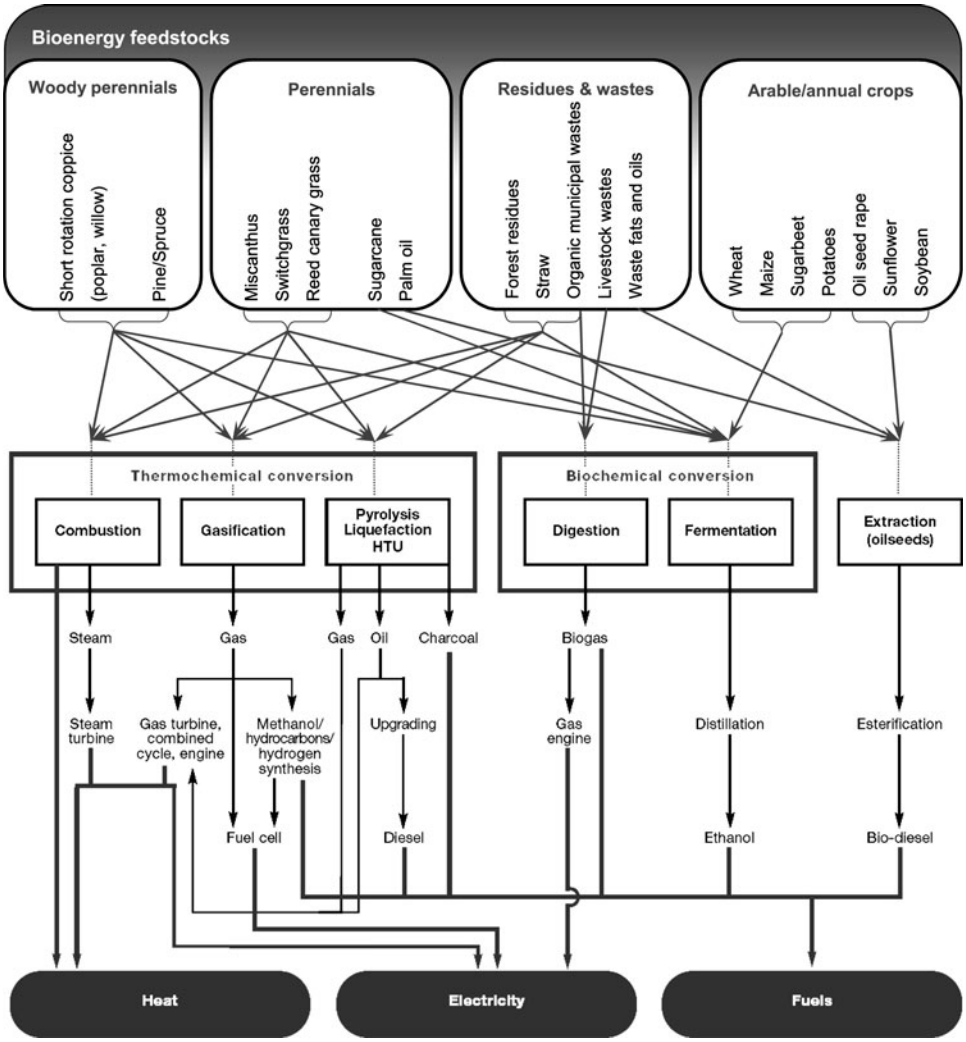

Fig. 1 Bioenergy feedstock and conversion routes, adapted from Plassat, 2005 and UNDP, 2000. HTU: hydrothermal upgrading

be exploited in a sustainable[4] way. The issue of sustainability being crucial in the field of bioenergy, the UN Executive Board for Clean Development Mechanisms released in December 2006 an official definition of "Renewable Biomass" including this sustainability dimension (UNFCCC, 2006). Among the five possible conditions where biomass can be defined as "renewable", the three that do not deal with residues or wastes have a first criterion mentioning that the land use shall not change except if land areas are reverted to forest. The second criterion implicitly linked to the first one states: "Sustainable management practices are undertaken on these land areas to ensure in particular that the level of carbon stocks on these land areas does not systematically decrease over time". This is a key element when comparing the CO_2 emissions from biofuels and fossil fuels. Indeed, the interesting fundamental carbon neutrality of combusted biomass is based on the fact that the emitted CO_2 from the plant originates from the atmosphere where it eventually goes back to. If land conversion to biomass production implies additional CO_2 emission through soil organic carbon losses, it may offset this carbon neutrality.

Highly dependent on the type of vegetation, the organic carbon stock is globally around 1.6 times higher in forest or permanent grassland soils than in crop soils (Antoni and Arrouays, 2007). Therefore, land-use

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[4] Based on the pillars of sustainability concept in the Brundland report, 1987.

change can lead to soil organic matter losses. Soil organic carbon content can also decrease in the long term as a consequence of the export of agricultural residues. Therefore, it is necessary to assess the net crop residue amount that would remain available for the bioenergy chain without degrading the soil quality in the long term (Saffih-Hdadi and Mary, 2008; Gabrielle and Gagnaire, 2008). Soil organic matter represents only a few percent of the total soil mass, but still constitutes a large organic carbon stock on a global scale, i.e. almost the same as the sum of the carbon stocks in the atmosphere and in the vegetation (Arrouays et al., 2002). Small but stable changes in this stock could critically impact the global carbon fluxes. Furthermore, soil organic matter plays a crucial role in soil quality. In an agricultural soil, whose main function is to provide nutrients and water to crops, soil organic matter permits the development of microorganisms decomposing organic matter into easy-absorbed mineral forms for the plant. It contributes to the soil cationic exchange capacity, which also influences the availability of essential minerals for the plant, and to the stability of the soil. Soil organic matter and forest biodiversity are among the precious resources whose conservation for future generations should not be jeopardised by land-use changes. For the producers, "Renewable Biomass" will no longer just mean "which can be grown" but also implies conditions for a sustainable production.

Biofuels are biomass materials directly used as solid fuel or converted into liquid or gaseous fuels that can be stored, so that the harnessed energy can be released through combustion when needed. This chemical reaction permits the release of the binding energy that holds electrons to a nucleus in the organic molecules, in order to produce work and heat. In a narrower sense, biofuels may be only perceived as liquid or gas transportation fuels derived from biomass. Many different biomass raw materials can be used to produce biofuels including energy crops, agricultural residues or forest products, for example.

Biomass gives way to a whole product chain in which residues or co-products can largely contribute to the environmental and economic optimisation of the whole biomass value chain. Besides bioenergy, biomass can serve as a source of biomaterial (building materials, papers, etc.) and chemical compounds (solvents, pharmaceutics, cosmetics, biodegradable plastics, etc.). This last field of activities based on biomolecules is called "green chemistry". The overall integrated biorefinery that aims at using all the biomass compounds within one refinery complex is summarised in Fig. 2. The power of the biorefinery concept is supported by economy of scale and by efficient use of all incoming bioresources. Using the petroleum industry as an illustrative example, ∼5% of the total petroleum output from a conventional refinery goes to chemical products; the rest is used for transportation fuels and energy. Most visions for integrated biorefinery do not expect this ratio to change (Ragauskas et al., 2006).

To conclude, among renewables, biomass gives way to diverse bioenergy chains. In comparison with all the other renewables, bioenergy firstly presents the

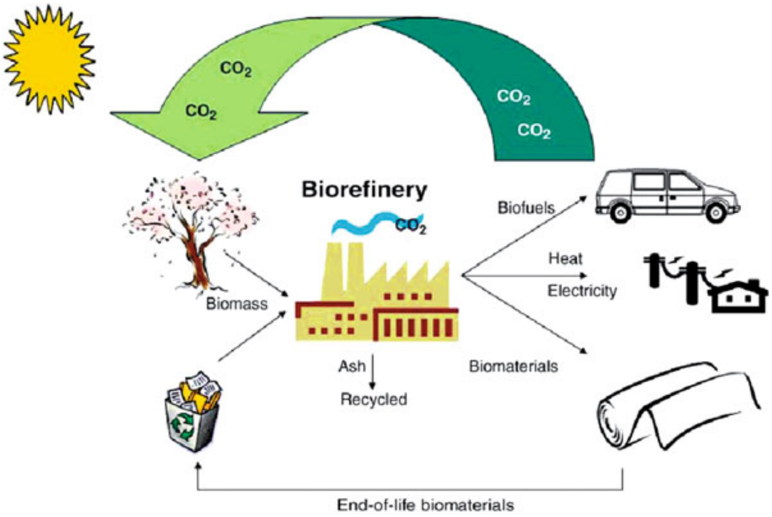

Fig. 2 The fully integrated agro-biofuel-biomaterial-biopower cycle for sustainable technologies (Ragauskas et al., 2006)

advantage that investments are generally lower. Furthermore, the diversity of raw materials and transformation processes offers a wide range of possibilities than can be adapted to different geographical locations, means and needs. Nevertheless, issues or challenges also arise when dealing with the development of bioenergy that will aim at finding the best cost/benefit equations, including externalities, depending on both the type and the amount of bioenergy produced. These equations appear to be especially difficult to solve in the case of transportation biofuels. Firstly, the reverse side of feedstock and technology multiplicity is that many options from worst to best biofuel chains exist, which finally creates an overall confusion and uncertainty about biofuels. This uncertainty is thereby hampering the development of production plants and new expensive technologies as it contributes to increasing investment risks. Secondly, whereas bioenergy chains, like the other renewables, were intrinsically thought to be advantageous first of all on a local scale, biofuels especially give way to international trade, that can raise the issue of externality displacement and imply the need for specific political and economic frameworks.

3 Transportation Biofuels

Biofuels are nowadays commonly classed as 1st, 2nd or 3rd generation biofuels, as shown in Table 1. First generation biofuels refer to those already considered as "traditional or conventional chains", whereas 2nd generation biofuels, requiring more complex and expensive processes, are not available yet on the market. The energy efficiency of a biofuel chain must be appraised considering two aspects, both dependent on feedstock type: the net energy yield per area unit and the energy cost for transformation processes. When considering plant biomass, the energy yield per hectare is a function of the type of plant, the climate, the soil properties and the crop management. C_4 plants, whose photosynthesis is more efficient, are especially energy cost-effective in humid tropical regions where water is not limiting, e.g. sugar cane in Brazil. On the other end of the spectrum, maize in the US necessitates considerable energy inputs.

There is among 1st generation biofuels no technological breakthrough that would lead to large differences in terms of energy efficiency. In temperate regions, oilseed crops typically generate lower yields per hectare than sugar or starch crops and are therefore more expensive to produce. But because oils seeds require less processing they still generally have positive global energy balances per unit of feedstock. Oilseed crops grown in tropical areas can thus be especially productive and competitive. Globally feedstock costs account for the majority of the eventual price of any 1st generation biofuel, while processing costs and a small proportion for transport represent most of the rest. For ethanol, feedstock comprises 50 to 70% of the production cost, while for biodiesel feedstock can be 60 to 80% of the production cost (Lang et al., 2001; Worldwatch Institute, 2007).

Table 1 Biofuel generations (Van der Drift and Boerrigter, 2006)

Biomass feedstock	1st generation biofuels	2nd generation biofuels	3rd generation biofuels
Vegetable oil	Pure Plant Oil (PPO, also called Virgin Plant Oil: VPO, or Straight Vegetable Oil:VGO) Fatty Acid Methyl Ester: FAME (e.g. Rape Seed Methyl Ester)		
Fermentable biomass	Biogas/Substitute Natural Gas		H_2
Starch/sugar	Ethanol/Ethyl Tertiary Butyl Ether (ETBE)		
Lignocellulose		Ethanol Fischer-Tropsch (FT) diesel* Dimethyl Ether (DME)* Methanol* Mixed Alcohols (MA)* Substitute Natural Gas: SNG	H_2*

Biofuels indicated with * are produced with synthesis gas (syngas, mainly H_2 and CO) as intermediate.

The split between 1st and 2nd generation biofuels lies in the fact that the latter are produced from lignocellulose, meaning that all types of vegetation and all parts of the plant are possible feedstock, whereas 1st generation biofuels only up-value specific parts of a few suitable plants. Hence, 2nd generation biofuels yield higher energy amounts per hectare than energy crops with a proportionally small specific organ of interest (such as seeds) as no part of the plant is left over. They also encompass a wider range of possible feedstock. Third generation biofuels are the follow-up of 2nd generation biofuels, from the same raw material up to H_2 production, whose energetic costs remain out of reach.

3.1 First Generation Biofuels

The production of 1st generation biofuels could rapidly be fostered as technologies ensue from the food industry. Pure plant oils or even cooking oils, also called yellow grease, can thus be directly used as fuel. However, complementary processes permit one to upgrade the biofuels in order to optimise their mixing with conventional fossil fuels without needing to adapt the motors.

3.1.1 Biodiesel

In a broad sense, biodiesel refers to pure and processed plant oils or animal fats. These oils and fats contain a mixture of triglycerides, free fatty acids, phospholipids, sterols, water, odorants and other impurities. Biodiesels are nowadays produced from a large range of oilseed crops, mainly rapeseed or canola, soybean and sunflower, palm oil and *Jatropha curcas* in tropical climates (see picture below). Other potential plant oil feedstock includes mustard seed, linseed, castor oil, peanut, cottonseed, coconut, *Lesquerella* spp. and micro-algae. There are as many different biodiesels as different oil compositions. Oilseed species vary considerably in their oil saturation and fatty acid content, characteristics that significantly affect the properties of the biodiesel produced.

Jatropha curcas fruit, Belize, February 2003 bessou©INRA

The boiling and melting points of the fatty acids, methyl esters and glycerides increase with the number of carbon atoms in the carbon chain, but decrease with increasing numbers of double bonds (Ma and Hanna, 1999). Saturated fatty acids are more compactable, which enhances the oil energy density. But if they contain lots of saturated fatty acids, oils and fats are solid at room temperature and cannot be directly used as fuel in a diesel engine in their original form except in warm climates. The disadvantages of vegetable oils compared with petroleum diesel fuel are their higher viscosity, lower volatility and the reactivity of unsaturated hydrocarbon chains (Lang et al., 2001). Because of subsequent problems such as carbon deposits in the engine, engine durability and lubricating oil contamination, they must be chemically transformed to be compatible and used on a long term with existing engines (Ma and Hanna, 1999).

The most widespread biodiesels are methyl esters produced from plant oils combined with methanol through transesterification. The two other routes, microemulsion and pyrolysis, are not worth it; pyrolysis notably is expensive for modest throughputs and processing removes any environmental benefits of using a biofuel (Ma and Hanna, 1999). Transesterification is an alkali-catalysed reaction that requires 107.5 kg of methanol per ton of vegetable oil and results in the production of 1004.5 kg of methyl ester and 103 kg of glycerol (Graboski and McCormick, 1998). In this three-step reaction, triglycerides are converted into diglycerides, then monoglycerides and finally reduced to fatty acid esters, enhancing the viscosity of the final biodiesel. The viscosity of vegetable oils and that of their final esters is of the order of 10–20 times and twice that of diesel fuel, respectively (Lang et al., 2001). A pre-step and catalysis make it possible to deal with the impurities such as free fatty acids and water to improve the reaction kinetics (Ma and Hanna, 1999). Methanol is preferred over ethanol because of its physical and chemical properties as well as comparative low cost (Ma and Hanna, 1999; Lang et al., 2001), although it introduces a part of fossil fuel into the biodiesel. For different esters from the same vegetable oil, methyl esters also appeared to be the most volatile ones (Lang et al., 2001).

Biodiesel used as an additive to diesel fuel can improve its lubricity. This property is becoming increasingly valuable as recent legislation has mandated further regulation on the sulphur content of diesel fuels; these cleaner diesel fuels exhibiting reduced lubricity as compared with their high sulphur predecessors (Radich, 2004; Goodrum and Geller, 2005). Some fatty acids such as ricinoleic (castor oil) and lesquerolic acids (*Lesquerella* spp.) could be especially efficient in enhancing the lubricity of a diesel fuel to an acceptable level at concentrations as low as 0.25% (Goodrum and Geller, 2005). Blending biodiesel with diesel fuel can increase the neat cetane number. Cetane number increases with increasing length of both fatty acid chain and ester groups, while it is inversely related to the number of double bonds and as double bonds and carbonyl groups move toward the centre of the chain (Graboski and McCormick, 1998; Tripartite Task Force, 2007). Highly saturated oils, with a low number of double bonds, hence provide the fuel with superior oxidative stability and higher cetane number (Worldwatch Institute, 2007). The average cetane numbers are 50.9 for soy and 52.9 for rapeseed esters. For the other esters listed in Graboski and McCormick, 1998, including sunflower, cottonseed and palm oil, cetane numbers vary in the 48–60 range. In comparison, the cetane index for petroleum diesel ranges from 40 to 52 (Radich, 2004).

The co-products of the entire chain are the meal left in the seed after oil extraction, which is sold as animal feed, and the glycerine from glycerol recovery, used in cosmetics. However, the rapid expansion of biodiesel has already saturated the market of glycerine in Europe, undercutting its ability to reduce the biodiesel price, as it could offset 5% of the production cost (Worldwatch Institute, 2007).

3.1.2 Ethanol

Ethanol, on the contrary to biodiesel, is a single-compound biofuel whose final composition does not vary with the type of feedstock. The feedstock is sugar and starch crops, which are basically equally processed through pre-treatment, fermentation by yeasts and other microbes, and distillation. The main sugar crops are sugar cane and sugar beet. Sweet sorghum could also become an interesting ethanol feedstock as a multi-use crop, whose seeds are edible and whose stalk contains sugar. The main starch crops used nowadays are maize and wheat; also potatoes, cassava and sorghum grain to a lower extent. Sugar crops typically yield more ethanol per hectare with an overall better energy balance than starch crops because (1) sugar crops yield higher sugar amounts per hectare compared with starch crops; and (2) sugar can be directly fermented, whereas starch long polymers have to be hydrolysed before being fed to yeast for the ethylic fermentation.

“Wet-milling” and “dry-milling” are the two current common methods to treat the starchy crop parts at their entry in the process chain. In the wet-milling, grains are soaked and chemically sub-divided into rich starchy parts of primary interest (grain endosperms) and other parts that contain more protein and fibres and constitute diverse co-products (maize oil and syrup, gluten feed, germ meal, vitamins and amino acids). These co-products can contribute up to 25% of the process economy (Worldwatch Institute, 2007). The dry-milling method only consists of grinding the unprocessed heterogeneous seeds into granules. It is therefore less expensive but also leads to less diverse co-product production. The main co-product is the dried distillers grain (DDG) fed to animals that can digest high proportions of fibres, and contributes up to 20% of the process economy (Worldwatch Institute, 2007). In both “wet” and “dry” processes, the starch is finally hydrolysed into sugar typically using a high-temperature enzyme process (Fulton et al., 2004; Cardona and Sanchez, 2007). CO_2 from fermentation can also be sold as a co-product to beverage industries. Indeed, in conventional fermentation, approximately one-third of the carbon available in the sugar is lost as CO_2 (Strege, 2007).

The fermented ethanol must be distilled until enough water is removed to make the final anhydrous ethanol suitable for blending with gasoline (<1% of water in temperate climates). Indeed, water in ethanol blended with gasoline makes the fuel more sensitive to frost and increases the risk of phase separation in both storage and vehicle fuel tanks, which can cause serious operating problems for the engines (Tripartite Task Force, 2007; Balat et al., 2008). To improve the ethanol quality as a blend in low percentage, ethanol (47% on a mass basis) can be converted into Ethyl-Tertio-Butyl-Ether (ETBE) by reaction with isobutylene (53%). An ETBE blend of 15% corresponds to a blend in volume of 7% ethanol.

Since they both contain oxygen, ethanol and biodiesel are better combustibles than the substituted fossil oils, reducing the emission of pollutants such as CO, hydrocarbons (HC), sulphur oxide[5] and particulates by up to half of these emissions, depending on the biofuel and the blend mix (Shahid, 2007; Luneau and Fayet, 2007; Murugesan et al., 2008). Exhausts from blends with vegetable oils also depend on the engine load (Murugesan et al., 2008). Conclusions are not univocal concerning NOx emissions[6], but biofuels would tend to lead to slightly higher emissions (Graboski and McCormick, 1998; Radich, 2004), notably with blends of 20% of ethanol or biodiesel in a car driven in the city (Luneau and Fayet, 2007). In Murugesan et al. (2008), NOx emissions are reported to be in the range of ±10% as compared with diesel, depending on engine combustion characteristics (2008). Biofuels increase the octane level[7] (thanks to ethanol, Harijan et al., 2007; Balat et al., 2008) and cetane number (thanks to biodiesel, Radich, 2004). On the other hand, both ethanol and biodiesel may cause corrosion and are sensitive to cold weather.

The primary asset of biofuels is the convenience that they can be used as blends with conventional fuels in existing vehicles. However, unmarked blends are limited to certain extents according to fuel and vehicle specifications. As an example, in Europe, these blends on a volume basis are: 5% ethanol or 15% ETBE blends with gasoline, and currently 5% biodiesel in diesel fuel (Wiesenthal et al., 2007), up to 20–30% for specific fleets (Plassat, 2005). Beyond these limits, engines have to be adapted so that their performances will not be affected in the long run. This is, for instance, the case with the flexible-fuel vehicles that can run on low- and high-level ethanol blends up to 85–100% (also written as E85, E90, E100; biodiesel blends are noted B20, B30).

Divergences in biofuel technical specifications have been introduced worldwide due to feedstock variances, climatic conditions in each country and region, and the characteristics of the local markets (Tripartite Task Force, 2007). In consequence, automotive sector and biofuel blend-related strategies have also diverged. Diverse biofuel and blend standards were adopted worldwide by the various agencies, ABNT/ANP[8] in Brazil, ASTM International in the US and CEN[9] in Europe. The Tripartite Task Force has been working on implementing a road map to come up with international compatible biofuel-related standards to help increase the use of biofuels and avoid adverse trade implications in the global market.

The water content of ethanol shows how critical it may be to agree on standardised blends. Phase separation due to water occurs more readily at lower levels of ethanol in gasoline. In Brazil and the US, where ethanol blends reach up to 5.7–10% and 20–25%, respectively, phase separation is not an issue, whereas it becomes an issue in Europe with lower ethanol blends in gasoline. Additional dehydration increases production cost and can reduce productivity at the mill by up to 7% (Tripartite Task Force, 2007). Considering the additional environmental and economic costs related to biofuel upgrading, international harmonisation should urge a maximum level of blend flexibility correlated with a focus on minimum processing and optimum engine modifications.

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[5] Sulphur oxides (SOx) contribute to acid rain and can be carcinogenic.

[6] NOx are precursors to the formation of tropospheric ozone.

[7] Gasoline high octane value indicates a smaller likelihood that the fuel combusts too soon (low auto-ignite tendency), provoking engine knock problems. A high tendency to auto-ignite, or low octane rating, is undesirable in a spark ignition engine (gasoline) but desirable in a diesel engine (high cetane number).

[8] Brazilian Petroleum, Natural Gas and Biofuels Agency.

[9] Comité Européen de Normalisation.

3.1.3 Biogas

Biogas is produced through methanisation, i.e. the anaerobic digestion by bacteria of biodegradable matter such as municipal solid or agricultural waste, liquid slurry, solid manure, or maize silage, for instance. The more dry matter and fatty acids in the substrate contents, the greater the biogas yield (Moras, 2007). Apart from about 55 to 70% of methane (52–65% in examples in Table 2), the actual fuel, biogas also contains substantial amounts of CO_2, 30 to 45%, small quantities of hydrogen sulphide and other trace gases such as ammonia[10]. The separation of these components of biogas via a gas scrubber is an expensive prerequisite in order to use the biogas as fuel or to mix it with natural gas.

Biogas is less considered as transportation biofuel, because its target vehicle fleet remains marginal due notably to onboard gas storage constraints. The primary interest of biogas remains its local development as fuel for heat and power plants in rural areas. About 25 million households worldwide currently receive energy for lighting and cooking from biogas produced in household-scale digesters, including 20 million in China, 3.9 million in India and 150 thousand in Nepal (REN21, 2008). Hence, the two prevalent types of digesters are the Chinese "fixed dome" and the Indian "floating cover" that only differ by the gas collection method (ITDG, 2000). Biogas production in specifically designed digesters is the most widespread technology, although capturing methane from municipal waste landfill sites has been considerably developed. In the US, waste management including the recovering of methane produced by landfills has made possible to reduce these methane emissions by 50% over the years and has become one of the largest holders of greenhouse gas emission credits (Kram, 2007). Although the reaction takes several days to finally degrade just about 10 to 15% of the initial material, biogas permits one to take advantage of cheap feedstock and to diminish greenhouse gas emissions. Moreover, the solid residue of the process can be dried and used as fertiliser that has a high nutrient content and whose pathogenic germs have been killed during the digestion process, notably due to temperatures of 35°C to more than 50°C (ITDG, 2000; Baserga, 2000).

Biogas as transportation fuel could receive more attention in the coming decades, especially for use in city fleets and trucks, as has been the case in Sweden and Switzerland for a long time. Table 2 gives the productivities of common feedstock and biofuels worldwide.

3.2 Current 1st Generation Biofuel Supply Worldwide

Combustible renewables and waste are mostly consumed directly; only about 1.8% is consumed by the transport sector, about 17.6% by the industries and 80.6% by other sectors, notably households (IEA, 2007a). Production of heat and electricity dominate current bioenergy use with two key industrial sectors for application of state-of-the-art biomass combustion for power generation: the paper and pulp sector and cane-based sugar industry (IEA Bioenergy, 2007).

Global fuel ethanol production more than doubled between 2001 and 2006, while that of biodiesel expanded nearly six-fold. The US and Brazil currently dominate world ethanol production, which reached a record 52 billion litres in 2007 (FAO, 2008a). Close to half the world's fuel ethanol was produced in the US from 14% of its national maize production in 2006 (Möller et al., 2007), and more than two-fifths in Brazil from sugar cane, roughly providing 21%[11] of its transport fuel consumption (OECD, 2006). The remaining production comes from Spain, Sweden, France and Germany. China's ethanol from maize, wheat and sugar cane is mostly destined for industrial use.

In 2006, Europe accounted for 73% of all biodiesel[12] production worldwide, mainly from rapeseed and sunflower seeds, with Germany as the leading producer (40%), followed by the US, France and Italy generating most of the rest (Worldwatch Institute, 2007). In 2007, the EU still accounted for 60% of global biodiesel production, that amounted to 6.2 billion litres in 2007 (FAO, 2008a), but biodiesel production has increased in all producing countries; it has doubled in the USA and in most of the producing

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[10] http://www.planete-energies.com (consulted on 10.03.2008).

[11] Various data: 13.2% in energy terms according to IEA 2006; 40% according to Xavier (2007).

[12] Biodiesel here does not take into consideration pure vegetable oils mostly directly consumed by farmers on the farm.

Table 2 Main feedstock, productivity per hectare, and co-products of 1st generation biofuels; FM: fresh matter

Biomass feedstock	Countries regions	Yields t FM ha^{-1}	Biofuels L ha^{-1}	Fossil equivalent (on energy basis) L ha^{-1}	Total energy output/Fossil energy input	Co-products	References
SUGAR CROPS			ethanol (1L = 0.791 kg)	1L ethanol = 0.665 L gasoline*	Indicative values	Crop residues left in the field are often considered as fertilizing co-products and are important for sustaining soil C content	Fulton et al., 2004 FNR, 2007
SUGAR CANE	Brazil	85	7.080	4.708		Yeast as cattle feed supplement;	Brazilian Ministry in Xavier, 2007
			6.500	4.323		Bagasse as feedstock for feed heat. Electricity, and cellulosic ethanol	Cardona and Sanchez, 2007
			6.800	4.522		Fructose as sweetener; invertase for food industry 23.8 t ha^{-1} bagasse (50% H_2O) = 7.854 k Wh electricity	Fulton et al., 2004
		73.5	5.476	3.641		Energy from cane trash	Moreira, 2000
		60.7	4.522	3.007	~8 [2.1–8.3]		Naylor et al., 2007 in FAO, 2008a Naylor et al., 2007 in FAO, 2008a
	India	66.5–71.3–	5.300	3.525		2.753–2.952 litres of cellulosic ethanol from 20.5–22 t ha^{-1} bagasse	Fulton et al., 2004 Kadam, 2000
	Thailand	55.7	–	–		104 kg sugar/t cane +45 kg molasses/t cane (+5 kg rice husk) = 10.2 L ethanol [national availability~1Mt yr^{-1} molasses]	Nguyen and Gheewala, 2008 Prasertsri, 2006
	Global	65	4.550	3.026			Rajagopal et al., 2007 in FAO, 2008a
SUGAR BEET	France	66.2	3.793	2.522		3.7 t ha^{-1} of sugar	ADEME/DIREM, 2002
		–	7.307 7.500	4.860 4.988		If only ethanol without sugar production 44 t green manure (10% dry matter)	

	Germany	55.5	5.439	3.617	∼2 [1.2–2.2]	(Stabilized) molasses as fertiliser Sugar beet pulp and dried slop as animal feed Slop to biogas	France Betteraves/Passion Céréales, 2007 FNR, 2007
	EU	–	5.500	3.658		Heat	Fulton et al., 2004
		68.9	–	–			JRC/EUCAR/CONCAWE, 2008
	Global	46	5.060	3.365			Rajagopal et al., 2007 in FAO, 2008a
SWEET SORGHUM	China	60	5.070	3.370	∼1 [0.9–1.1]	3–4 t sorghum grains	USDA, 2007
		90	7.605	5.057			
STARCH CROPS							
MAIZE	Brazil	10	3.570	2.374		Wet-milling process : fibre as substrate for enzymes production, CCDS as feed for non-ruminants, gluten meal (60% protein. high fat) and gluten feed (20% protein, low fat), corn oil, different chemicals and food-related products as vitamins and amino acids	Brazilian Ministry in Xavier, 2007 Fulton et al., 2004 Cardona and Sanchez, 2007 Graboski, 2002 Naylor et al., 2007 in FAO, 2008a
	USA	–	3.100	2.062			Levelton, 1999
		8.7	3.471	2.308			
		9.4	3.751	2.494	∼1.5 [1.4–1.8]		
	Canada	7.2	3.384	2.250		Dry-milling process: DDGS as feed for ruminants	
	China	5	2.028	1.349		(27–35% protein)	USDA, 2007
		5	1.995	1.327		4.8 t ha^{-1} DDGS	Naylor et al., 2007 in (FAO, 2008a)
	France	–	3.600	2394			France Betteraves/Passion Céréales, 2007
	Global	4.9	1.960	1.303			Rajagopal et al., 2007 in FAO, 2008a

(continued)

Table 2 (continued)

Biomass feedstock	Countries regions	Yields t FM ha^{-1}	Biofuels L ha^{-1}	Fossil equivalent (on energy basis) L ha^{-1}	Total energy output/Fossil energy input	Co-products	References
WHEAT	France	7.5	2.600	1.729		2.8 t ha^{-1} DDGS	FB/Passion Céréales, 2007
		9	2.550	1.696		DDGS can be used as animal feed or for fuel	ADEME/DIREM, 2002
	Germany	–	2.560	1.702		Glycerol	FNR, 2007
		7.44	2.753	1.831	~2	Straw for fertiliser or fuel	Öko-Institut, 2004
	EU	–	2.500	1.663	[1.2–4.2]		Fulton et al., 2004
		5.2	–	–			JRC/EUCAR/CONCAWE, 2008
	Global	2.8	952	633			Rajagopal et al., 2007 in FAO, 2008a
CASSAVA	Brazil	13.6	1.863	1.239			Naylor et al., 2007 in FAO, 2008a
	Nigeria	10.8	1.480	984	~2.85		
	Thailand	19	–	–	2.1 in China		Nguyen et al., 2007
	Global	12	2.070	1.377			Rajagopal et al., 2007 in FAO, 2008a
RICE	Global	4.2	1.806	1.200	–		Rajagopal et al., 2007 in FAO, 2008a
BARLEY	Global	–	1.100	732	–	DDGS can be used as animal feed or for fuel	Fulton et al., 2004
SORGHUM GRAIN	Global	1.3	494	329	–	Straw for fertiliser or fuel	Rajagopal et al., 2007 in FAO, 2008a
OIL PLANTS			Pure vegetable oils	rapeseed oil 1 L = 0.961 diesel fuel			ITDG, 2000
				sunflower oil 1 L = 0.851 diesel fuel			ADEME/DIREM, 2002 FNR, 2007
SUNFLOWER	France	3.34	1.522	1.461	4.7	Meal or cake as animal feed	ADEME/DIREM, 2002
RAPESEED		2.44	1.178	1.000	7.5		
	Germany	3.4	1.480	1.420	3–5		FNR, 2007

METHYL–ESTERS			Biodiesel (methyl-ester) 15°C (1 L = 0.9 kg)	1 L biodiesel = 0.88–0.95 L diesel = 0.85 L diesel = 0.89 L diesel			Worldwatch Institute, 2007 ITDG, 2000 Lang et al., 2001
RAPESEED	Germany	3.4	1.550	1.380			FNR, 2007
	France	3.34	2.184	1.944	∼2.5		ADEME/DIREM, 2002
	EU	–	1.200	1.068	[1.3–3.7]		Fulton et al., 2004
		3.11	–	–			JRC/EUCAR/CONCAWE, 2008
SUNFLOWER	France	2.44	1.690	1.504	3	Meal or cake as animal feed or converted to biogas, steam and electricity	ADEME/DIREM, 2002 Fulton et al., 2004
	EU	–	1.000	890		Glycerol sold or converted to biogas then steam	JRC/EUCAR/CONCAWE, 2008
		2.44					Fulton et al., 2004
SOYBEAN	USA	–	500	445			
		2.7	552	492			Naylor et al., 2007 in FAO, 2008a
	EU	–	700	623	∼3		Fulton et al., 2004
		–	400	356	[1.5–3.5]		Fulton et al., 2004
	Brazil	2.4	491	437			Naylor et al., 2007 in FAO, 2008a
		2.8	–	–			JRC/EUCAR/CONCAWE, 2008

(continued)

Table 2 (continued)

Biomass feedstock	Countries regions	Yields t FM ha^{-1}	Biofuels L ha^{-1}	Fossil equivalent (on energy basis) L ha^{-1}	Total energy output/Fossil energy input	Co-products	References
PALM OIL	Brazil	–	5.000	4.450			Fulton et al., 2004
	Malaysia	–	6.000	5.340			Fulton et al., 2004
		20.6	4.736	4.215	~9 [8.7–9.7]	Glycerol sold or converted to biogas then steam	Naylor et al., 2007 in FAO, 2008a
	Indonesia	17.8	4.092	3.642			Naylor et al., 2007 in FAO, 2008a
	Global	19	–	–			JRC/EUCAR/CONCAWE, 2008
JATROPHA	India	1.42	577	514		Husks and cakes as fertiliser or for fuel	Reinhardt et al., 2007
	Global	–	2.000	1.780	–	Detoxified meal or cakes as animal feed Meal to produce biogas Shell as fuel Glycerol	Fulton et al., 2004
WASTED VEGETABLE OIL	Global	–	–	–	[4.8–5.8]	Meal or cakes as animal feed	Rajagopal et al., 2007 in FAO, 2008a
Biomass feedstock	Countries regions	Yields m^3 t FM ha^{-1}	Biofuels m^3 ha^{-1}	Fossil equivalent (on energy basis) m^3 ha^{-1}	Total energy output/Fossil energy input	Co-products	References
DIGESTIBLE MATERIALS			Biogas	(% of CH_4) m^3 methane (LHV: 36 MJ m^{-3}			
MAIZE SILAGE	Germany	170–202	7.800–8.300	(52) 4.000–4.300			(FNR, 2008)
	France	200	6.660–8.500	(52) 3.500–4.450	–		www.pleinchamp.com (03.03.08)
GRASS SILAGE	France	211	2.000–2.600	(54) 1.100–1.400			Poiret, 1996
	Germany	172	2.500–3.200	(54) 1.400–1.700			Moras, 2007
SLURRY							
Bovine	Germany		25 m^3.t^{-1}	(60) 15 m^3.t^{-1}			
	Global		26 m^3.t^{-1}	(60) 15.6 m^3.t^{-1}			

Pig	Germany		$28\ m^3.t^{-1}$	(65) 18.2 $m^3.t^{-1}$	–		
	Global		$36\ m^3.t^{-1}$	(65) 23.4 $m^3.t^{-1}$			
Chicken	Global		$44\ m^3.t^{-1}$	(60) 26.4 $m^3.t^{-1}$			
MANURE						CO_2 (25–45% of biogas) Digested biomass as fertiliser	
Bovine	Germany		$45\ m^3.t^{-1}$	(60) 27 $m^3.t^{-1}$			Moras, 2007
	Global		$25\ m^3.t^{-1}$	(60) 15 $m^3.t^{-1}$			FNR, 2008
Pig	Germany		$60\ m^3.t^{-1}$	(60) 36 $m^3.t^{-1}$	–		
Chicken	Germany		$80\ m^3.t^{-1}$	(60) 48 $m^3.t^{-1}$			
BIOMASS WASTE							
Green waste	Germany		$100\ m^3.t^{-1}$	(61) 61 $m^3.t^{-1}$			
	Global		$60\ m^3.t^{-1}$	(61) 36.6 $m^3.t^{-1}$			
			$151\ m^3.t^{-1}$	(61) 92 $m^3.t^{-1}$	–		
Maize straw	Global		$402 \times 10^3 L.t^{-1}$	–			
WASTED OILS	Global		$800 \times 10^3 L.t^{-1}$	–	–		
MISCANTHUS (>3 years)	Germany	15	6.081 L ha^{-1}	4.044 L ha^{-1} gasoline			Nguyen et al., 2007
CEREAL STRAW	2nd generation	6	2.390 L ha^{-1}	4.590 L ha^{-1} gasoline	20		FNR, 2007
STRAW OR WOOD–BTL	biofuels stand	–	4.030 L ha^{-1}	3.900 L ha^{-1} diesel	–		

Notes: energy ratios are “approximate” values and range given in Worldwatch Institute (2007), except those in italics.
FM: fresh matter; CCDS, corn condensed distiller’s solubles; DDGS, dried distiller’s grains with solubles.
* In Nguyen et al. (2007), 1 L ethanol = 0.89 L gasoline based on fuel economy, i.e. taking into consideration that vehicle performance is enhanced due to ethanol’s higher octane value.
Indicative provisional figures are given for 2nd generation biofuels in order to give a quick comparison point.

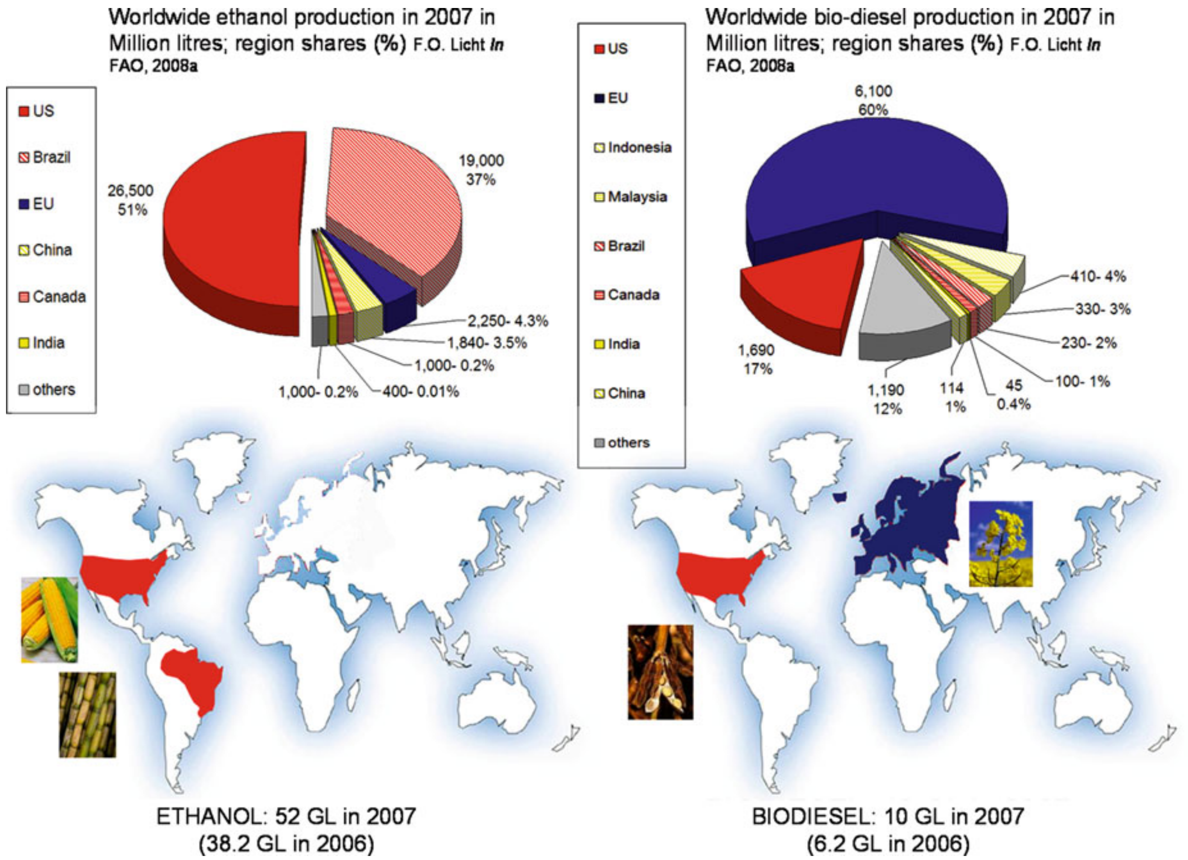

Fig. 3 Worldwide ethanol and biodiesel productions in 2007 in Million litres; region shares (%). Drawn from data of F.O. Licht[13] in FAO 2008a

countries in Asia and Oceania, and more than tripled in Brazil. Figure 3 show the production shares of ethanol and biodiesel worldwide in 2007.

Despite the growth in biofuel consumption and a comparatively slower growth in oil consumption, biofuels still do not represent a significant share in worldwide liquid fuel supply; about 0.9% by volume, 0.6% by transport distance travelled (Worldwatch Institute, 2007). Within Europe, biofuels are essentially domestically produced and consumed, except in Sweden, where since 2004 all petrol has been blended with 5% ethanol, mainly originating from Brazil and wine production sites in Southern Europe; only 20% is produced nationally (Van der Drift and Boerrigter, 2006). International trade in biodiesel is minimal as yet: only 10% of biofuels produced in the world are sold internationally, with Brazil accounting for roughly half of those sales (IEA, 2006). However, trade is very likely to increase, notably due to the fact that only some regions of the world may be able to produce large biomass feedstock (see part 6).

3.3 Towards 2nd and 3rd Generations of Biofuels

Second generation biofuels are produced via biochemical (hydrolysis and fermentation) and thermochemical (pyrolysis or gasification) treatments. The biochemical or so-called "wet process" is very similar to the 1st generation ethanol except for the feedstock, which is not specific. Indeed, 2nd generation biofuels are all produced from lignocellulose, i.e. all

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[13] Christoph Berg is Managing Director at the commodity analysts F.O. Licht. F.O. Licht monitors the global soft commodity markets.

kinds of vegetal biomass, as lignocellulose forms the basic structure of vegetal cell walls. Cell walls make up a substantial portion of the dried biomass: about 60–80% and 30–60% in the stems of woody and herbaceous plants, respectively, and about 15–30% in their leaves (Möller et al., 2007).

Lignocellulose consists of intricate assemblages of cellulose, hemicellulose and lignin, whose proportions and molecular organisation vary depending on the type of biomass. A typical range is 40 to 55% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin (Worldwatch Institute, 2007). The other minor components of cell walls are proteoglycans and pectins that glue together all the lignocellulosic compounds. The conformation of glucose residues in the crystalline cellulose core of cell-wall microfibrils forces the hydroxyl groups into radial orientation and the aliphatic hydrogen atoms into axial positions. It leads to the creation of strong interchain hydrogen bonds between adjacent chains in a cellulose sheet, which make cellulose resistant to enzymatic hydrolysis, and weaker hydrophobic interactions between cellulose sheets that contribute to the formation of a water layer near the hydrated cellulose surface, protecting cellulose from acid hydrolysis. Furthermore, the microfibrils are embedded in the matrix of hemicelluloses and lignin, the latter also contributing to making cells walls hydrophobic and more resistant against enzymatic attack (Möller et al., 2006). Other molecules such as, for instance, waxes or inhibitors of fermentation, which naturally exist in the cell walls or are generated during conversion processes, also contribute to biomass recalcitrance (Himmel et al., 2007). This recalcitrance is the primary barrier to producing ethanol from lignocellulosic feedstock, commonly referred to as cellulosic ethanol. Indeed, expensive pre-treatments are necessary to break down this resistance, and reaching a cost-effective cell wall saccharification, i.e. the degradation of cell walls into monosaccharides, is the key that could really permit cellulosic ethanol to enter the market.

Pre-treatments include physical methods such as milling and grinding, high-pressure steaming and steam explosion, and biological (lignin- or/and cellulose-degrading organisms) or chemical methods (alkali or acid treatments, solvents) to solubilise parts of the hemicelluloses and the lignin. So far, methods such as ammonia fibre explosion (AFEX), wet oxidation and liquid hot water (LHW) treatment seem to be more successful for agricultural residues, whereas steam pre-treatment has resulted in high sugar yields for both forestry and agricultural residues (Hahn-Hägerdal et al., 2006). Monosaccharides from cellulose (glucoses) and hemicelluloses (pentose sugars) are then released through acid- or enzyme-catalysed hydrolysis, and finally fermented. Concentrated or dilute acid hydrolysis methods are more mature but very energy-intensive and present the disadvantage of potentially also degrading the monosaccharides. Enzymatic degradation, on the contrary, is more specific and perceived by many experts as a key to cost-effective saccharification, but none of these methods is currently cost-effective (Möller et al., 2006). As an example, hydrolysis of pre-treated lignocellulosic biomass requires one hundred-fold more enzymes than hydrolysis of starch (Tolan, 2006 in Möller et al., 2006).

Researchers have therefore been focusing, on the one hand, on improving the yields of pre-treatment and lowering their costs, and on the other hand, on developing integrated processes that make it possible to protect, separate and use the other compounds, such as the C5-sugars and the lignin, or the co-products such as furfural and fermentation gases. Traditional fermentation processes relied on yeasts and microbes that only convert C6-sugars (mainly glucose) into ethanol (Fulton et al., 2004). Researchers have already succeeded in producing several new yeast strains and bacteria, such as engineered *E. coli*, *K. oxytoca* and *Z. mobilis* (Balat et al., 2008), that exhibit varying degrees of ability to convert the full spectrum of available sugars into ethanol.

The introduction of simultaneous saccharification and fermentation (SSF) permitted a gain in efficiency of a 13% higher overall ethanol yield than separate hydrolysis and fermentation (SHF) (72.4% versus 59.1% of the theoretical maximum yield) (Öhgren et al., 2007). This gain is due to the fact that hydrolysed sugars are immediately fermented in the case of SSF, whereas their accumulation leads to enzyme inhibition in the case of SHF (Fulton et al., 2004). More recently, the simultaneous saccharification and co-fermentation of hexoses and pentoses (SSCF) has proved to be further advantageous as the hexose sugars continuously released by enzymatic hydrolysis increase the rate of glycolysis, so that pentose sugars are fermented faster and with higher yield (Hahn-Hägerdal et al., 2006). This makes it possible to lower the cost as both operations can be done in the same tank, added to the fact that enzyme manufacturers have recently reduced costs substantially thanks to biotechnology (Solomon et al., 2007; Balat et al., 2008). Nevertheless, further advances in discovering new hydrolases, new fermentation enzymes and organisms with process-tolerant traits such as tolerance to alcohol, pH and inhibitors, and advances in product recovery technology are required to reach commercial viability (US DOE, 2006a). Producing enzymes with combined tolerant traits is a real challenge, considering, for instance, that the majority of organisms cannot tolerate ethanol concentrations above 10–15% (w/v) (Balat et al., 2008). Moreover, optimal temperature and pH conditions vary depending on the enzymes and microorganisms involved in the different process stages, which can hamper the efficiency of the batch SSF or SSCF (Cardona and Sanchez, 2007; Öhgren et al., 2007). "Consolidated biomass processing" (CBP), the logical end point in the evolution of biomass conversion technology, would require a unique microbial community to produce all the enzymes for the saccharification and fermentation within a unique reactor vessel, but it has not been achieved yet (Fulton et al., 2004). For robust and complete conversion of polysacchaarides locked in biomass, the ultimate ethanologens will have to produce at least a dozen enzymes of different catalytic activities. Engineering such a yeast strain requires (1) screening thousands of combinations of biomass-degrading enzymes to identify the appropriate set of enzymes, then (2) managing to ensure that this strain is capable of simultaneously expressing the genes for all necessary enzymes (Hector et al., 2008).

Current biomass-conversion technologies were developed empirically and are based on limited understanding of the biological and chemical properties of biomass (Himmel et al., 2007). Therefore, all research efforts also rely in parallel on fundamental research to understand and characterise the cell walls of a very wide range of biomass feedstock better. This feedstock encompasses perennial grasses, short rotation coppices, cereal straws, and other biodegradable residues or waste. According to Möller et al. (2007), poplar, willow, miscanthus (see picture below) and wheat straw are the main relevant feedstock in Europe. In the US, attention is especially paid to maize stover, wood waste and switchgrass, whereas sugar cane producers are obviously more interested in converting the sugar cane bagasse. Research worldwide includes breeding programmes to develop new varieties with interesting phenotypes in terms of growth and resistance, but also in more specific biorefinery-related terms, i.e. regarding the cell wall composition. Research also includes genetic engineering. As an example, "Spartan maize" has been genetically modified within a research programme at the Michigan State University to express cellulase and hemicellulase in the plant's leaves and stover (Sticklen, 2007). Transgenic alfalfa has also demonstrated lower amounts of lignin, leading to drastic reduction of pre-treatment costs (Chapple et al., 2007). However, reduction in lignin content also leads to reduced biomass by up to 40%, which emphasises the need to determine whether cell wall manipulation may compromise the plant's structural integrity or susceptibility to pests and pathogens (Chapple et al., 2007).

Miscanthus at Estrees-Mons,
November 2008 bessou©INRA

Figure 4 shows the expected time frame for research advances and economically viable implementation of cellulosic ethanol over the next 5 to 15 years in the US. Considering the potential to sustainably harvest more than 1.3 billion metric tons of biomass from U.S. forest and agricultural lands by the mid-21st Century

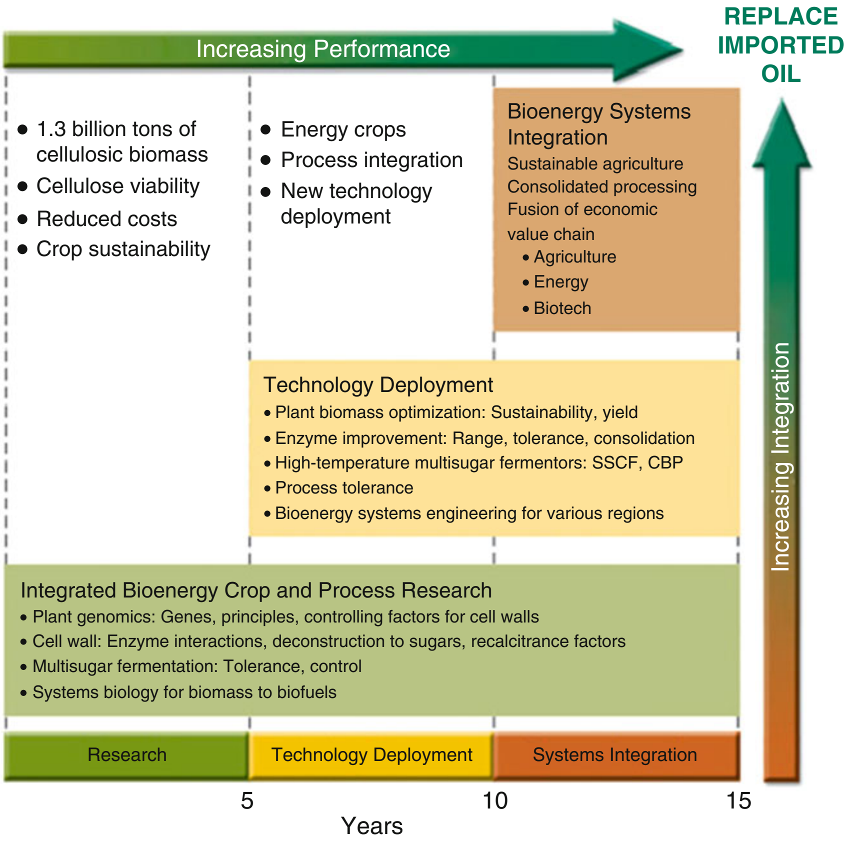

Fig. 4 Phased development of bioenergy systems over the next 5 to 15 years (US DOE, 2006a). SSCF: simultaneous saccharification and co-fermentation; CBP: consolidated biomass processing

(Perlack et al., 2005), these projections illustrate the needed co-increase of technological performances and systems integration, and detail the research fields. Within 10 years, dedicated energy crops with composition and structure better suited for breakdown into sugars for fermentation, high yield and robustness will be essential in contributing to achieving energy security.

While large deployment may not occur before ten years (US DOE, 2006a; BIOFRAC, 2006; FNR, 2006), or even fifteen years (Möller et al., 2007), several pilot and demonstration plants have already been built worldwide. Some twenty of such plants have been implemented since 1985 in the US, Canada, Brazil, Europe and Japan and about a dozen cellulosic ethanol commercial plants were being developed in 2007–2008, essentially in the US (Solomon et al., 2007) or under discussion in Canada and China. Steam pre-treatment with the addition of a catalyst for hydrolysis and improved enzymatic digestibility is the closest technology to commercialisation and has been widely tested in pilot-scale equipment (Hahn-Hägerdal et al., 2006). Considering the state-of-the-art technology in 2006, an estimated capital investment for a 220-million-litre cellulosic ethanol plant would approximate US $ 300 million, with the largest capital components for feedstock pre-treatment (17%) and simultaneous saccharification and fermentation (15%), and energy utilities (36%). The production cost could then approximate US $ 0.57 per litre with 40% related to the annualised capital charge and 46% to the feedstock and other raw materials (Solomon et al., 2007). In another estimate, production and transport of feedstock would represent about 21% and 26% of the total annual plant costs, respectively (Kaylen et al., 2000). In recent simulations, production costs, mostly based on the laboratory scale, range from 0.28 to 1.0 US $ per litre of cellulosic ethanol (Hahn-Hägerdal et al., 2006). In 2006, the production cost of dry mill ethanol from maize was US $ 0.44 per litre (Balat et al., 2008).

Scientists mostly argue that the technology is not mature yet for commercial production (Solomon et al., 2007; Cardona and Sanchez, 2007), whereas some industrials may be ready to take the chance. Still, all agree that tremendous increase in production volumes is the determinant techno-economic factor to reach commercial viability. About 86% of operating costs appeared to be proportional to the size of the plant (Kaylen et al., 2000). A drastic increase in production volumes and an "on-site" enzyme production, provided with governmental funds in the first development phase, appear to Murray Burke[14], Vice President and General Manager of the SunOpta BioProcess Group, the essential challenges to reach commercial production. As costs are highly linked to feedstock, whose price is volatile, diversification of feedstock, maximisation of ethanol yields and optimisation of the use or commercialisation of co-products must also be achieved. Current pilot plants can produce a few million litres a year, possibly integrated with an ethanol from-grain plant, which can be a near-term solution (Hahn-Hägerdal et al., 2006), but a ten-fold capacity increase appears to be necessary for a complete switch of these plants to cellulosic ethanol plant.

Whereas lignin cannot easily be converted through biochemical processes, it can be burnt. Therefore, thermochemical processes are especially more effective in the case of plants with a high content of lignin, up to 30–35% of the biomass in some plants (Fulton et al., 2004; Möller et al., 2006). The main criteria for biochemical conversion of lignocellulosic feedstock are the quantity of sugars and the structure of the lignocellulose; in the case of thermochemical conversion, the main criteria are rather the biomass' bulk density, moisture and ash contents, and the calorific value (Möller et al., 2007). In a rough overview, agricultural residues and grasses with intrinsically higher sugar content and lower lignin content are generally more suitable for enzymatic conversion, whereas dense woody biomass with higher amounts of lignin and lower amounts of ash is comparatively more oriented toward thermochemical conversion. Ash can indeed lead to the slagging or fouling of heat-transfer surfaces during gasification. However, improvement of current technologies will notably permit one to reach efficient conversion ratios for a mix of the cheapest and most available feedstock within the supply area of an implemented technology (Worldwatch Institute, 2007).

The thermochemical pathway is referred to as Biomass to Liquid (BtL) as an analogy with the conventional fossil Gas to Liquid pathway (GtL). Nowadays, 8% of the syngas produced worldwide is converted into transportation fuels through GtL processes; the overall production of syngas corresponding to almost 2% of the total worldwide primary energy consumption. Thereby, thermochemical technologies are well developed but have to be adapted to biomass feedstock in qualitative terms as well as in terms of plants' scale, considering that biomass availability might appear to be a limiting factor (Van der Drift and Boerrigter, 2006). The core process is gasification, that involves using heat to break down the feedstock molecules and produce a synthetic gas or syngas (also called "bio-syngas" when biomass is the feedstock), and whose compound-mix of hydrogen, carbon monoxide and dioxide, water vapour, methane and nitrogen varies depending on the process (Fulton et al., 2004).

There are two major types of gasifier that were selected because of their high efficiency in producing H_2 and CO, although they still produce a different ratio of gases at different temperatures and with a differing level of cleanliness (ITDG, 2000; Van der Drift and Boerrigter, 2006). The fluidised bed gasifier, typically operated at 900°C, has already been developed and demonstrated to produce heat and power from biomass. It requires, though, a catalytic reformer downstream to treat the produced gas so that it can fulfil the requirements to be converted into biofuels. On the contrary, the entrained flow gasifier, typically operated at 1300°C, makes it possible to produce syngas without a catalytic reformer but needs energy-intensive pre-treatment of the biomass, such as torrefaction or pyrolysis, in order to reach a sufficient conversion rate. Torrefaction at 250–300°C or flash/slow pyrolysis at 500°C both turn solid biomass into a biocoal or a bio-oil/char, respectively, that can be easily transported and fed to the gasifier. In all processes, syngas has to be further conditioned via gas cleaning and the H_2/CO ratio adjusted to be fed to a synthesis reactor and converted into final biofuels, such as Fischer-Tropsch diesel and naptha (basic gasoline), dimethyl ether (DME), methanol, mixed alcohols or hydrogen. Hydrothermal upgrading (HTU) is another

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[14] Speech at the Platts Cellulosic Ethanol Conference in Chicago on October 31, 2006.

process that makes it possible to transform biomass into a “biocrude” liquid by dissolving the cellulosic materials in water under high pressure but relatively low temperature. Bio-oils produced via pyrolysis or HTU can be subsequently upgraded into diverse hydrocarbon liquids and fuels (Fulton et al., 2004).

The diverse options are not incompatible; fluidised bed gasification can even occur as pre-treatment to feed an entrained flow gasifier. Choices are guided by the type of available biomass and the desired biofuels. Research projects are currently focusing on improving the pre-treatments, adapting the scale, and developing integration options such as polygeneration and mature biorefinery concepts to make the processes economically viable. Polygeneration refers here specifically to the up-value of all produced gases, and in particular of methane used as biogas to provide heat and power. Mature biorefinery (Fig. 5) is the combination of biochemical and thermo-chemical treatments that will permit one to produce as much biofuel (54% ethanol from sugars, 10% FT-diesel and 6% FT-gasoline from lignin and other residues) and heat/power (5% electricity also from lignin and other residues) as possible from lignocellulosic biomass (100%) with a minimum of energy input (21% captured for process energy or lost and <5% agricultural inputs: e.g. farming costs, feedstock transport) (US DOE, 2006a).

The frontier between 2nd and 3rd generation biofuels is conceptual and is not due to differences in biomass feedstock or radically new conversion processes. Still further technological breakthroughs will be needed to permit the economic viability of completely integrated biorefinery complexes, as well as technological revolutions in the transportation sector to introduce hydrogen as a competitive fuel for automotives. Hydrogen (H_2) is a fuel whose combustion produces only water. Although water vapour is the most significant greenhouse gas, its equilibrium in the atmosphere seems to be ensured by the natural water cycle. Hydrogen has been used by the aerospace industry since the 1960s and is nowadays

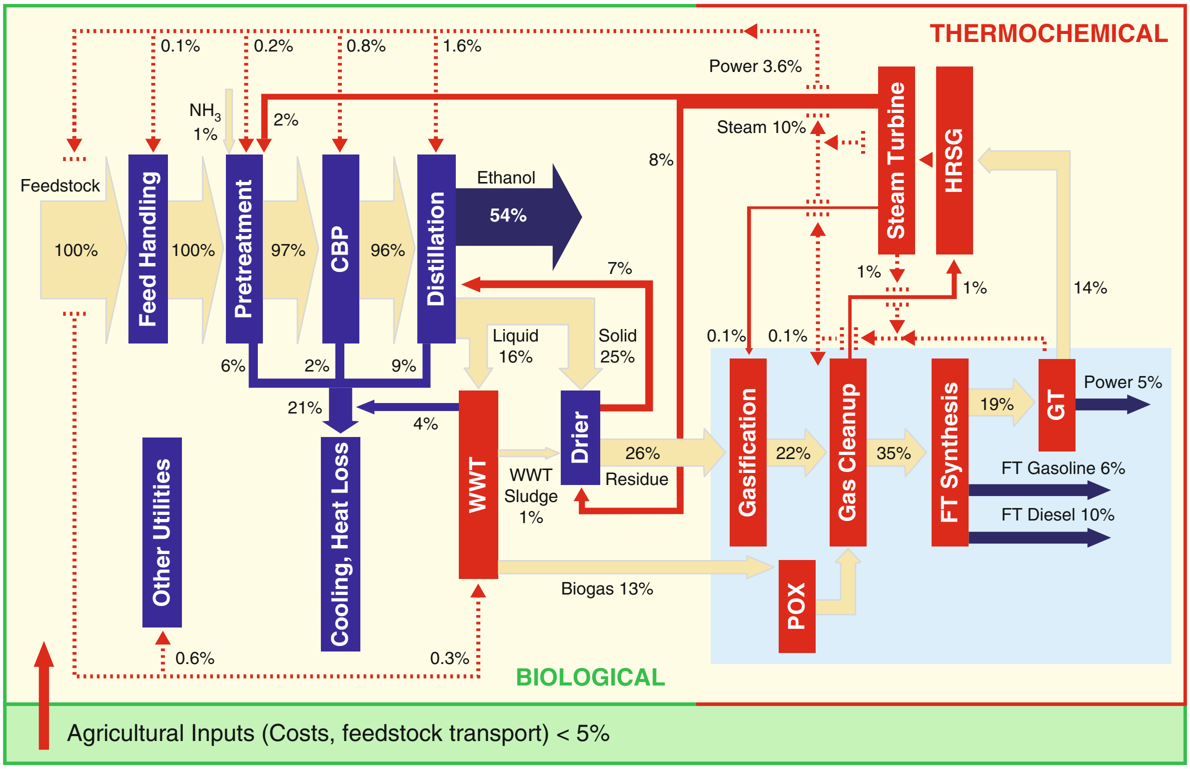

Fig. 5 Mature biomass refining energy flows: example scenario in US DOE, 2006a. “Envisioning Mature Biomass Refineries,” presented at First International Biorefinery Symposium, Washington, DC (July 20, 2005). Dotted arrows from above indicate energy inputs needed to run machinery

especially used in the petrochemical industry to make ammonia fertilisers, to upgrade lower quality fractions in the refining of fossil fuels, and also to produce glass, lubricants, refined metals and processed foods (Zeman, 2007). According to Shell, the world market for distributed and centralised hydrogen is estimated at approximately 45 million tons per year. However, hydrogen is not to be found in nature under this diatom form and has to be produced from hydro-carbonates or water, requiring considerable energy inputs.

Hydrogen is designated as a 3rd generation biofuel, when it is produced from biomass via the thermo-chemical processes described above. However, this term would not be appropriate when talking about hydrogen coming from the conversion of fossil fuels, even if the processes ensured the storage of all emitted greenhouse gas during the conversion. 95% of today's hydrogen is produced from fossil combustible, most commonly methane (Demirbas, 2007), via steam reforming that releases CO_2 into the atmosphere. Production via water electrolysis is three to four times more expensive and has a low energy yield (CEA, 2004). Hydrogen from renewables for fuel cell-driven vehicles might be a long-term solution, but its introduction needs breakthroughs in technology and cost and would require intermediate steps, to make a gradual growth of both fuel availability and number of vehicles possible. An effective intermediate step will be the use of hydrogen as a component in fuel production processes from biomass. This is applicable for today's fuel routes via synthesis gas, but will also be a serious option for future biorefineries (BIOFRAC, 2006). The development of a profitable hydrogen chain will take longer, especially considering the gas' inherent limit in terms of compression and storage on board. Although hydrogen contains three times as much energy as gasoline per unit weight, 4.6 litres of hydrogen compressed at 700 bars are needed to substitute 1 litre of gasoline (CEA, 2004). Moreover, as it is a flammable very small molecule, it requires specific hydrogen-proof material to be stored and transported. Currently hydrogen transportation is 50% more expensive than natural gas transportation, notably because one volume unit of hydrogen contains three times less energy than the same volume unit of natural gas (CEA, 2004).

Considering the risks and following costs implied in the development of new biofuel chains, industries' investments are significantly subordinated to the commercial perspectives that global policies underpin. These policies tend to respond to global issues and inevitably affect trade, as economic incentives often appear as efficient levers to reach targets.

4 Political and Economic Frameworks

4.1 Climate Change and Greenhouse Gas Emission Trends

Lately biofuels have been fostered worldwide in a double context of energy insecurity and climate change. Except for a few exceptional cases, such as the Brazilian Pró-álcool Programme launched in 1975, it was not until the awareness of the risks associated with the depletion of fossil resources was drastically raised that biofuels and other renewables were widely given attention as real potential energy sources. Since the late 1980s, the more explicit the conclusions of the Intergovernmental Panel on Climate Change on the reality of climate change and the impact of anthropogenic greenhouse gas emissions[15] have become, the more concrete the international policies and instruments to promote renewables have appeared. Needs for action and cooperation have been expressed within the framework of international agreements; such as the Framework Convention on Climate Change (UNFCCC) in 1992 and the Kyoto Protocol in 1997. Although they might not have federated enough stakeholders, notably the Kyoto Protocol which only entered into force in 2005 without some of the main CO_2 contributors, they gave way to the establishment of effective frameworks and national action plans.

The global average surface temperature on the Earth increased about 0.7°C between the late 1800s and 2000, with a rate of about 0.2°C per decade (IPCC, 2007) in the past three decades. However, taking into account the effects of orbital variations on climate, absent human influence, the natural trend would be toward a cooler climate, as peak warmth of the current interglacial period (Holocene) occurred 8–10 thousand years ago. Examination of prior interglacial periods reveals a strong correlation between the CO_2 and CH_4 concentrations in the

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[15] By default examining greenhouse gas emissions includes "six" gases: CO_2, CH_4, N_2O, SF_6, PFCs and FCs.

atmosphere and temperature records. Nevertheless, in the past the temperature changes usually preceded the changes in gas concentrations. Today, anthropogenic greenhouse gas emissions are overwhelming and have reversed the order so that greenhouse gases are driving temperature increases. The climate system has not come to equilibrium with today's climate forcing and more warming is "in the pipeline". "Humans now control global climate, for better or worse" (Hansen, 2006). In other words, the IPCC stated in its last report: "Most of the observed increase in global average temperatures since the mid-20th century is very likely (probability >90%) due to the observed increase in anthropogenic greenhouse gas concentrations. Discernible human influences now extend to other aspects of climate, including ocean warming, continental-average temperatures, temperature extremes and wind patterns" (IPCC, 2007). Pre-industrial global atmospheric concentrations of CO_2, N_2O and CH_4 have increased markedly as a result of human activities since 1750[16] and now far exceed pre-industrial values determined from ice cores spanning many thousands of years.

Global increases in CO_2 concentration are due primarily to fossil fuel use and land-use change, while those of CH_4 and N_2O are primarily due to agriculture. If CO_2 emissions continue to increase by 1.5 to 2% per year, doubled CO_2 will be reached in approximately the year 2050. Encompassing the whole range of the six IPCC emission scenarios[17] from the lowest to the highest emissions, global warming could reach 1.8 to 4°C by 2100 (IPCC, 2007). A global warming of 2 to 3°C over the pre-industrial temperature would already "make the Earth a different planet" (Hansen, 2006). As a very critical issue, sea level rise illustrates how climate change can lead to exponential and irreversible impacts due to accumulation phenomena and positive feedbacks. IPCC scenarios give estimates of a sea level rise between 38 cm and 59 cm by the end of the 21st century relative to 1980–1999, due mostly to thermal expansion and excluding future rapid dynamical changes in ice flow. There is still no consensus on the long-term future of the ice sheet or its contribution to sea level rise. It is not possible to say how long it would take for sea level to change as feedbacks can lead to highly non-linear responses, nevertheless "it is almost inconceivable that under the business-as-usual scenario climate change would not yield a sea level change of the order of meters on the century timescale" (Hansen, 2007). Given the populations in 2000, a sea level rise of 6 m would displace 35 million inhabitants throughout the world and trouble is brewing for many species.

The distance that climate zones have moved so far is small, but the rate of movement of isotherms is now pole-ward at 50 km per decade and will double this century if we follow the business-as-usual scenario, surely causing the extinction of lots of species (Hansen, 2006). The IPCC stresses that: "Continued greenhouse gas emissions at or above current rates would cause further warming and imply many changes in the global climate system during the 21st century that would very likely be larger than those observed during the 20th century. [...] Sea ice is projected to shrink in both the Arctic and Antarctic under all scenarios[18]. It is very likely that hot extremes, heat waves and heavy precipitation events will continue to become more frequent. Even if the concentrations of all greenhouse gases and aerosols had been kept constant at year 2000 levels, a further warming of about 0.1°C per decade would be expected" (IPCC, 2007). An alternative scenario aims at limiting the CO_2 peak at 475 ppm in 2100 before it should slowly decline thereafter and also requires a reduction of non-CO_2 forcing gases in order to hold warming to less than 1°C. The 500 ppm scenario could make it possible to hold warming to less than 2°C. From today's perspective, the 2°C target is only achievable if global emissions are reduced below 10 $GtCO_{2eq}\ yr^{-1}$ in the longer term, meaning more than halving the 1990 level. In 2004, 51 $GtCO_{2eq}$ were added to the atmosphere, and the rise in 1990 emissions alone also produced an additional annual steady flow of 39 $GtCO_2$ due to climate time-lagged response to greenhouse gas emissions. If this development continues, it will be impossible to stay within the aforementioned limit for temperature increase (Fischedick et al., 2007); another decade of business-as-usual would eliminate the Alternative Scenario (Hansen, 2006).

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[16] Pre-industrial concentrations/in 2005: CO_2 (280/379 ppm); N_2O (270/319 ppb); CH_4 (715/1774 ppb), IPCC, 2007.

[17] SRES: special report on emission scenarios IPCC.

[18] Sea ice melting does not directly cause sea level rise like ice on continents; however, it can lead to the extinction of species that rely on these relatively scarce habitats. It also contributes to ocean thermal expansion.

Together, the 25 countries with the largest greenhouse gas emissions account for approximately 83% of global emissions. The largest emitter is the United States, with 21% of global emissions, followed by China with 15%. It follows that most of the remaining countries contribute little to the build-up of greenhouse gases in the atmosphere; 140 countries contribute only 10% of annual emissions (Baumert et al., 2005). The largest percentage increase since 1976 occurred in 2004, when more than 28 $GtCO_2$ were added to the atmosphere from fossil fuel combustion alone. Emission growth rates are highest among developing countries, where collectively CO_2 emissions increased by 47% over the 1990 to 2002 period. Among the major developing country emitters, growth was fastest in Indonesia (97%), South Korea (97%) and Iran (93%). During the same period, emissions also increased mainly in Canada (+20%) and Australia (+22%), whereas emissions in most developed countries did not change. During the 2003–2004 period, the CO_2 growth of 50% in China accounts for more than half of the worldwide CO_2 increase.

The Kaya Identity[19] model (Fig. 6) gives some clues to understanding the energy-related CO_2 emissions by using four factors. Emission intensity or "carbon intensity" is a function of "energy intensity" and "fuel mix". Energy intensity reflects both the level of energy efficiency and the overall economic structure of a country. An economy based on heavy industrial production, for instance, is more likely to have higher carbon intensity than one where the service sector is dominant. However, energy-intensity levels are not well correlated with economic development. In several countries, it can be seen that declines in intensity were accompanied by significant increases in GDP, leading to increases in absolute CO_2 levels. The most notable case is China, where the effect of significant intensity declines, although China still heavily relies on the coal industry, was more than offset by substantial GDP growth. Likewise, the US decline in carbon intensity (17%) was offset by increases in population and GDP, giving a significant greenhouse gas emission growth in the US of 13% over the 1990 to 2002 period.

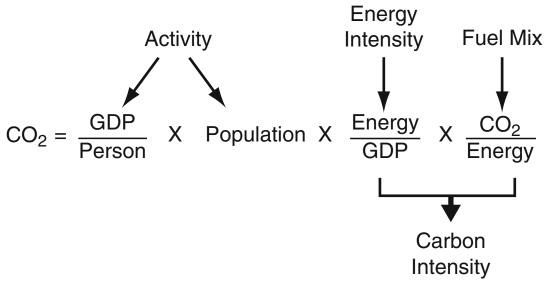

Fig. 6 Kaya Identity model

Whereas in agrarian economies with little heavy industry or energy production, land-use change, especially tropical deforestation, represents a larger share of CO_2 emissions, in a majority of countries, economic growth has, finally, the strongest influence on emission levels. This is the case in countries as diverse as the US, India, Australia and Iran (Baumert et al., 2005). Given the diversity of large emitting countries, it is simply not possible to adequately address the climate change problem without engaging both developed and developing countries, while adapting mitigation instruments to the specificity of influencing factors in the diverse countries. Fixed emission "caps" in particular may be impracticable in developing countries where economic growth is robust. Furthermore, differentiated per capita greenhouse gas emission targets rather than absolute emissions would reduce the effects of population growth on the commitments of Parties. Total emissions of CO_2 in 2004 show, for instance, China (3.7 tCO_2/capita) being far below the US (19.6 tCO_2/capita) and EU-27 (8.7 tCO_2/capita) (EEA, 2008). Also, projections of carbon intensity tend to exhibit less uncertainty than absolute emission forecasts (Baumert et al., 2005).

Between 1990 and 2004, European global greenhouse gas emissions had decreased from most sectors, particularly energy supply, agriculture and waste management; except from transport, which increased by nearly 26%. Had transport sector[20] emissions followed the same reduction trend as in society as a whole, total European Union-27 greenhouse gas emissions during

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[19] Model developed by the Japanese energy economist Yoichi Kaya in Environment, energy, and economy: strategies for sustainability, co-authored with Keiichi Yokobori as the output of the 1993 Tokyo Conference on Global Environment.

[20] The transport sector presented here consists of road transportation, domestic civil aviation, railways, national navigation and other transportation. It excludes emissions from international aviation and maritime transport (which are not covered by the Kyoto Protocol or current EU policies and measures). Road transport is by far the biggest transport emission source.

the period 1990–2005 would have fallen by 14% instead of 7.9% (EEA, 2008). Only Germany, France and Portugal have managed to stabilise transport emissions in recent years. Nevertheless, EU-15 greenhouse gas emissions from transport are still expected to increase a further 35% above 1990 levels by 2010 if only existing policies and measures are used (EEA, 2006a). The transport sector represents the most significant climate policy challenge at two levels. First, transport contributes the lion's share of the emission increase of the European Union and in spite of the voluntary agreement to reduce the carbon content of travel for new vehicles, there does not appear to be in the near future a technological solution of a magnitude that could offset the effect of increased traffic and increased onboard equipment on CO_2 emissions. Second, further efforts to mitigate emissions in other sectors will be difficult to accept if governments do not undertake meaningful efforts in the transport sector (Barbier et al., 2004).

In the EU-25, despite an annual 1 t decrease in the average greenhouse gas emissions per capita of CO_{2e} between 1990 and 2004 and some successful decoupling of greenhouse gas emissions and economic growth, total EU-23 greenhouse gas emissions rose in 2004 by 0.3% compared with 2003 and were 5% below the 1990 level, the highest level since 1997. With existing policies and measures, and without additional ones, EU-23 greenhouse gas emissions are projected to keep on increasing and to be 2.1% below the 1990 level by 2010, meaning that the EU-15 Kyoto commitment of 8% emission reduction from this base-year level by 2008–2012 would not be reached, although the eight new member states had in 2004 emissions of only 76.8% of those in 1990 (EEA, 2006a).

At the UNFCCC meeting in Bali, December 2007, representatives of 180 countries agreed on a 'Bali roadmap' with the aim to achieve by the end of 2009 a global post-2012 climate change agreement to limit emissions, and address other issues such as adaptation to climate change, after the end of the Kyoto Protocol commitment period (2008–2012), whose targets will not be achieved. It should include both developed and developing countries, but with the largest emission reduction effort expected by the developed countries (indicatively in the range of 25 to 40% emission reductions by 2020 from 1990 levels). The European Council agreed in March 2007 on an integrated energy and climate change strategy. It endorses an EU objective of a 30% reduction in greenhouse gas emissions by 2020 compared with 1990 levels, provided that an international agreement can be reached with other industrialised countries. Without such an agreement, the EU would still pledge to a firm independent commitment to achieve at least a 20% reduction (EU, 2007). The EU Commission proposed to split the overall emissions reduction target into two: one for the sectors covered by the European Emissions Trading Scheme (ETS) and one for the non-trading sectors in which transport is included (EEA, 2008).

4.2 Biofuel-Related Policies

4.2.1 European Policies

In Europe, several directives have been released; notably in 1997, the Energy White Paper and Action Plan, and the Green papers on Energy Supply (2000) and Energy Efficiency (2005). These policy instruments notably set indicative objectives in terms of consumption of renewables, e.g. on the share of green electricity (EU, 2001), the promotion of biofuels or emissions trading (EU, 2003), and the use of waste and disposal (2005). In parallel, other directives were released, which notably deal with energy efficiency improvement, economic incentives and eco-labels. However, none of the given targets were binding ones and the results have so far not been convincing. The White Paper (CEC, 1997) on the share of RES in total energy had proposed a common framework for action aiming to achieve the indicative objective of 12% for the contribution of renewables to the EU gross inland energy consumption by 2010, i.e. to double the share of renewables compared with 1997, including a tripling of biomass use. In 2003, the total amount of renewables used averaged only 6% of the EU gross inland energy consumption (EU DG-TREN, 2006a), about two-thirds of this contribution coming from biomass, i.e. 4% of EU total energy needs (CEC, 2005). Even if renewables consumption can widely differ between Member States, the challenge to reach the global objective remains entire; only 7% of the necessary growth of bioenergy production has been achieved globally (Fagernäs et al., 2006). In 2005, the indicative target[21] of 2% market share for biofuels stated in the EU Council Directive on "Biofuels" (EU, 2003) was not reached; biofuels apparently merely attained 1.4% of market share within the EU-25 (EU DG-TREN, 2006b). This share was better than in 2003 (0.6%) but if this trend continues, the 2010 target of 5.75% share will not be achieved: the forecast indicates a 4.2% share by 2010 (CEC, 2008).

Considering the need for a drastic reduction of greenhouse gases from transport and the still very low incorporation of biofuels, the European Union has decided to put into force a new directive that fixes mandatory targets, as was agreed during the European Union Summit in March 2007: 20% of the global energy consumption of the European Union has to be renewably sourced, including a minimum binding target of 10% within the transport sector (only consumption of gasoline and diesel are considered) for each Member State by 2020 (CEC, 2008). This directive proposal was published in January 2008 and should be followed up by concrete Member States' action plans by the end of March 2010. It will replace the former 2001/77/CE and 2003/30/EC directives after the 31st of December 2011. As a further incentive for investors, the Directive indicates that the share contribution by 2nd generation biofuels to the 10% binding target would count for double in comparison with the other biofuels (Article 18). For biofuels and other bioliquids, the directive sets up three conditional criteria of sustainability for their production, so that their consumption can be taken into account to fulfil the binding target and allow financial support (Article 15), although no sanction has been planned for non-compliance with these criteria or the non-fulfilment of both targets:

- a minimum saving of 35% of greenhouse gas emissions compared with the substitute fossil fuels,
- biomass feedstock must not be produced on soils within ecosystems considered to have a high value in terms of biodiversity: i.e. undisturbed forests, protected areas and specific permanent grasslands that shall be geographically identified by the Commission,
- biomass feedstock must not be produced on soils with high organic carbon contents: specific humid areas, notably virgin peat soils, and forests wider than 1 ha with canopy covers superior to 30%.

Concerning agricultural feedstock, supplementary environmental criteria of the European regulation related to good agricultural practices (CE 1782/2003 Article 5 and Annex III point A) remain applicable. Member States shall require economic operators to show that the environmental sustainability criteria set out have been fulfilled. In particular, the Directive stipulates the method to calculate the greenhouse gas emissions throughout the production chain and states that emission reduction due to the co-products shall be handled either by system extension in the case of co-generated electricity from agriculture, or by energy allocation[22] in all other cases. It also gives minima of emission reductions for each biofuel chain (Annex VII) that shall serve as reference.

However, the method does not specify how to take into account the N_2O emissions from the agricultural phase, and cannot at the Community level take into consideration the regional variability (see part 5). Furthermore, no independent certifying authority will be involved in the control of the respecting of the criteria, and biofuels are not included in the guarantee of origin system that will ensure the traceability of electricity, heating and cooling produced from renewable energy sources (Article 6–10). It is also mentioned that it would be technically and administratively unfeasible to apply EU environmental requirements for agriculture to biofuels and other bioliquids from third countries. In fact, since the proposal does not include any derogation for countries where the situations are constrasted with regard to continuous forest, peat soil or grassland resources as well as to certification access, establishing these biofuel sustainability criteria at a multilateral level could be perceived as a discriminatory measure according to the regulation of the World Trade Organization (Pons, 2008). Much remains to be done in order to establish multilateral agreements between the Community and third countries defining international standards of sustainability criteria, the greenhouse gas emission calculation method and certification control.

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[21] Minimum indicative targets from the European Council Directive 2003/30/EC of 8 May 2003: 2% in 2005 and 5.75% in 2010 share of biofuels of all petrol and diesel for transport purposes placed on the market calculated on the basis of the energy content. (about 3% and 8.6% for ethanol; 2.2% and 6.4% for biodiesel when calculated on a volume basis).

[22] Based on the low calorific values.

4.2.2 US Policies

For the last twenty years, the US government has also been putting into force several policies related to renewables in a view to reduce its dependency on imported oil. Starting with the Energy Tax Act of 1978, the US government has continuously maintained national tax incentives to encourage ethanol fuel production and use. Increases in ethanol excise tax exemption about every two years during the 80s–90s and loan guarantees to build up production facilities have notably fostered the growth of domestic maize-based ethanol (MacDonald et al., 2004). In 2000, the Biomass Research and Development Act directed the departments of Energy and Agriculture to integrate their biomass Research and Development and established the Biomass Research and Development Technical Advisory Committee (BTAC), which advises the Secretary of Energy and the Secretary of Agriculture on strategic planning for biomass Research and Development. In 2002, this Committee set up a challenging goal requiring biomass to supply 5% of the nation's power and 25% of its chemicals, and biofuels to meet 20% (10%) of transportation fuel consumption by 2030 (2020). These contributions would represent all together 30% of the current petroleum consumption (Perlack et al., 2005). The 2002 Farm Bill also established new programmes and grants that support increased use of biofuels and biobased products as well as advanced biorefinery development (US DOE, 2006a). The need to substitute MTBE[23], which has been banned[24] in a growing numbers of US States due to its toxicity in high blends (formerly 15% in California, for instance), has also contributed to underpinning ethanol blends. However, biomass currently accounts for merely 4% of total energy consumption (BRDI, 2006), and biofuels, mostly maize ethanol, for around 2 to 3% of domestic transportation motor fuels (Kojima and Johnson, 2005; US DOE, 2006a).

With the growing energy consumption, US dependence on imported oil has reached severe levels. Between 1984 and 2005, crude oil imports increased 194%. In 2005, about 65% of crude oil and petroleum products were supplied by imports, representing 30% of the total US trade deficit. The overall demand for transportation fuels has increased 19% in the past ten years, with the vast majority of this growth reliant on imported petroleum (BRDI, 2006). During the last three years, the Government has hence especially insisted, with the Energy Policy Act of 2005, the American Competitiveness Initiative and the Advanced Energy Initiative (2006), on providing an aggressive strategy for tackling long-term energy challenges (US DOE, 2006b) and has shown ambitious goals in terms of energy efficiency and diversification, i.e. the increase in domestic production of conventional fuels as well as the development of new nuclear power generation, hydrogen and renewables.

In terms of bioenergy, the Energy Policy Act of 2005 (EPAct), a US $ 14-billion national energy plan, notably aims at fostering research programmes and partnerships between industries and academic institutions in order to develop advanced processes in bioproduct production. In this sense, it includes amendments to the Biomass Research and Development Act, focusing on four new technical areas for research and development activities: (1) develop crops and systems that improve feedstock production and processing, (2) convert recalcitrant cellulosic biomass into intermediates that can be used to produce biofuels and products, (3) develop technologies that yield a wide range of bioproducts that increase the feasibility of fuel production in a biorefinery, and (4) analyse biomass technologies for their impact on sustainability and environmental quality, security, and rural economic development.

The "Initiatives" strategies also particularly emphasise the role of technology development and innovations. The President's Advanced Energy Initiative provided a 22% increase in funding for clean-energy technology research at the Department of Energy in two vital areas: 1. "Changing the way we fuel our vehicles"; and 2. "Changing the way we power our homes and businesses". In 2007, a total budget of US $ 150 million was allocated to the DOE to fund biomass research and help to reduce the costs of producing advanced biofuels and ready technologies for their commercialisation.

Finally, biofuel production objectives are also underpinned by renewable content requirements for

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[23] Methyl tertiary butyl ether: fossil oxigenate additive to gasoline.

[24] Contamination of groundwater by MTBE due to leaking tanks is especially severe in the US. Despite the decision to phase it out, the quantities of MTBE used in the US have not decreased due to its technical advantages that actually help to produce a cleaner burning gasoline (http://www.acfa.org).

motor vehicle fuels. Called Renewable (or Alternative) Fuel Standard (RFS), the EPAct's provision requires gasoline sold in the US to be mixed with increasing amounts of renewable fuel on an annual average basis, up to at least 28 billion litres per year of biofuels by 2012 blended into the nation's fuel supply. In 2007, the US produced about 24.2 billion litres of ethanol and 1.7 billion litres of biodiesel, four times more ethanol than in 2000 and 80% more biodiesel than the previous year (US Government, 2008). In December 2007, President G.W. Bush signed the Energy Independence and Security Act, which notably responds to his "Twenty in Ten" challenge, a regulation to reduce gasoline consumption by 20% in ten years. This Act includes a new Renewable Fuel Standard, requiring fuel producers to supply at least 136 billion litres of renewable fuel in the year 2022, but also a Vehicle Fuel Economy Mandate, specifying a national mandatory fuel economy standard corresponding to a gain of 17 kilometres per litre by 2020. Several states in the US have adopted biofuel blend mandates; Louisiana, Montana, New Mexico, Oregon and Washington states, for instance, require ethanol (mostly 10 %) in gasoline and/or biodiesel (2 to 5%) in highway diesel fuel with effective dates in the future. California is, moreover, developing a Low Carbon Fuel Standard for transportation fuels with a goal to reduce the carbon intensity by at least 10 % by 2020. US policies undoubtedly boosted the biofuel supply and were massively followed by the member states as illustrated by the Governors' Ethanol Coalition, that includes 32 member states out of 50, as well as international representatives from Brazil, Canada, Mexico, Sweden and Thailand (US DOE, 2006a).

Although biofuels were initially thought to contribute to lowering US energy dependence on imports, the US still imported about 1.7 billion litres of ethanol in 2007[25]. Even if all maize grain grown in the US were converted into ethanol, it would have satisfied just about 15% of the transportation needs (US DOE, 2006a). To reach the 30% vision of the BTAC, one billion dry tons of biomass feedstock would be needed annually, from the potential dedicated 1.3 billion dry tons from forestlands and agricultural lands, provided that large-scale bioenergy and biorefinery industries, including cellulosic ethanol plants, exist (Perlack et al., 2005). Therefore, the government especially focuses on the development of cellulosic ethanol (1 litre of non-grain-based ethanol is counted as 2.5 litres of grain-based ethanol to fulfil the RFS). This focus was confirmed when, on May 5, 2009, United States president Barack Obama signed a presidential directive on developing advanced biofuels, i.e. 2nd and 3rd generation biofuels. Cellulosic ethanol could enable greater greenhouse gas savings, which appears to be a crucial means for the US to drastically lower its greenhouse gas emissions, while it is besides not particularly willing to commit itself within the international emission reduction targets plan.

4.2.3 Chinese Policies

As Chinese economy and consumption levels boom, Chinese energy policy is likely to significantly affect the worldwide energy market and is fatally expected to play a growing and major role in greenhouse gas emissions. In 1975, China became a net oil importer. Today, it depends on coal for around 70% of its primary energy and the main role of coal within the energy structure will remain unchanged for a long time to come. Nuclear power and renewables account for about 7% of primary energy consumption, the rest comes from fuel oil used in the transportation sector, whose consumption is growing rapidly (SCIO, 2007). Considering that China's energy efficiency is about 10% lower than that of the developed countries, and its per unit energy consumption of energy-intensive products is about 40% higher than the advanced international level, priority is given to the up-grading and widening of the domestic energy-grid by implementing more efficient and cleaner technologies. The 11th Five-Year Plan for National Economic and Social Development of the People's Republic of China outlines that the per-unit GDP energy consumption by 2010 will have decreased by 20% compared with 2005, and the total amount of major pollutants discharged will have been reduced by 10 percent (SCIO, 2007).

In terms of renewable energies, national targets are to reach contributions of 10% and a further 15% of total energy consumption by 2010 and 2020 (SCIO, 2007). The Renewable Energy Law of the People's Republic of China[26] (09/11/2005, Article 16) as well as China's Energy Conditions and Policies

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[25] http://www.worldwatch.org (25/04/2008).

[26] Online: http://www.ccchina.gov.cn/en/Public_Right.asp?class=17.

(28/12/2007, pages 17 and 37) directly but briefly endeavour the production of biofuels. The primary option for renewables is hydropower. This is notably illustrated by China's National Climate Change Programme, indicating that current measures are expected to lower greenhouse gas emissions by 2010 by 500 Mt CO_2 thanks to hydropower, 60 Mt CO_2 thanks to wind, solar, geothermal and tidal energy and only 30 Mt CO_2 thanks to bioenergy, essentially for heat and power (NDRC, 2007).

Over the past two decades, China's vehicle market has been the fastest growing in the world (+12% each year, Latner et al., 2007). China's consumption of crude oil totalled 323 million tons in 2005, including net crude-oil imports of 119 million tons. Consistent with new car use, the annual average growth rate for gasoline and diesel consumption during the period from 1990 to 2004 reached 6.8% and 10.1%, respectively. Thus, China views biofuel as a necessary strategic component to reach independence of imported oil (Latner et al., 2006). The development of biofuels, started in the late 80s, led to the first ethanol production in 2002. In 2004, the first recorded ethanol production was 300 000 tons and it increased more than four-fold within two years to reach 1.3 million tons in 2006, and an estimated 1.45 million tons in 2007. Ethanol is primarily converted from maize (>80%). Biodiesel, which was not introduced in the development programme until 2006, is mostly produced from animal fat or waste vegetable oils.

The 11th Five-Year Plan for biofuels that had suggested an implementation plan leading to a production of 5.2 million tons of biofuels by 2010 was not approved for food security concerns (Latner et al., 2007). China is already a net importer in all the major edible vegetable oils, the largest importer in the world, and a net sugar importer (Latner et al., 2006, 2007). As ethanol already accounts for 40% of the industrial maize use, higher demand for ethanol could turn China from a net exporter of maize into a net importer (Latner et al., 2006). Therefore, the government focuses more on the use of other crops such as cassava, sorghum, and the use of feedstock grown on non-arable lands, notably cellulosic feedstock for ethanol and *Jatropha* for biodiesel. It has already launched an E10 mandate in nine provinces that will be expanded to some of the other thirteen provinces. It is not clear today whether the ambitious government's target of a biofuel share of 15% of total transportation fuels by 2020 [about 12 million tons of biofuel (Latner et al., 2006)] remains on the agenda, since the Plan has been rejected. A realistic target would be 3 to 4 million tons of biofuels by 2010 (Latner et al., 2007), but the Chinese government needs to draw up new policies to ensure that its biofuel targets can be achieved efficiently and economically, said a researcher within the Chinese National Development and Reform Commission (Stanway, 2008).

The European Union, the United States of America and China are major emitters of greenhouse gases, and therefore show a growing political will to reduce their emissions, notably by developing cleaner energy sources. Nevertheless, many other countries, even some minor polluters, are implementing national strategies and policies to develop biofuels playing a more or less important part within renewables development plans. This is notably the case in some South American countries such as Colombia and Peru, but also in Asian countries such as India, Japan and Thailand. Industrialised countries may emphasise more their role in greenhouse gas reduction and energy diversification, while developing countries promote biofuels especially as an opportunity to foster rural development and save foreign exchange. In all cases, biofuels are perceived as a means to contribute to energy security, when concerns are growing with the surge in oil price (Kojima and Johnson, 2005). However, the greatest barrier that has hampered biofuel large-scale commercialisation is their high cost of production compared with conventional fuels, two to four times higher (VIEWLS in Pelkmans et al., 2006). Thereby, biofuel policies notably consist of implementing economic incentives to counterbalance high production costs and make biofuels competitive.

4.3 Economic Incentives

Biofuels cannot be the panacea that petroleum has been for decades. The feedstock has to be produced (feedstock prices account for from two-thirds up to 90% of the total costs of 1st generation biofuels, Wiesenthal et al., 2007) and transformed, while petroleum just has to be looked for and exploited, which until resources started to become scarce, was largely cost-effective. Moreover, prices of agricultural commodities, especially those of crude materials, are highly volatile due to fluctuations in price inelastic supply and demand, meaning that a small shift in supply or demand results in a large price change. Supply may widely vary following climatic hazards and demand on such competitive markets, as agricultural commodities have only limited substitutes, and can be severely impacted by diverse factors, e.g. large purchase by governments (Clem, 1985). These fluctuations are further influenced by growing spill-over effects from one market to another, as global markets have become increasingly intertwined across all commodities and between commodities and the financial sector. Greater price uncertainty implies higher risk and growing speculations, which in turn can initiate a vicious cycle of even more enhancing price volatility. On the other hand, this uncertainty tends to limit opportunities to access credits and result in the adoption of low risk production technologies at the expense of innovation and entrepreneurship (FAO, 2007), which is notably critical in the development of second generation biofuels. Facing the high cost of biofuels, even Brazil's ethanol infrastructure model, that relies on an optimum combination of the very productive sugar cane and favourable climatic conditions, required huge taxpayer subsidies over decades before it could become viable (Xavier, 2007). Today, Brazil continues to maintain a significant tax differential between gasoline and ethanol (Kojima and Johnson, 2005).

Figure 7 gives an overview of the current and foreseen production costs of biofuels compared with petroleum products. Except for sugar cane-based ethanol and animal fat-based diesel, drastic cost reductions are still necessary for biofuels to become clearly competitive by 2030. Tax incentives, administered pricing, restrictive trade policies, credits and numerous other economic incentives are in force worldwide to underpin the production or consumption of biofuels, notably by making them artificially competitive.

Demand-side instruments, such as tax incentives and obligations (e.g. mandatory blends), are the most common mechanisms that have proven to be efficient in pushing biofuels onto the market (Wiesenthal et al., 2007). Tax incentives are tax provisions that grant special tax relief designed to encourage certain behaviours by taxpayers. Tax exemption on biofuels and higher excise taxes on fossil fuels permit one to compensate for the higher biofuel production costs (Table 3) and create or enlarge a favourable price for biofuels relative to fossil ones, providing a strong incentive for the consumer to prefer biofuels over fossil ones. Hence consumer acceptance was shown in a study of the United States General Accounting Office to be essential to the use of alternative fuels in the case studies of Brazil, Canada and New Zealand (US GAO, 2000). In the same study, it was also shown that the expected decline in ethanol use in the US, if tax exemption were eliminated, would be at least 50%.

In 2004, nine European Union member states had partly or completely detaxed biofuels: Austria, the Czech Republic, France, Germany, Italy, Lithuania, Spain, Sweden and the United Kingdom. All European Union Member States that achieved a high biofuel share had a full tax break in place and high fossil fuel tax levels. However, the reverse case does not seem true, which indicates that tax exemption is not a sufficient condition to reach a high share of biofuels (Pelkmans et al., 2006). Moreover, tax exemption implies budget losses for the governments, some 1140 million Euros in 2005 ($US 1419 million[27]) in Germany (Wiesenthal et al., 2007). These losses can be particularly critical in developing countries, where gasoline taxes are often a significant source of tax revenue more supported by high-income groups. Furthermore, tax expenditures aiming at favouring biofuels may fall under little scrutiny, while public expense on biofuels might need to be weighed against other social priorities (Kojima and Johnson, 2005).

Although tax incentives appear to be necessary to create and maintain a minimum biofuel demand, they are not sufficient to reach significant levels of biofuel consumption in most countries. In particular, ethanol tax incentives in the US, for instance, have failed in enhancing US energy security because they have not created enough usage to reduce petroleum imports and the likelihood of oil price shocks (US GAO, 2000). Thus, currently implemented schemes are mixed, i.e. some kind of tax incentives and/or obligations apply in parallel (Wiesenthal et al., 2007). In France, reductions of the interior consumption tax (Art. 265 bis A from the Duty Code 2006) by an average of 28 € ($US 35.2[24]) per hectolitre of biofuel blended with fossil fuels is combined with a supplementary tax (General Tax on Polluting Activities, TGAP, Art. 32 from the Finance Law 2005) on diesel and gasoline sales, which do not contain a minimum share of biofuels (Luneau and Fayet, 2007).

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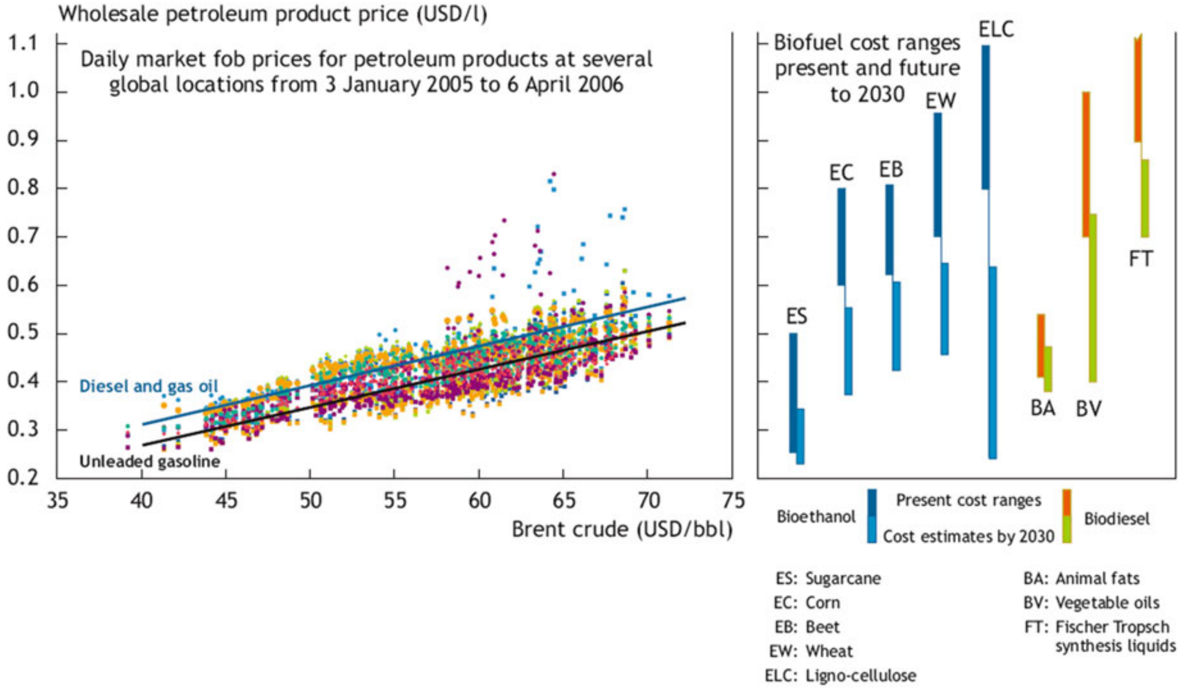
[27] Mean annual foreign exchange rates from the US Board of Governors of the Federal Reserve System annual databases.



Daily wholesale gasoline and diesel prices (USD/l free on board) in 12 locations versus the crude oil price compared with the production cost ranges for biofuels without any government support schemes, both now and as anticipated by 2030.

Fig. 7 Biofuels current and prospected production costs without support schemes and petroleum products price evolution (IEA, 2007b)

Table 3 Production costs of ethanol and biodiesel and prices of petroleum-based fuel in major biofuel-producing countries, 2004. Data from OECD (2006)

		Gasoline	Biodiesel	
Countries/Community	Ethanol	(US$ per energy-equivalent litre*)		Diesel
US	0.82 (wheat) 0.45 (maize)	0.54 (with tax) 0.38 (without tax)	0.62 (vegetable oils)	0.57 (with tax) 0.37 (without tax)
Canada	0.85 0.50	0.68 0.40	0.50	0.68 0.39
EU-15	0.88 0.68	1.32 0.40	0.68	1.29 0.40
Poland	0.80 0.51	1.2 0.39	0.82	1.1 0.38
Brazil	0.35 (sugar cane)	0.84 0.39	0.65	0.49 0.38

Notes: * Biofuel prices accommodate differences in energy content. Ethanol is assumed to contain 0.66 the energy of 1 litre of gasoline, and biodiesel is assumed to contain 0.89 the energy of 1 litre of diesel. Note: when not specifically mentioned, the numbers follow the same order as for the US, i.e. 1st: wheat, 2nd: maize; and 1st: with tax, 2nd: without tax.

In Germany since 2007, a tax exemption system has been replaced by an obligation for fuel suppliers to provide a certain share of their total sales as biofuels. Obligations encompass mandatory blends, i.e. an obligation to add a certain % biofuels to fossil fuels, obligation to bring a certain quantity of biofuels on the market (e.g. the 10% share proposed in the European Union Directive CEC, 2008), and an obligation to bring a certain biofuel quantity on the market including a tradable renewable fuels certificate system (e.g. the Renewable Transport Fuels Obligation in the UK, the Green Power Certificates System in Flanders) (Pelkmans et al., 2006). In a broader sense, fuel standards, an authorised quota system for biofuel producers or filling station obligation (e.g. a mandate to fuel distributors to offer at least one renewable fuel in Sweden, Pelkmans et al., 2006) can also be perceived as obligation incentives, since they aim at introducing given quantities of biofuels on the market. Indeed, a major interest of obligation is the long-term visibility that they may offer to industries willing to produce biofuels, especially to those taking the risk to implement new technologies. This market prospect remains relative, though, as it depends on the governmental politics for the quota amounts (Pelkmans et al., 2006). Furthermore, in an obligation scheme, fuel suppliers will pay for the additional costs, meaning that they have an incentive to opt for the lowest cost biofuels, e.g. imported or low-blend biofuels. Fuel standards and low blends do not make any biofuel visibility possible, and obligations do not push industries to go beyond mandate targets (Pelkmans et al., 2006).

Finally, if high blends or certain technologies are to be promoted, neither obligations nor tax reductions are the appropriate instruments (Wiesenthal et al., 2007). It can be noticed that second generation biofuels will count for double compared with first generation ones, when considering US or European obligation systems, which in that case is a further incentive towards these biofuels. However, supply-side instruments may be more efficient in promoting specific biofuels. Moreover, while most of the initiatives in the biofuels field have been focusing on conversion and end-use sectors so far, there is a primordial need to support raw material producers to expand and secure feedstock supply in order to/and thereby be coherent with increasing biofuel share policies (European Commission, 2007). As an example, the E5 mandate in India was suspended in 2004, two years after its implementation, due to the lack of ethanol supply.

Supply-side instruments, mainly capital grants and feedstock support, have had limited success in pushing biofuels so far (Wiesenthal et al., 2007). Indeed, support for production facilities for 1st generation biofuels does not drastically impact the cost of biofuel due to the fact that capital does only represent a marginal part of the total cost in comparison with feedstock (0.01 € per litre with 10 million € support for a 15–20 million € investment to build a typical large biodiesel plant, Pelkmans et al., 2006). However, capital grants play an important role in fostering the development of the not yet mature second generation of biofuels, firstly as an incentive to build up demonstration plants, but also given that capital costs account for much more of the total cost when compared with 1st generation biofuels, some 60% and above (JRC/EUCAR/CONCAWE, 2006). The same pattern is observed with feedstock support. Although feedstock represents a large part of the total production costs, the energy crop premium indeed only lowers 1st generation biofuel costs by 0.01 to 0.04 € per litre. But as capital grants or loan programmes, feedstock subsidies appear to be an efficient instrument in order to support special types of biofuels (Wiesenthal et al., 2007).

In Europe, agricultural subsidies are defined by the Common Agricultural Policy (CAP). In 1992 following the Mac Sharry reform, liquid biofuels appeared as a way to compensate for the set-aside obligation that aimed at tackling the issue of overproduction and corresponded to 15% of the arable crops area in 1993–1994. In exchange, farmers were entitled to compensation payments (area and headage payments), and were allowed to grow non-food crops on set-aside lands (regulation n° 1765/92). In France, rapeseed methyl ester was favoured because it permitted the cultivation of the greatest area of set-aside lands for a given amount of public financial support considering its low yield per hectare (3.3 t/ha on average in 2005) (Sourie et al., 2005).

In 2003, the Fischler reform established "an income support for farmers", the single payment scheme (SPS), which replaces area and headage payments, cutting the link between subsidies and production. As a result, farmers can respond freely to increasing demand for energy crops (CEC, 2005). In particular, crops, which were eligible for direct payments only as non-food crops grown on set-aside lands, can from now on be grown on any kind of land. Moreover, in the past, only a limited range of energy crops could benefit from support, whereas this new reform has paved the way for farmers to grow more energy crops, including short rotation coppice and other perennial crops (CEC, 2005). Non-food crops including energy crops can be grown on set-aside lands under the condition that the use of the biomass is guaranteed by a contract between the farmer and the processing industry or by the farmer if the processing occurs on the farm (CEC, 2006a). In these cases, the 2003 reform also introduced a 45 € /ha premium for energy crops grown on non-set-aside land, the so-called Carbon Credit, with a budgetary ceiling of Maximum Guaranteed Area of 2 Million hectares subsidised in the EU (regulation1782/2003, amendment[28] in 2007). In 2006, this energy crop premium was already applied to some 1.2 to 1.3 Million hectares (Wiesenthal et al., 2007). In a further push to encourage the production of feedstock for renewable energy production, the Commission also proposed allowing the Member States to grant national aid of up to 50 percent of the costs of establishing multi-annual crops on areas on which an application for the energy crop aid has been made[29].

Finally, in November 2005, a major reform of the sugar regime was agreed. It aims at a progressive cut in price support of 36% over four years and the reduction of EU sugar subsidised exports from the current level of 7.6 Mt to the agreed Uruguay Agreement[30]'s limit of 1.4 Mt (OECD/FAO, 2007); meanwhile, "partial compensation" was introduced in the form of a direct decoupled payment. Sugar for the chemical and pharmaceutical industries and for ethanol production is excluded from production quotas. The lower sugar production quotas and the lower sugar price paid to EU farmers are likely to foster the production of sugar beet for ethanol, which is also eligible for the energy crop premium. The new US Farm Bill will also emphasise the role of agricultural subsidies to foster the development of biofuel with increased support to farmers. Despite the willingness of the former US President, G. Bush, to privilege direct payments upon coupled ones[31] or even the recent CAP reform, feedstock subsidies still imply market distortion.

As Mrs. Corre, Director General of the European Union of Ethanol Producers (UEPA), pointed out, a balanced trading framework is a pre-requisite to ensure the viability of the nascent industry and offer win-win opportunities, especially to developing countries. Also, biofuel producers need a self-running market with long-term visibility, and a 2–3-year perspective based on a yearly-tailored fiscal budget does not offer that. Internalising the external benefits of fuel ethanol is the greatest help of all which could solve the problem (in EurObserv'ER, 2006). However, these externalities are not easy to quantify and to be given any value and the cost/benefit analysis also includes a lot of socio-economic factors that are in constant evolution, e.g. economic incentives, the cost of an oil barrel, etc. Taking the example of greenhouse gas emissions, which are given an economic value within the Emissions Trading Scheme (ETS), the assessment of CO_2 equivalent emissions throughout biofuel chains, first of all, still lacks precision (see part 5).

Transportation biofuels are not included in the current Emission Trading Scheme for CO_2 permits. The abatement cost of using biofuels to reduce greenhouse gas emissions, by substitution of fossil ones, is indeed very high due to their high production costs. Therefore, unless a given biofuel permits drastic greenhouse gas reductions with low costs (Fig. 8: Cane ethanol in Brazil, $ US/ton GHG reduction = $ US 25) the price of the ton CO_2 equivalent, i.e. of the tradable permit, would be too high within the current ETS "pricing engine" (Fig. 8: Grain ethanol in IEA countries, $ US/ton GHG reduction = $ US 200–500) and biofuels would not be a favoured solution for greenhouse gas reductions compared with green heat and power if permits were the only incentive (Fulton et al., 2004). This system makes emission reductions possible wherever abatement costs are the lowest, i.e. this trading scheme cannot lead to efficient greenhouse gas reductions where they are the highest as long as production costs are not significantly reduced.

Clean Development Mechanism (CDM) projects that involve an industrialised country buying carbon credit for a developing country, which uses the payment to produce biofuels or dedicated vehicles, etc., might be a better option to foster biofuels, depending on their greenhouse gas reduction potential. Also, reduction of tariff barriers within international trade for biofuels as environmental goods (under the classification of OECD, 2003 in Fulton et al., 2004) is an interesting instrument given the wide range of biofuels' potential and production costs worldwide.

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[28] http://ec.europa.eu/agriculture/capreform/infosheets/energy_en.pdf.

[29] European Commission Press releases, IP/06/1243, Brussels, 22 September 2006.

[30] URAA, Uruguay Round Agreement on Agriculture in Marrakech, 1994.

[31] Communication of Charles E. Hanrahan, Senior Specialist at the Library of Congress in Washington, on 01/2008 at the Agroparistech.

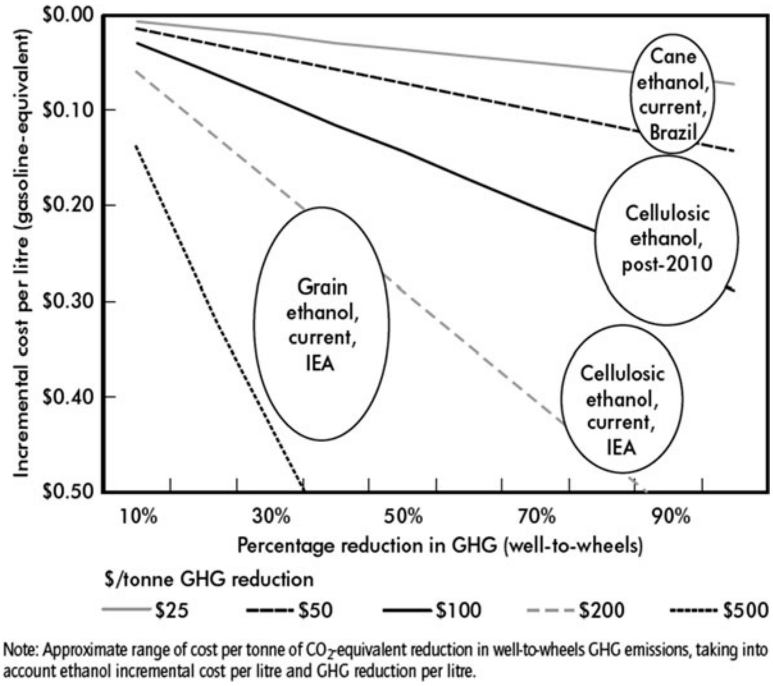

Fig. 8 Biofuels incremental cost per ton of greenhouse gas reduction (Fulton et al., 2004)

However, again, these opportunities are still hampered by the lack of data and agreement on real biofuels' environmental benefit.

Finally, the assessment of past biofuel success stories indicates that a portfolio of policy instruments, including supply- and demand-side instruments, is necessary to bring biofuels onto the market (Wiesenthal et al., 2007). This implies a need for interdisciplinary integration and harmonisation within ministries and governments at all levels. All over the world projected or implemented mandatory blends are on the agenda: the E10 mandate in Thailand and China, planned B2 and E10 mandates in Latin America, the possible ethanol blending mandate in Japan, the Brazilian mandatory B2 blend introduced in 2008 and to be increased to B5 in 2013, etc. However, these targets are far from being reached and illustrate a lack of policy harmonisation in terms of bioenergy strategy on a global level.

Furthermore, difference in blend standards can impact international trade as biofuel producers from different countries might not be supported by subsidies or taxes in the same way when considering various blend characteristics needed to get this help, as claimed by the European Biodiesel Board (EBB) in Bioenergy Business, 2007. This also stresses the need for harmonisation across several sectors and especially involving the automotive sector. Collaboration with car manufacturers has appeared crucial to ensure biofuel compatibility with engines, that was necessary to offer biofuel producers and consumers warranty (Wiesenthal et al., 2007). The introduction of policies on vehicle technology standards, so that all new vehicles would be compatible with a specific mixture of biodiesels, would permit one to lower the production costs of such vehicles and further incite biofuel consumption (Fulton et al., 2004). It would also pave the way to more flexibility and more coherence with other policies, which is lacking; as the example of the Spanish biofuel domestic surplus shows (Bioenergy Business, 2007).

Despite existing policies and measures, the major uncertainty factor, to decide which biofuel path should be fostered or not and to define better who should bear the additional costs, is due to diverging results on the energetic and environmental balances of biofuel chains. Calculations and results can drastically differ among studies and the lack of transparency behind hypotheses or the data quality also lead to some confusion. Therefore, growing doubts about the real ability of first generation biofuels to reduce overall greenhouse gas emissions and growing awareness of negative impacts of biofuel production on biodiversity, water and soil, point to the need for great caution in promoting biofuels further. Better knowledge of life-cycle greenhouse gas emissions from all energy uses of biomass, and strong sustainability criteria for biomass production, also addressing knock-on effects due to indirect land-use change, are needed to fully judge the benefits and limitations of biomass use (EEA, 2008).

5 Biofuels and Greenhouse Gases

5.1 Assessing the Environmental Impacts of Biofuels

5.1.1 Life Cycle Assessment of Biofuel Chains

The Life Cycle Assessment Tool

Life cycle assessment (LCA) is a holistic methodology developed in the 1980s[32], which assesses the potential environmental impacts of a product considering every step of the commodity chain from "the cradle to the grave". When comparing biofuels and fossil fuels, LCA appears to be an inescapable tool because the production of biofuels must be optimised, considering the environmental impacts throughout the whole commodity chain in order to avoid pollution trade-offs between ecological compartments or processing steps. This is, indeed, very important in "eco-design"; not to solve one environmental problem merely by shifting it to another stage in the product's life cycle (Guinée, 2002) and this is particularly crucial when it comes to decisions on national and international levels on global issues such as energy and climate change. The LCA methodology consists of 4 steps: "goal and scope definition", "inventory of extractions and emissions", "impact assessment" and "interpretation". An iterative approach should ensure that the system boundary and the inventory have been correctly adjusted, so that a comprehensive inventory of emissions makes it possible to correctly and completely characterise the selected impacts. A complementary sensitivity analysis then allows eliciting the weight of input data uncertainty and model assumptions on the final LCA results. Although the LCA tool has been standardised by the ISO norms 14 040 series (40/41, 43, 47–49, 1997/98, 2000/01[33]), there are several methods to apprehend the impacts and their characterisation, and no ready LCA adaptable to various cases. This firstly implies that the whole analysis has to be started from zero each time, but also that results might considerably vary between studies due to diverging background assumptions. LCA has been performed a lot during the last decade, notably in order to compare the environmental performances of bioenergy chains with one another or with fossil fuel chains. In this sense LCA can serve for decision-making but under some conditions on its construction that should lead to consensus on the LCA results.

Figure 9 shows a simplified scheme of a representative system boundary for a bioenergy chain from the extraction of raw materials to the combustion of the biofuel, the so-called Well-to-Wheel (WtW) system boundary. Delineating the system boundary is a decisive step of the "goal and scope definition" step of LCA, although it might evolve through iterative analyses of result sensitivity to assumptions. There is nowadays a global consensus on this WtW system boundary, although some divergences still appear, notably concerning the accounting of energy invested in farm machinery and infrastructure capital (Farrell et al., 2006). A distinction should be observed, though, between WtW assessments, that elicit the impacts of the fuel combustion, and Well-to-Tank (WtT) assessments, that assume total fuel combustion without further impact assessment. Renewable energies, upstream in the chain, are not included in the system boundary; essentially solar energy for photosynthesis, but also the so-called indirect energies such as human work, for example.

Within the system boundaries, from the first extraction to the last emission, all elementary flows are accounted throughout every step of the "tree of the product life cycle" in accordance with a quantified functional unit, i.e. the function that the studied product

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[32] By BUWAL, Bundesamt für Umwelt, Wald und Landschaft (Swiss federal office) and SETAC, Society of Environmental Toxicology and Chemistry (international scientific society).

[33] ISO 14040:2006 and ISO 14044:2006 replace the previous standards (ISO 14040:1997, ISO 14041:1999, ISO 14042:2000 and ISO 14043:2000). The new editions have been updated to improve the readability, while leaving the requirements and technical content unaffected, except for errors and inconsistencies.

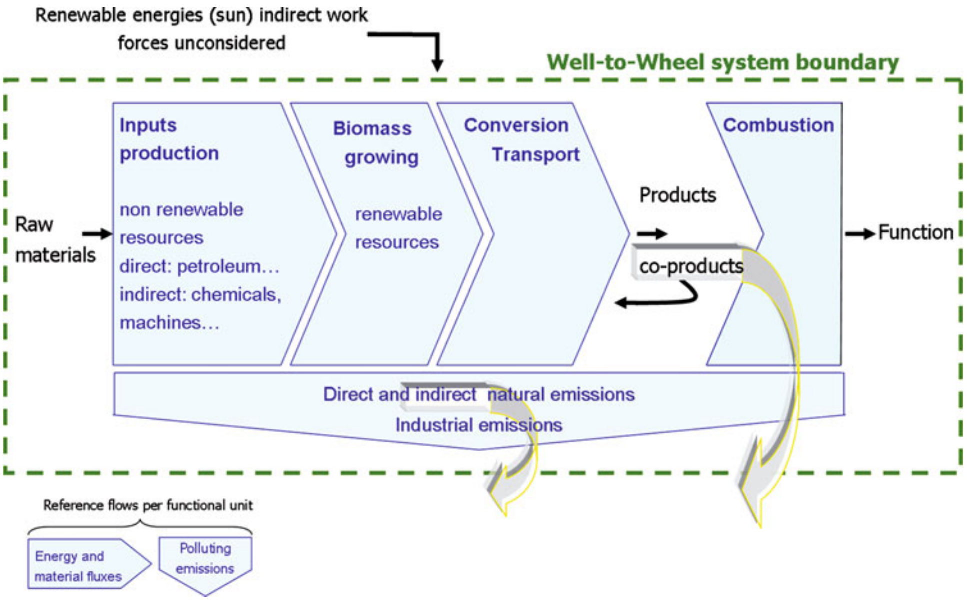

Fig. 9 Biofuel LCA simplified well-to-wheel system. LCA: life cycle analysis

is to fulfil, e.g. to provide 1 MJ. The “tree of the product life cycle” corresponds to the succession of the unit-processes. The blue boxes represent the main classes of processes within bioenergy chains, each one encompassing several unit-processes. For each unit-process, data are recorded on the inputs, the emissions, waste flows, and other environmental exchanges that are typically assumed to be linearly related to one of the product flows of the unit-process. All unit-process are linked through intermediate product flows, which makes the process system model linear with respect to the quantity of function it provides (Rebitzer et al., 2004). Inputs include raw materials and energy. Outputs are the products, the work or energy output and the polluting emissions. “Products” include the product of interest and all co-products; i.e. all the products, included biomaterials, waste or energy, that are concomitantly produced although all processes aim at optimising the production yield of the one product of interest.

A distinction can be made between co-produced materials that are directly generated from part of the feedstock such as straw or meal, and by-products such as glycerine or heat that are sub-products of other production processes (Malça and Freire, 2006). In this review all these “secondary” products are included in the “co-products”. Direct emissions are emissions occurring during the production of the biomass due to natural biochemical and physical mechanisms within the soil-plant-atmosphere ecosystem. Indirect emissions can also occur on a wider space and time scale, following further reactions affecting the substances previously emitted, or upstream in the chain due to land-use changes. Indirect emissions also encompass methane emissions through fodder digestion by livestock. The term indirect emissions will be, in this review, reserved for these “natural second-order” emissions, whereas emissions through cultural operations, transport, conversion processes, storage, etc. will be referred to as industrial emissions (Fig. 10).

Energy ratios are a critical aspect of bioenergy chain assessments, since an important matter is to determine the fossil primary energy savings. Nevertheless, there is still a lack of consensus concerning the definition and designation of energy efficiency indicators to be used in a life-cycle perspective in particular to characterise renewable energy systems (Malça and Freire, 2006). The respective definition and use of among others “energy efficiency”, “overall energy balance”, “gross/net energy requirement” and “energy renewability efficiency” (Malça and Freire, 2006) will also be clarified when comparing bioenergy chain assessments.

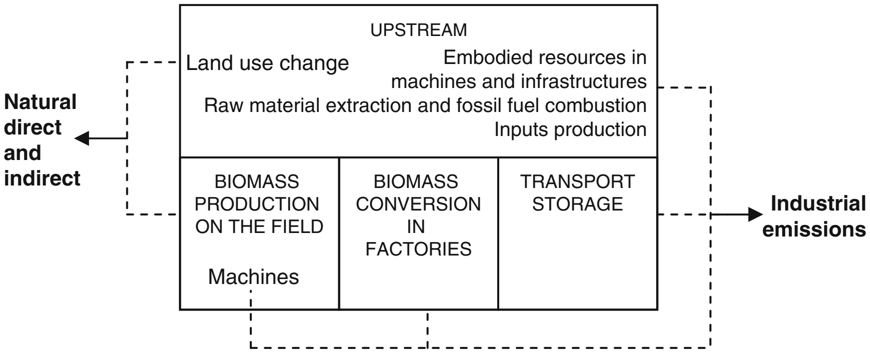

Fig. 10 Natural and industrial emissions of a bioenergy chain (without combustion)

Biofuels versus fossil fuels

Advantages of biofuels over fossil fuels depend on the environmental impacts that are considered. If e.g., savings of fossil resources as well as greenhouse gases are given the highest ecological importance, all biofuels compare favourably with their fossil counterparts if competition for other uses of the resource is not considered (Quirin et al., 2004), whereas taking into account the impacts such as acidification, eutrophication or ozone depletion reverses this trend. Indeed, these drawbacks are linked to intensive agricultural production that notably leads to nitrogen compound emissions responsible for those bad impacts on the environment. The relative advantages of biofuel chains between one another depend on the feedstock. Figure 11 illustrates the results of a comparative study of 109 biofuel chains by the IFEU, i.e. the ranges of greenhouse gas and primary fossil energy savings by substituting fossil fuels with the biofuels assessed in some 64 studies (Quirin et al., 2004).

Comparison on the basis of a unit MJ energy content of biofuels (Fig. 11a) makes it possible to compare all the chains, including biofuels from residues, whereas the comparison on a hectare basis (Fig. 11b) introduces a land-use perspective. Amongst 1st generation biofuels, bioethanol from sugar cane, molasses and sugar beet, as well as biogas from wastes (here compared with gasoline, but also advantageous compared with natural gas) show the best combined performances in terms of both greenhouse gas and primary fossil energy savings (Fig. 11a). Greenhouse gas emissions are partly linked to the combustion of fossil primary energy input throughout the chain; both savings are therefore connected and their ranges appear within similar orders of magnitude.

Due to the high energy content of oilseeds and less complex processes, relatively less primary energy input is necessary to produce a MJ of biodiesel or pure vegetable oil compared with a MJ of ethanol, whose distillation and dehydration are energy-intensive. In comparison, the primary energy input in refining gasoline or diesel is not significantly different between the two fuels. Therefore, diesel substitutes can save the most fossil energy. To a lesser extent, they also mostly emit less greenhouse gases than ethanol from starch crops and ETBE. Greenhouse gas savings vary a lot among biofuel chains and biodiesel roughly performs better than the worst ethanol chains but is worse than the best ethanol candidates. Ranges in energy and greenhouse gas savings of biodiesel chains are less connected than those of ethanol, since the conversion processes consume less energy. Greenhouse gas emissions depend therefore more on the feedstock types and the cropping systems. In the case of sugar crops, energy inputs are partly compensated for by higher yields per unit of agricultural input.

Further savings are also due to the co-products. This is notably the case with sugar cane, whose stalk or bagasse are burnt to produce energy input, or in the case of rapeseed biodiesel compared with pure rapeseed oil. The latter is not refined and would by itself lead to higher saved fossil energy and greenhouse gases. However, the production of biodiesel co-produces glycerine that substitutes fossil-based glycerine. This co-product can be used as a substitute for chemical glycerine or as animal feed. The savings from displacing this otherwise produced glycerine are included within the system of the biodiesel chain, and can be particularly significant in the case of substituted chemical glycerine, whose manufacturing is very energy-intensive (JRC/EUCAR/CONCAWE,

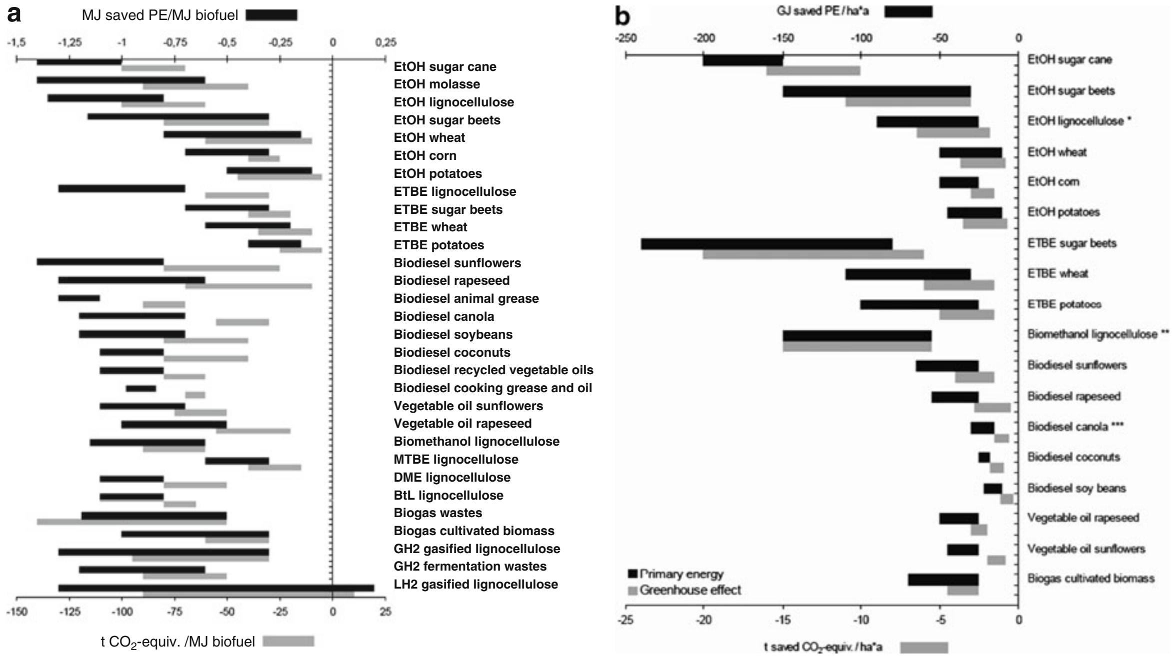

Fig. 11 Results of the energy and greenhouse gas balances of the analysed biofuels as compared to their fossil counterparts (gasoline for ethanol, biomethanol, biogas and H_2; MTBE for ETBE; diesel for biodiesels, vegetable oils, DME and BtL) in MJ or GJ saved primary energy and saved metric tons CO_2 equivalent per MJ of biofuel (**a**) and per hectare a year (**b**). Negative values imply advantages for the biofuels; the zero mark means that the CO_2 equivalent emissions are balanced when the total life cycle (biofuel minus fossil fuel) is considered (Quirin et al., 2004)).
* The spectrums for ethanol from lignocellulose are not unrestrictedly comparable with the others, since lignocellulose from biomass and that from organic residues are put together here.
** Only from cultivated biomass
*** Canadian brand name of summer rapeseed

2006). Nevertheless, these savings are no longer true as soon as the glycerine market is satisfied. There are many diverse uses of glycerine, even as feedstock for energy (IEA, 2007b), but there are also many industrial processes that lead to glycerine co-production (Russi, 2008). Assuming that large-scale biodiesel production will cause a saturation of the glycerine market (Larson, 2006), some authors do not consider any savings from glycerine co-production (Russi, 2008), which equal using economic allocations for co-product handling.

From a hectare perspective (Fig. 11b), ethanol from sugar crops performs better than ethanol from starch crops, and much better than the bio-substitutes of diesel, due to higher crop yields. ETBE makes more significant savings possible than ethanol because of its high energy-related hectare yield that permits one to displace higher amounts of fossil fuel; for each MJ of ethanol that replaces gasoline, 3 MJ of ETBE[34] replace MTBE. However, on an energy-unit basis ethanol is slightly more interesting than ETBE, as ETBE requires a further processing step compared with ethanol. ETBE is, though, here compared with MTBE, which is produced with more energy input than gasoline but emits a little less greenhouse gas, as the bulk of the energy is provided by natural gas for MTBE rather than heavier hydrocarbons in the case of gasoline (JRC/EUCAR/CONCAWE, 2006).

When comparing gasoline blended with MTBE, ETBE or ethanol (50% ex-wheat, 50% ex-sugar beet) combined with isooctane that compensates for the ethanol higher density, ETBE permits more CO_2 equivalent reduction per MJ of ethanol (Croezen et al., 2007; Higgins, 2007). Blending in ethanol or ETBE reduces the petroleum base fuel requirement for butanes and reformate octane numbers. Compared with a LCA in which ethanol and ETBE simply replace MTBE, these modifications in the refinery operations cause an additional 3% GHG savings in the case of ethanol, and about 20% in the case of ETBE. In the case of

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[34] 3 MJ of ETBE are produced from "1 MJ of ethanol and 2 MJ of isobutylene".

ethanol, its high vapour pressure requires a counterpart reduction of the petroleum base fuels' vapour pressure that partly offsets the savings due to the ethanol's higher octane numbers. In the case of ETBE, less volume of petroleum base fuels is needed for the same volume of ethanol (5%) with an even lower octane number requirement. Although this advantage for ETBE is to some extent undone by the higher GHG emissions related to its production and that of extra isobutylene, ETBE finally results in a higher net GHG reduction of 61 kg CO_2-eq/GJ ethanol converted into ETBE, against 37 kg CO_2-eq/GJ ethanol when 5 vol% ethanol is added in pure form (Croezen et al., 2007). Overall more greenhouse gas reduction is possible though, when engines are modified to run with higher blends of pure ethanol.

Results on 2nd generation biofuels are still theoretical and provisional, since their production is still at a pilot stage and technology keeps evolving, which is notably illustrated by the wide spectrum of lignocellulose or H_2 biofuel chains (Fig. 11a). Moreover, 2nd generation biofuels can proceed from a wider range of feedstock, diverse organic residues and theoretically all possible biomass. Regarding the spectrum of possible pathways, 2nd generation biofuel assessments are still scarce.

Mature 2nd generation biofuels are expected to have less impact on the environment. This firstly lies in the fact that the whole plant is transformed, increasing the energy yield for the same mechanical and chemical treatment, which implies that the environmental load is lower for the same energy unit. Furthermore, in the case of perennial grasses, the environmental advantages could be even more consistent. The biomass of perennial grasses has higher lignin and cellulose contents than the biomass of annual crops (Lewandowski et al., 2003). Perennial energy crops and short rotation forestry or coppice (SRF/SRC) generally have less impact on: soil erosion, nutrient inputs into the ground and surface water, pesticide pollution, and water abstraction. In contrast to annual crops, perennials require only one cultivation activity, i.e. preparation for planting, over a 10- to 20-year duration, and minimal nitrogen inputs (Heaton et al., 2004). Typical energy input/output ratios vary between 1/10 and 1/20 (IEA, 2006).

Perennials also have more extensive root systems present throughout the year, thus providing increased resistance to soil erosion and a more effective means of trapping nutrients and preventing nitrogen loss to drainage water. Production and turnover of belowground storage organs can furthermore add organic matter and carbon to the soil (Heaton et al., 2004). In addition, most nutrients also remain on the land under SRC or with perennial grasses, when the harvest takes place after the nutrient-rich leaves have dropped. As a result, soil carbon and quality tends to increase over time, especially when compared with conventional farming (IEA, 2006). Finally, annual crops on average need better quality land than perennials to achieve good productivities, whereas perennials can be grown on marginal lands, thereby achieving other potential benefits such as soil quality improvement or in some cases adding to biodiversity (Lewandowski et al., 2003; EEA, 2006b). However, perennial grasses and SRC, like other crops, may also contribute to ozone depletion, acidification and eutrophication if they are fertilised.

5.1.2 Limits of the LCA Tool

Data Quality

When compiling results from diverse biofuel LCAs, it appears that results widely differ between studies. This variation can be explained following two intertwined tracks: the intrinsic limits of LCA methodology and the lack of scientific background knowledge. LCA was first established for industrial production; so that differences between industrial and agricultural systems originate many methodological problems for agricultural LCA. The fact that industrial systems are mostly independent of their local environment has led to a site-independent methodology for LCA. However, the life cycle steps in close contact with the environment (such as agriculture or land filling) are site-dependent by nature (Kodera, 2007). Thus, in essence, LCA implies a site-independent model of a biofuel chain at a given period of time, which is merely compatible with the dual agricultural-industrial nature of biofuel chains. The first consequence is the difficulty of collecting data sets of representative quality; i.e. relevant, transparent, precise, complete and reproducible, while data collection and compilation are already often the most work- and time-consuming steps in LCA (Rebitzer et al., 2004). On the one hand, agricultural data sets are time- and site- (soil-climate) dependent, which implies uncertainty in extrapolation and modelling, and further variability in biofuel chain assessments. On the other hand, industrial data sets are not systematically accessible and transparent for outsider assessors.

Now, the attempt to make an exhaustive inventory should not be at the cost of data quality. According to Delucchi (2004), using literature-review estimates with an aggregated approach of processes instead of primary data from an appropriated input/output flow model for each process can amount to a percent or two of direct fuel-cycle GHG emissions. Three further percentage points in the fuel-cycle analysis would be related to data uncertainty with regard to estimates of the energy intensity of fuel production and the energy efficiency of motor vehicles (Delucchi, 2004). Comparing the life-cycle inventory for refinery products among several databases, Jimenez-Gonzalez and Overcash (2000) have shown that the variability in estimated emissions to the atmosphere, and waterborne and solid waste are approximately 50–150%, 1000% and 30%, respectively (in Lo et al., 2005). Also, models based on international or national average values might not be useful or adaptable to more specific local production conditions for decision-making on regional levels. Moreover, a LCA steady-state model cannot take directly into account the variation in market demand or the technological advances, although they can be introduced through economic allocation or as prospective assumptions. Both of these factors are crucial for bioenergy; likewise social aspects, which are also not encompassed in LCA.

The varying quality of input data makes the comparison of diverse scenario outputs more difficult. The lack of transparency and homogeneity in background assumptions between different biofuel chain assessments may hide the fact that data might not always be reliable. To deal with data quality and uncertainties, tools exist and could, if systematically associated with the results, enlighten comparisons between assessments. A "pedigree matrix" permits one to establish data quality indicators (DQIs) that give scores to data sets (1 to 5) in function of their reliability, their completeness, and their temporal, geographical and further technological correlations linked to the goal and scope of the study (Weidema and Wesnaes, 1996). These scores make it possible to distinguish processes and flows for which input data quality is poor, and to focus on these inventory parts to compare their impacts on output data among different assessments. This qualitative approach is to be completed with statistical indicators, such as coefficient of variation, that highlight the data uncertainty: i.e. the basic uncertainty linked to typical measurement errors or normal fluctuation of the variables, and an "additional uncertainty" related to the data not being of the optimal quality as reflected by its pedigree scores. This additional uncertainty can be calculated or estimated. "Default uncertainty matrices" for different specific types of data or domains could also serve as references, when the uncertainties cannot be directly assessed (Weidema and Wesnaes, 1996). Such tools might often appear to be necessary in the case of agricultural systems for which data such as probabilistic distributions and the correlations of key parameters are particularly rarely available Basset-Mens et al. (2006a).

Co-product Handling

The complexity of bioenergy chains evidences limits of LCA. Indeed, LCA methodology leaves some degree of liberty, when it comes to dealing with the handling of co-products and the complexity of a wide range of environmental impacts. The LCA methodology stipulates that the product and co-products should be handled separately as long as possible, through sub-division of processes, in order to avoid problems of burden allocation. This is, however, impossible in the case of bioenergy chains, where product and co-products come from the same feedstock and are chemically linked. In this case, methods of co-product handling are suggested but none is mandatory.

The expansion of the system boundary implies that the process leading to the production of a co-product is taken into account as a co-function of the system, either by additive substitution or by subtractive substitution. In both cases, the flows corresponding to the production of the co-product are taken into account in accordance with energy and environmental loads of the co-product, when produced through a fossil chain; either as supplementary loads in the reference scenario (additive substitution), or as loads to be subtracted from the bioenergy chain (subtractive substitution).

The ISO 14 041 (1998) stipulates, where allocation cannot be avoided, that the inputs and outputs of the system should be partitioned between its different products or functions in a way which reflects the underlying physical relationships between them (physical allocation or apportioning). Physical allocation implies that environmental loads and energetic costs are partitioned between the product and the diverse co-products in accordance with defined mass or energy ratios. Although mass ratios are easily measurable and therefore more frequently reproducible across studies, they do not reflect a fair share in burdens. Indeed, since the biofuel justifies all the energy and material expenses, it should support the main share of the total burdens implied by its production, which is not the case, for instance, when a co-product weighs more than the biofuel itself. Energy allocation credits may be closer to the logics of the functional unit of biofuels; however, differing calculation on an energy content or energy consumed basis can also introduce further variations among studies. When physical relationships cannot be distinguished, then a financial allocation is the remaining option and consists of defining ratios in function of the market value of each product. This last option can be quite relevant in the sense that economics will still be the underlying driving factor of a biofuel chain development, while LCA does not in itself take into account economic factors. But it also may create a bias if market values are punctually considered. An economic analysis is necessary to speculate on the price evolution of the diverse products.

As explicitly recommended by the ISO norms, expansion of the system boundary is the first solution that should be examined in order to address the issue of co-product handling. The introduction of the substitutes within the system boundary permits one to elicit the real impact of the co-product production in a specific context. For instance, Prieur and Bouvart (2006) showed with the example of BtL from wood, that the relative greenhouse gas savings compared with the fossil chain could vary between around 68% and 104% when considering a substitute co-produced for electricity either from a French mix (mainly from nuclear power) or average European mix (more from coal), respectively. Variability in this case is even wider between the two types of substitute than between one of the two substitutions and mass or energy allocations.

Biofuel chains are not equally sensitive to co-product handling. Comparing the diverse co-product handling scenarios (three allocation ratios: mass, energy or market value; and expanding the system), the burden of primary energy that comes to ethanol varies from 89.7–95.6% of total primary energy in the case of sugar beet, whereas it varies from 42.7–91.2% in the case of wheat (Malça and Freire, 2006). Kim and Dale (2002) also found that burdens of corn ethanol widely vary following the co-product handling, within the comparable range of 48–80% for both dry and wet processes. In these studies, mass allocation tends to lower the biofuel burden, whereas system expansion maximises it. Methodological choices (e.g. system boundaries and allocation methods) have a large influence which may very well override many other types of uncertainty (Björklund, 2002 in Malça and Freire, 2006). Following a pragmatic approach, it may be very relevant to introduce substitute products that are representative for the specific local production at stake. However, system expansion requires that an alternative way of generating the exported function exists and that data are available and collected. Such ways may not be found, or on the contrary, some co-products can possibly substitute a wide range of products (Malça and Freire, 2006; Kodera, 2007).

The lack of a clear standard for choosing substitution products may lead to arbitrary choice and inconsistent calculations and results even if the methodology remains unchanged (Kodera, 2007). Ink has been spent on looking for a consensus on this methodological issue, but there is no single procedure to deal with the diverse co-products, not even for each biofuel chain. The ISO norm (14 041/1998) states that when an allocation issue arises, a sensitivity analysis on this allocation parameter should be done. Hence, each biofuel LCA should explore a range of possibilities. Scenarios of allocation ratios and substitution means should be elaborated and compared, taking into consideration the most relevant co-products substituting chains on the local scale.

Impact Characterisation

A lot of published LCAs for biofuel are not in fact LCAs stricto sensu. Indeed, they may be assessments based on a life-cycle approach and guided by the LCA ISO norms, but rarely assess all the potential environmental impacts (Quirin et al., 2004; Blottnitz and Curran, 2007). By its holistic nature, LCA would require assessing all the potential impacts linked to the flows inventoried; nevertheless, the attempt at an exhaustive impact characterisation also faces methodological as well as scientific constraints. Life cycle impact assessment (LCIA) is the step that actually permits one to quantify the potential environmental impacts. It consists of classifying and aggregating the results of the inventory into category indicator results (Eq. (1)) that characterise how the environmental mechanisms for the chosen impact categories are modelled, and what the contributions are of the involved flows to these impacts.

$$\text{Impact Category Indicator}_i = \Sigma_j\,[\text{Characterisation Factors}\ (_{ij}) \times \text{Emission/Extraction Inventory}\ (_j)] \quad (1)$$

where "j" denotes the substances or resources (Brentrup et al., 2004).

Traditionally in LCIA, characterisation factors linearly express the contribution of a mass unit of an emitted substance to a given impact category. Depending on the scope and goal of the assessment, different impact assessment methods or approaches can be used, while the inventory remains the same. They actually differ in the choice of impact categories, the logic and approaches underpinning the characterisation (models and indicators). The main discussion here lies in the consideration of either midpoint impacts, or endpoint impacts, i.e. damage-oriented assessments.

Midpoints are considered to be points in the cause-effect chain (environmental mechanism), between stressors and endpoints (Bare et al., 2000 in UNEP, 2003). Midpoint impact categories are, for instance, climate change, stratospheric ozone depletion, human toxicity, acidification, eutrophication, etc. In damage-oriented assessment, impact pathways connect the inventory results across midpoints to one or more of the damage categories; i.e. classes of endpoints defined as areas of protection (AoP) that can be impacted both in their intrinsic and functional values (Jolliet et al., 2004). There exist different classifications of AoP that are actually connected; e.g. human health, the natural environment and manmade environment, human health, biodiversity (or ecosystem health), and natural resources (Heijungs et al., 2003). Life support functions (LSF), climate regulation, hydrological cycles, soil fertility and biogeochemical cycles, are classes of midpoints, just like areas of protection are classes of endpoints (Heijungs et al., 2003). Depending on the state-of-the-art knowledge, the representation of a pathway link may vary from a fully quantitative description, involving new contribution indicators such as disability-adjusted life years (DALYs), for instance, to a short qualitative description of the expected causal impact on subsequent pathway links (Jolliet et al., 2004).

Some LCA practitioners argue that endpoints are the elements of an environmental mechanism that are in themselves of value to society (Udo de Haes and Lindeijer, 2001 in UNEP, 2003). Others fear that uncertainties, due to a lack of sufficient data and robust models, may be extremely high beyond midpoints (UNEP, 2003). Hence midpoint approaches, such as CML (Heijungs et al., 1992), Eco-indicator 95 (Goedkoop, 1995) and EDIP (Wenzel et al., 1997), have been the main widespread approaches historically, whereas endpoint ones, such as EPS (Steen, 1999) and Eco-indicator 99 (Goedkoop and Spriensma, 2000) are more recently gaining interest, since they imply the analysis of trade-offs between and/or aggregation across impact categories. They also permit an integrative estimate of environmental externalities by monetary valuation of welfare losses due to impact on the AoP, which are fundamentally linked to societal values. Endpoint LCA results can be interpreted in light of marginal impact costs or distance-to-target performances, and can in this sense clearly serve to help design market-based internalisation instruments (e.g. taxes) (UNEP, 2003). Endpoint assessments are hence more directly understandable and useful for decision-makers; however, they lose in transparency when weighting is required to compare across categories, which does not systematically elicit links between midpoints and endpoints.

Therefore, both midpoint and endpoint approaches provide useful information with a trade-off between reliability and relevancy, and should be, to some practitioners' mind, conducted in parallel to determine how the results are affected (UNEP, 2003). In the case of biofuel LCAs, midpoint assessment appears to be relevant enough, though, given that impacts in terms of "climate change" and "fossil resources" are of central importance, and that uncertainties linked to data quality and co-products may already be a significant source of misinterpretation. Furthermore, a biofuel LCA is to be compared with a fossil fuel LCA, while weighting[35] across cat-

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[35] Weighting and normalisation are two non-mandatory steps in LCA methodology.

egories or other subjective scoring is not suitable when it comes to comparative assertions (ISO 14 042).

A more crucial issue is to improve impact characterisation, notably in order to account for the location and time of the emissions, waste generated, and resources depleted better, as well as the geographical zone and time period over which the contributions to different impacts should be considered (Pennington et al., 2004). Linear characterisation (Eq. (1)) aggregates the environmental loads at the time of the assessment, and neither takes into account the substance background concentration nor its temporal and geographical dependency on exposure and fate (UNEP, 2003). This implies that all impacts, irrespective of the moment and the place that they occur, are equally included (Udo de Haes et al., 1999). The fundament, referred to as the "less is better" principle, is that a pollutant remains a pollutant even if it is emitted in a place where it will not cause any harm; as such its emission shall be considered as contributing to potential impacts independently of site and time (Heijungs et al., 2003). It follows that impact categories are assumed to be independent of one another, and unless a precise scientific background permits one to justify the hypothesis to partition the contribution of the same substance to several impacts, the substance flow shall contribute in its entirety to each impact (Guinée, 2002). Firstly, these assumptions only lead to calculating potential impact scores, not actual damage (Khalifa, 1999; UNEP, 2003). Potential impacts thus represent the worst-case scenario, where some redundancy of a substance contribution is preferred to the risk of not considering its contribution to one impact. However, practitioners should be aware that double-counting may lead to poor decisions and that their models should try partitioning burdens as far as the state-of-the-art knowledge on the causality chain permits it (Reap et al., 2008).

Secondly, this dose-response modelling is not sufficient to describe complex environmental mechanisms, especially those where thresholds intervene (Khalifa, 1999; UNEP, 2003; Pennington et al., 2004). Simplifying assumptions and available scientific knowledge influences the accuracy of the indicators, which may vary among impact categories due to discrepancies between models and the corresponding environmental mechanisms. Hence the applicability of the characterisation factors depends on the accuracy, validity and characteristics of the models used (UNEP, 2003; Basset-Mens et al., 2006a). Lack of knowledge on the dose-response determinisms may jeopardise the reliability of the impact assessment. This is particularly true in the case of impacts on biodiversity or natural habitats, for example, for which the mechanisms are especially complex, and may also include other determinisms than chemical or physical ones. Khalifa (1999) emphasises that impact assessment within LCA is notably lacking in reliability for the impact categories of eutrophication, photochemical ozone, ecotoxicity, loss of habitats and biodiversity, as existing thresholds and non-linear dose-response notably are not considered. The reliability of the assessment is also in essence lower when it comes to local-range impacts that are more specifically dependent on the local ecosystems than global impacts. Truncations and assumptions about global homogeneity and steady-state conditions introduce the most severe errors in impact assessment. Indeed, first setting arbitrary time horizons skews results in favour of short- or long-term impacts, thus ignoring spatial variation, local uniqueness and environmental dynamics discounts the influence of environmental stress concentrations, leading to inaccurate estimates of potential damages (Reap et al., 2008). Spatial information in LCA is actually mandatory in order to contribute to solving the poor accordance between potential impact as calculated in LCA and the expected occurrence of actual impact (Khalifa, 1999; Heijungs et al., 2003; Potting and Hauschild, 2005).

Sophisticated LCIA have been developed in order to improve the level of detail, regarding in particular the temporal and spatial dimensions of the impacts. Nevertheless, only a few integrated approaches have been proposed so far (UNEP, 2003) and they were essentially developed within an endpoint characterisation, whereas regionally differentiated midpoints would also be better indicators (Heijungs et al., 2003). In particular, models have been used to determine regionalised fate and exposure factors, in order to account for background load and a priori tolerance of ecosystems to the emissions (Potting and Hauschild, 2005). For instance, Potting et al. (1998) used the RAINS model (IIASA) to produce acidification factors to be used within LCA in order to simulate acidification discrepancy better across 44 regions in Europe. This model integrates information on emission levels for each region with information on long-range atmospheric transport in order to estimate patterns of depositions and concentrations for comparison with critical loads and thresholds for acidification, eutrophication and photochemical ozone creation. These critical load functions (weighted for the size of the ecosystems) are used to construct so-called "protection isolines" for the grid element that consist of all combinations of S and N deposition for which a given fraction of ecosystems does not exceed critical loads, and thus in RAINS terminology is assumed to be protected against the adverse effects of acidification. Regional acidification factors were calculated by reducing one by one the emission levels of each separate region by 10%, and then relating the result to the reference situation (the initial emission level and area of unprotected ecosystems). Hence acidification factor ($AF_{s,i}$ in ha/tons) directly relates a change in emission of substance (s) in a region (i) to the change in unprotected ecosystems in its total deposition areas. A similar approach was notably used to calculate regional factors for terrestrial eutrophication (with NH_3, NO_x).

Impact characterisations are integrated over an infinite period of time, since the variation in emissions on the regional scale during the time period for integration is considered as marginal when compared with the total contributing emissions (Potting and Hauschild, 2005). Indeed, LCA steady-state assumption is founded on the "multiple sources-multiple receptors" character of present environmental problems, i.e. the temporal variation in the contribution from a single source emission is usually to a large extent cancelled out against the high background exposure from all sources together. Moreover, the large impact area of an emission and the overlapping with impact areas of neighbouring sources make the precise location of a source of less importance, which makes it possible to determine site-dependent factors on a regional scale (Potting and Hauschild, 2005). However, these approximations will not be true for local (exposures within the first kilometres from the source) or time-dependent impacts (such as very slow emissions or synergic impacts) (Potting and Hauschild, 2005). Modelling the combined impacts of agricultural inputs, the climate and the hydrological functioning of catchments, Basset-Mens et al. (2006a) determined N apparent fate factors that describe the part of leachable N that actually contributes to the annual stream nitrate flux. This study emphasises that, on the regional scale, these nitrate fate factors can imply large variations compared with the results of a standard LCA methodology. In the case of pig production, the eutrophication result was reduced by 5% to 32%, and the climate change impact varied between "no change" and an increase of 200% (Basset-Mens et al., 2006b).

Temporal and spatial dimensions are tightly intricate together. Especially meteorological conditions influence the determination of fate and exposure factors. Emission timing at different rates and locations defines site-specific emitting, fate and exposure conditions. For instance, the acidification factors calculated for the reference years 1990 and 2010 show that the difference between different calendar times can be notable (Potting and Hauschild, 2005). This would also be particularly relevant in the case of the region-specific fate factors for airborne nitrogen compounds causing aquatic eutrophication by Huijbregts and Seppälä (2000). These region-specific fate factors were modelled given European emission and meteorological data from 1985 to 1995. Regional NH_3 and NO_x fate factors express the fractions of these airborne emissions that actually end up in the aquatic environment, taking into account both direct deposition in the freshwater and marine environments and run-off from terrestrial systems into the aquatic environment. Now, for a short time frame, these regional fate factors may, in essence, vary depending on punctual variations in precipitation patterns compared with those used to model the fate factors. The characterisation factors used to assess the impact from a given process should also relate to the calendar time in which that process takes place (Potting and Hauschild, 2005).

However, time-dependent environmental processes may necessitate time horizons for impact integration that are not in accordance with an optimum for those site-dependent factors. Thus, time-dependent factors add to the continuing discussion within the LCA community on selecting integral limits and valuing impacts distributed in time (Reap et al., 2008). Furthermore, depending on the impact, the extraction/emission region might not be the same as the region where impact/damage occur. For both time and space, it would be necessary to distinguish factors at the extraction/emission point and impact/damage point (Heijungs et al., 2003). Thus, scale precision for spatial and temporal discrepancies might remain constrained by a geographical scale large enough to cover most of the impacts from an emission source. Site-dependent factors already mostly encompass characteristics that are relevant at a country-based level. Now, these factors appear to vary also on the regional scale. In the life cycle region-specific assessment method proposed by Yi et al. (2007), where "affected regions" are distinguished from "emitting regions", regional damage factors considerably vary within the 9 defined areas and are 0 to 3 times higher than the national average.

To conclude, LCA is a powerful tool but there remain theoretical and methodological open questions that can lead to diverging results and interpretations, in particular in the biofuel chain cases. While some practitioners work on sophisticating LCA (UNEP, 2003), some others look at ways to simplify it (Rebitzer et al., 2004). On the one hand, some limits (methodological, scope of the impacts: site independency, etc.) should be overcome to make LCA a better tool for decision-making purposes; on the other hand, it already is a challenging exercise (lots of data needed, various sources of uncertainty, crucial lack of scientific knowledge for impacts, etc.). A way to solve this puzzle may be to produce local specific LCAs, based on local inventories and a better knowledge of the local receptive environment. Although the requirement of additive site-dependent data is often put forward as an objection against spatial differentiation in LCA (UNEP, 2003; Potting and Hauschild, 2005), local data sets and fitting models with local receptor parameters can make it possible to diminish the uncertainty of the results.

Uncertainty affects all the assessment steps: from the input data uncertainty, through model uncertainty, up to uncertainty on the chosen uncertainty formalisms (e.g. determinations of probability distributions, etc.) and may, if completely represented, even entail a broad interval of imprecision that could finally make the results of comparative LCAs indistinguishable (Reap et al., 2008). As illustrated in a review by Boer (2003), comparison between LCAs of diverse commodity chains across different case studies is hampered by the lack of international harmonisation on LCA methods among the studies, whereas a within-case-study comparison of diverse chains using the same LCA methodology appears suitable to track down the main differences in potential environmental impacts. Biofuel chains should be hence compared on a regional case-study-based scale, and further extrapolation across regions should be limited to LCAs that use harmonised methods.

5.2 *Focus on Greenhouse Gas Emissions from Agriculture*

"Climate change" is often presented as an example of well-established midpoint impact characterisation, notably because its global dimension particularly fits the "multiple sources-multiple receptors" background assumption. However, when assessing biofuel chains, it appears that greenhouse gas emissions in the field can show highly variable temporal and spatial patterns, and that these emissions contribute a significant share of the total greenhouse gas balance of the whole chain. Emissions during agricultural production contribute, for instance, 34%–44% to the greenhouse gas balance of corn ethanol in the US (Farrell et al., 2006) and up to more than 80% in the case of pure vegetable oils (ADEME/DIREM, 2002). Focusing on the agricultural phase only, many factors imply variations among biofuel chains. They are linked to the ecosystem characteristics and the cropping systems, both dependent on local conditions. After briefly describing the greenhouse gas emissions linked to agricultural activities, two aspects of the current limits in the assessment of biofuels' greenhouse gas balance will be detailed, i.e. the determinism of N_2O emissions and CO_2 emissions due to land-use change.

5.2.1 Overview of Greenhouse Gas Emissions from Agriculture

Agricultural activities contaminate the environment through three main impact pathways: land-use changes, the use of farm machines, and the use of inputs, e.g. fertilisers, that are sources of many diverse pollutants throughout their life cycle. Industrial emissions concerning the agricultural phase in a biofuel LCA encompass all the emissions linked to the production and use of machines for agricultural operations and of inputs such as fertilisers or pesticides. Briefly, the more intensive the use of machines and inputs, the higher the overall emissions of pollutants. In Farrell et al. (2006), agricultural practices across six compared corn with ethanol LCAs are responsible for 45% to 80% of all petroleum inputs and related emissions. This illustrates how the intensity of cropping systems influences the overall chain performance, but also that the lack of transparency in primary energy inputs adds to the global confusion when comparing

LCAs. More attention is paid here to emissions that are the results of natural reactions within the coupled biogeochemical cycles of C and N. In general, CO_2, N_2O and CH_4 are by-products of the microbial activity, which is characterised by a transfer of electrons, hence depending on soil redox potential, dissolved organic carbon content, and the concentrations of the relevant electron acceptors (Li, 2007). These reduction-oxidation reactions are influenced by both the natural conditions and the agricultural activities, meaning that resulting emissions can vary widely and are therefore hard to predict.

Focusing on greenhouse gas emissions, in 2005 direct CH_4 and N_2O emissions from the agriculture sector worldwide accounted for about 5.1–6.1 $GtCO_{2eq}\ yr^{-1}$, equivalent to 10–12% of the total anthropogenic emissions of greenhouse gases (Smith et al., 2007). This includes 3.3 $GtCO_{2eq}\ yr^{-1}$ of CH_4 (50% of total CH_4 anthropogenic emissions[36]) essentially due to enteric fermentation from livestock (27% of agricultural greenhouse gases in Baumert et al., 2005 of a total of 6.2 $GtCO_{2eq}\ yr^{-1}$ in 2000), to rice cultivation on wetlands (10% of agricultural greenhouse gases in Baumert et al., 2005, and to manure management (7% of agricultural greenhouse gases in Baumert et al., 2005), and 2.8 $GtCO_{2eq}\ yr^{-1}$ of N_2O (60% of total N_2O anthropogenic emissions) (Smith et al., 2007) produced by microorganisms in the soils (40% of agricultural greenhouse gases in Baumert et al., 2005). Annual CO_2 emissions by agricultural lands are low compared with overall anthropogenic CO_2 emissions. The net flux between the atmosphere and the agricultural land, not considering energy-related emissions, is estimated to be approximately balanced at around 0.04 $GtCO_{2eq}\ yr^{-1}$ (Smith et al., 2007), while energy-related CO_2 emissions accounted for around 9% of global agricultural greenhouse gases[37] in 2000 (Baumert et al., 2005), although this share can be higher in industrial countries within intensive agricultural systems.

Moreover, land-use change and forestry (LUCF) are responsible for around 13% of global anthropogenic greenhouse gas emissions, i.e. some 5.4 $GtCO_{2eq}\ yr^{-1}$ on average during the period from 2000–2005, respectively (Houghton, 2008). The carbon flux includes emissions due to land clearing, emissions from forest products (including woodfuel) (80% in Duxbury and Mosier, 1993), and emissions from the oxidation of soil organic matter in the years following initial cultivation on former forest land (20% in Duxbury and Mosier, 1993). On the other hand, the carbon sinks accounted for are re-growing forest (vegetation and soils) after agricultural abandonment, reforestation, harvest and fire suppression. The assessed flux does not take into account the influence on carbon stocks of agricultural or silvicultural practices that do not imply changes in area, such as changes in species, no-till agriculture and thinning of forests, for instance. Finally, the assessment does not consider the indirect effects of fertilisation by N deposition or increased atmospheric concentration of CO_2, that could partly counterbalance the rise in CO_2 emissions (Houghton, 2003).

Agriculture is indirectly responsible for a large part of these emissions due to land clearing to convert lands into croplands or grasslands, primarily deforestation in developing countries driven by the conversion of forest to agricultural lands (Houghton, 2003; Baumert et al., 2005); 60% of released carbon due to land-use change between 1850 and 2000 came from the tropics and during the 1990s, the net carbon flux outside the tropics has actually turned into a net sink (Houghton, 2003). Nevertheless, anthropogenic CO_2 emissions from land-use change and the forestry sector are subject to *extraordinary uncertainties*[38], notably linked to the varying availability and quality of regional land-use data and to uncertainties in estimating forest growth rates and carbon stocks in ecosystems affected by various human management practices (Houghton, 2003). Thus, estimates of the carbon fluxes are uncertain in the order of ±150% for large fluxes, and ±50 $MtC\ yr^{-1}$ for estimates near zero (Houghton, 2003).

For the 1990s, IPCC estimates of CO_2 from land-use change ranged between 12% and 28% of world total CO_2 emissions (in Baumert et al., 2005). This sector also includes N_2O and CH_4 emissions, although no reliable global estimates make it possible to assess the share of these emissions that are linked to land-use change and forestry (Baumert et al., 2005). In order to assess the greenhouse gas emissions due to

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[36] These shares are followed in the report by the mention "*medium agreement, medium evidence*", and the same for the balanced CO_2 net flux by agricultural soils "*low agreement, limited evidence*".

[37] The remaining 6% of agricultural greenhouse gases by subsector are undifferentiated sources of CH_4 and N_2O.

[38] As written by the authors Baumert et al., 2005, p. 91.

agricultural production better, a deeper understanding of the local determinism of CO_2, N_2O and CH_4 contributions is needed, which also takes into account the impacts of land-use change and agricultural practices on these emissions. We focus here, within the framework of biofuels from energy crops, on N_2O and CO_2 emissions.

5.2.2 N_2O Emissions

Global Budget

N losses from agricultural fields are a very critical issue for mainly two reasons. First, they represent a net loss of nutrient for the plant and a consequently supplementary cost in terms of fertilisation. Second, all the Nr[39] leaks outside of the soil-plant system are sources of pollution. The mechanisms of N losses are diverse, and so are their impacts on the environment. The determinism of N losses and the characterisation of their impacts are complex especially because of difficulties in considering changes in spatial and temporal scales between emission sources and final impacts, as reactive Nr is widely dispersed by hydrologic and atmospheric transport (Galloway et al., 2003). Furthermore, little is known about how to quantify synergic or antagonistic processes occurring between midpoint impact (e.g. acidification) and endpoint damage (e.g. water toxicity), inducing further uncertainty in indirect emissions that are linked to primary direct emissions.

A wide range of experiments and studies have been focusing on how to improve N fertilisation efficiency in order to firstly reduce the source of these losses as far as possible. In this sense, much progress has already been achieved during the last decades, notably by better adapting the type, doses and applications of fertilisers to the crop needs and pedo-climatic conditions. However, many questions still remain, especially concerning the determinisms of gaseous N losses at the field scale. The imbalance between total inputs and outputs of N in agricultural systems has puzzled scientists for more than 50 years (Wrage et al., 2001). ^{15}N balances show deficits in N fertiliser recovery that vary between 1% and 35% (Recous et al., 1988).

Special attention has increasingly been paid to nitrous oxide (N_2O), since it is an important agricultural greenhouse gas. Indeed, due to its long residence time in the atmosphere and its high relative absorption capacity per mass unit, its 100-year global warming potential is about 298[40] times that of CO_2 per mass unit (Forster et al., 2007). Considering its current concentration in the atmosphere, N_2O is the fourth largest single contributor to positive radiative forcing (after CO_2, CH_4 and tropospheric ozone[41]) (Denman et al., 2007). Its radiative forcing averaged 8.5% of total radiative forcing for the period 1750–2000, when CO_2 contributed to 85% of this total radiative forcing (Forster et al., 2007). N_2O is also the main source of stratospheric NO[42], that catalyses the photolysis of O_3 (Conrad, 1990).

Nitrous oxide is naturally produced in soils through the microbial processes of denitrification and nitrification. Nitrification is the aerobic oxidation of ammonium into nitrate, and denitrification is the anaerobic reduction of nitrate into nitrogen gas (N_2). N_2O is an obligate intermediate in the reaction sequence of denitrification and a by-product of nitrification (IPCC, 2006). Hence, the availability of inorganic Nr in the soil appears to be one of the main controlling factors of these reactions, and the intensification of agricultural activities leading to more use of N fertilisers enhances N_2O emissions by the soils (Mosier et al., 1998; IPCC, 2006).

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[39] Nr means reactive nitrogen compounds, i.e. all inorganic and organic N compounds except N_2, that is a non-reactive N compound.

[40] The former GWP in the second IPCC assessment report was 310 eq CO_2 per kg, 298 includes the indirect negative radiative forcing due to the destruction of stratospheric ozone.

[41] Radiative forcing ($W.m^{-2}$), or global warming potential, refers to the change in the radiative balance on Earth's surface that is normally ensured by the natural greenhouse effect whose dominant contributing gases are water vapour (60–70% in Duxbury and Mosier, 1993), CO_2 (25% in Duxbury and Mosier, 1993) and O_3. A positive radiative forcing (warming) occurs when the concentration of greenhouse gases increases; a negative radiative forcing (cooling) when precursors that lead to the destruction of greenhouse gases are released into the atmosphere. Halocarbons are also main contributors to radiative forcing to an extent similar to that of tropospheric ozone (Forster et al., 2007). They are not mentioned amongst the first single contributors though, because they encompass several gas contributors.

[42] $NO_x = NO + NO_2$ which are in photochemical equilibrium. NO_x is mostly firstly emitted in the form of NO (Conrad, 1990). NO_x is a common anthropogenic pollutant (Duxbury and Mosier, 1993).

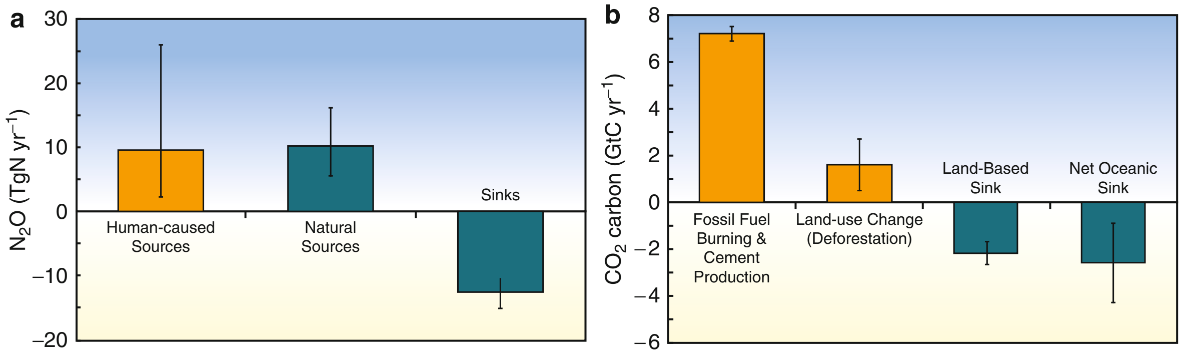

Fig. 12 Global budgets of N_2O (14) and CO_2 (15) (Denman et al., 2007)

Agriculture is the single biggest anthropogenic N_2O source (Denman et al., 2007), being the third overall most important source after soils under natural vegetation, especially land at tropical latitudes due to more rapid N cycling (Duxbury and Mosier, 1993), and N_2O release by oceans. Compared with CO_2 (Fig. 12), there is only one significant known sink of N_2O, i.e. its destruction in the stratosphere after an average residence time of 114 years in the atmosphere, and no robust evidence of soil N_2O sink strength. The amount of N_2O that is absorbed by soils, i.e. "consumed" by denitrification, is subject to extreme uncertainty. Net N_2O uptake by soils has been observed under different conditions, making it difficult to identify a set of conditions promoting N_2O uptake. However, factors opposing diffusion of N_2O in soil generally seem to increase its consumption, as well as low Nr and O_2 availability[43] (Chapuis-Lardy et al., 2007). N_2O uptake is often masked by larger N_2O production and may be indirectly accounted for in global budgets, provided that emission factors are based on all measured fluxes without discarding negative measurements.

More understanding of N_2O consumption by soils is needed to take into consideration its contribution to the global N_2O budget better; especially since the current global estimated sources and sinks of N_2O are not balanced (Chapuis-Lardy et al., 2007; Goldberg and Gebauer, 2008). Reported sources are larger than summed sinks and atmospheric increase. Considering that oceanic N_2O source may be underestimated[44] by at least two-fold (Bange, 2006), it is additionally likely that some N_2O source is overestimated or N_2O sink underestimated (Goldberg and Gebauer, 2008). Moreover, N_2O stratospheric lifetime seems to be shorter than previously thought, which also indicates that sinks may have been underestimated (Chapuis-Lardy et al., 2007). Recent bottom-up and top-down estimates of total N_2O sources in the 1990s agreed on averages of 17.7 (8.5–27.7) and 17.3 (15.8–18.4) MtN yr^{-1}, respectively (Denman et al., 2007). With a bottom-up approach, total direct and indirect annual N_2O emissions from agricultural soils in the 1990s, including synthetic fertiliser, manure and biological N fixation would average 3.5 MtN yr^{-1} (2% of N input) (Mosier et al., 1996), 4.2 MtN yr^{-1} (Mosier et al., 1998; i.e. IPCC, 1997) or 5.4 MtN yr^{-1} (Denman et al., 2007). A FAO statistical model, considering most of the factors[45] influencing median values of N_2O measurements, gives estimates

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[43] Soil humidity favours denitrification up to N_2O reduction, while NO_3^- is preferred as an electron acceptor over N_2O (Granli and Bøckman, 1994).

[44] Bange emphasised that estimates used in global budgets are out of date. Moreover, due to increased release of anthropogenic Nr into the ocean, N_2O emissions by marine microorganisms could increase up to 1.6 MtN-N_2O per year (in Galloway et al., 2008).

[45] Climate, crop type, fertiliser type, application rate, mode and timing of application, soil organic C and N content, soil pH, soil texture and drainage, measurement technique, frequency of measurements, length of measurement period. This analysis does not include organic soils; neither did the one from Mosier et al. (1996). Organic soils are considered in the IPCC guidelines. They appear to be a great source of N_2O, because of high soil organic content and low drainage, which implies reducing conditions (IPCC, 2006). Total areas of organic soils (histosols) ∼1.2% of ice-free land area (online 03.02.2009: http://soils.ag.uidaho.edu/soilorders/histosols.htm).

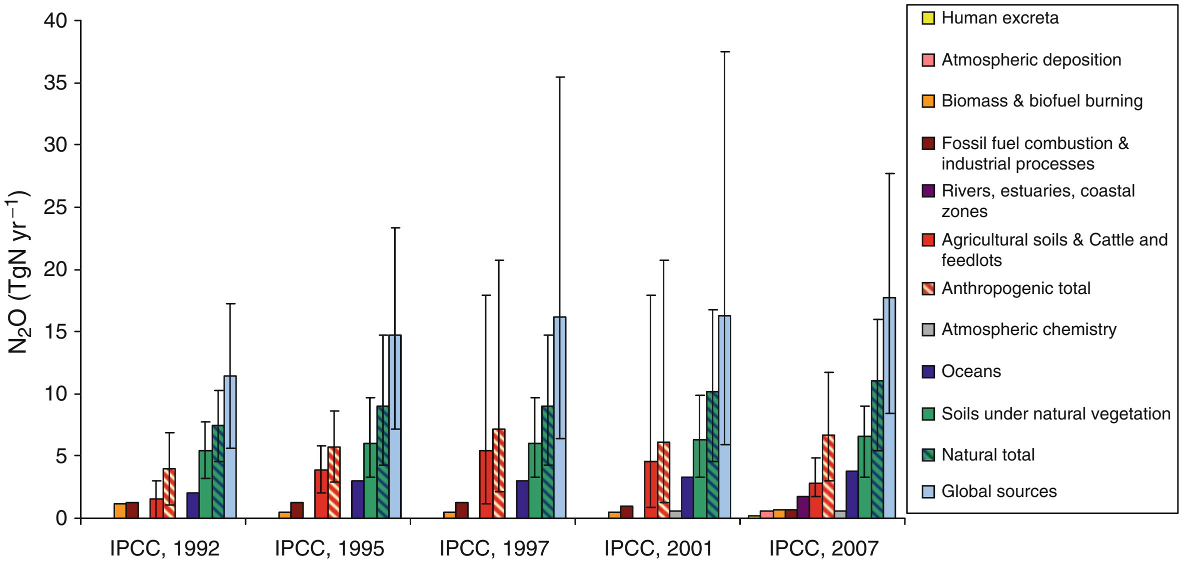

Fig. 13 IPCC assessments of total annual N_2O emission during the 1990s. Drawn from IPCC data in Mosier et al., 1998 and Denman et al., 2007. Note: for the 1997 assessment 0.9 Tg yr^{-1}(Mt yr^{-1}) of emissions by agricultural soils are subtracted to the total 6.3 Tg yr^{-1}(Mt yr^{-1}) to prevent double counting with part of indirect agricultural emissions already accounted for within emissions by oceans. Emissions from human excreta, rivers and atmospheric deposition can also be partly considered as agricultural indirect emissions

for total direct N_2O emissions[46] of 3.5 MtN yr^{-1}, with a 34% share occurring in developed countries, respectively (i.e. 3.3–3.4% of N input) (FAO, 2001). Total top-down assessed agricultural N_2O emissions of 4.3 to 5.8 MtN yr^{-1} (3.8–5.1% of N input) (Crutzen et al., 2008) are to be compared with the bottom-up estimate 6.3 MtN yr^{-1} (Mosier et al., 1998) that also encompasses 2.1 MtN yr^{-1} from animal waste management. IPCC assessments of global N_2O budgets have been continuously evolving, notably due to improvement in considering the diverse direct and indirect sources and refining emission factors; still, the uncertainty on anthropogenic N_2O remains remarkably significant (Fig. 13).

Origins of Uncertainties

Uncertainties in estimating N_2O emissions from agricultural fields originate in the difficulties (1) of identifying all the primary sources; the contribution of biological N fixation[47] is especially hard to quantify (Mosier et al., 1998; IPCC, 2006), (2) of following the fate of nitrogen throughout the whole nitrogen cascade that implies several processes and "actors" (Duxbury and Mosier, 1993; Galloway et al., 2003), and (3) of capturing and characterising the spatial and temporal high variability in emissions (Parkin, 1987; Mosier et al., 1996). This variability is due to multiple involved processes that each respond differently to various environmental and soil factors (Farquharson and Baldock, 2008). Moreover, these factors can interfere at three control levels: (1) in the rate of nitrification and denitrification, (2) in the proportions between the gaseous end products of these reactions, and (3) in the consumption of these gases in the soil before escaping to the atmosphere (Firestone and Davidson, 1989).

On a global basis, about 120 MtN from new Nr (fertilisers and cultivation-induced biological fixation) and 50 MtN from previously created Nr (crop residues, deposition, etc.) are added annually to agroecosystems (Galloway et al., 2003). Within the primary cycle of Nr (dashes lines in Fig. 14), only half of the N input is harvested in the crop (Duxbury and Mosier, 1993; Galloway et al., 2003), while the other half is lost by

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[46] Data for fertilisation IFA/IFDC/FAO (1999), land-use in FAO, 2001.

[47] Symbiotic rhizobia in root nodules are able to denitrify. This can lead to N_2O emissions, possibly 4 kg N.ha^{-1} for improved pastures; legumes could increase N_2O emission two- to threefold compared with unfertilised fields in Mosier et al. (1996). This denitrification by rhizobia could also lead to net N_2O consumption depending on local factors.

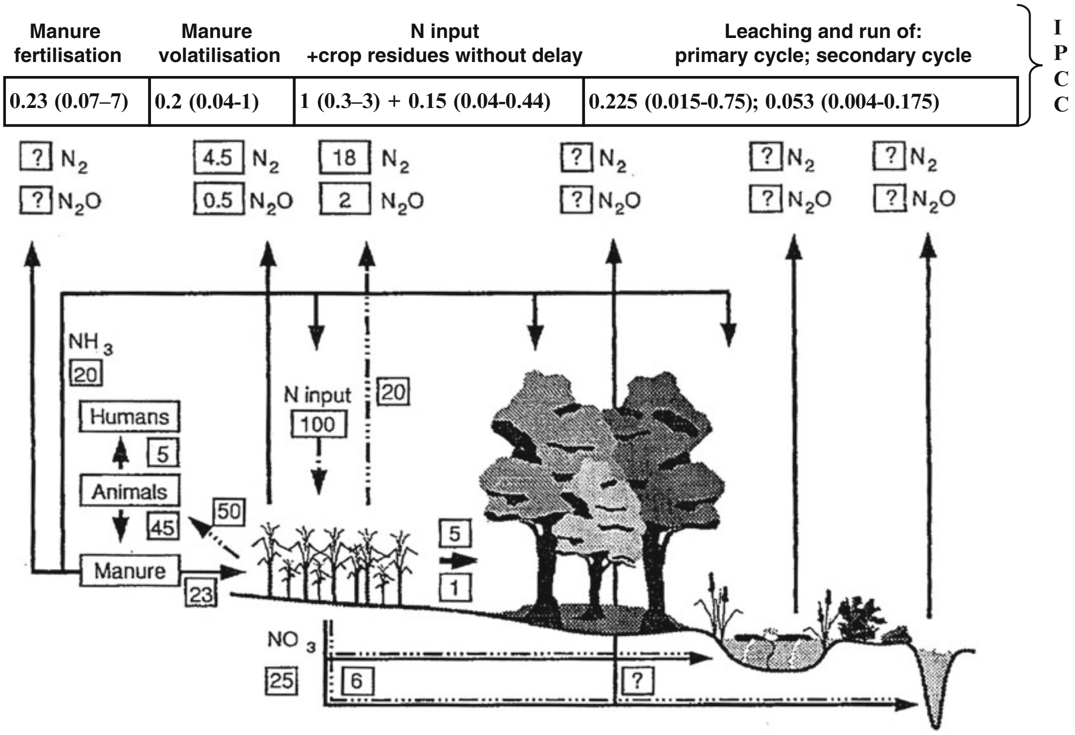

Fig. 14 A simplified flow of fertiliser N through the environment (Duxbury and Mosier, 1993) and N_2O emissions with ranges into brackets (N_2O-Nkg) estimated with the IPCC Tier 1 method (IPCC, 2006) considering some US average data on the diverse fractions as given by Duxbury and Mosier (1993)

a combination of leaching (19–26% of input in Smil, 1999), run-off and gaseous losses through direct emissions (15–35% of input in Smil, 1999), primarily from denitrification (Duxbury and Mosier, 1993), volatilisation and nitrification. Secondary N flows, shown by the solid lines (Fig. 14), encompass N_2O emissions from nitrification and denitrification through two indirect pathways; (i) following volatilisation of NH_3 from urea, ammonia or manure application, of NO_x, and the subsequent re-deposition of these gases and their products NH_4^+ and NO_3^- into soils and waters; and (ii) after leaching and run-off of Nr, mainly NO_3^-.

Throughout the whole Nr life cycle, only a small amount (about 4 MtN from the initial 170 Mt in Smil, 1999) will accumulate in the agroecosystems, while the rest will eventually transfer back into the atmosphere (Duxbury and Mosier, 1993; Galloway et al., 2003), including the 21 MtN temporarily stored through human consumption of grain (64%), and meat (CAFOs[48] 20%) (Galloway et al., 2003). Despite current knowledge, it is still not possible to reliably predict the fate of a unit of Nr that is applied or deposited in agroecosystems (Mosier et al., 1996), and the total amount of Nr lost through denitrification in agroecosystems is poorly known. Agroecosystems receive about 75% of the Nr created by human activity (Galloway et al., 2003). In the mid-1990s, the fate of only 35% of Nr inputs in the terrestrial biosphere was relatively well known: 18% was exported to and denitrified in coastal ecosystems, 13% deposited into the ocean, and 4% directly emitted as N_2O; the remaining 65% either accumulated in soils, vegetation and groundwater or was denitrified into N_2, but the uncertainty of those estimates remains large on every scale (Galloway et al., 2008), and further uncertainties appear when trying to assess all the direct and indirect N_2O emissions. In the field, direct N_2O emissions from N-fertilised agricultural fields have been found to vary between 0.001% and 6.8% of the N applied. Case studies combining diverse measurement techniques confirmed that uncertainty in N_2O fluxes found in the literature was indeed due to diverse combinations of controlling factors and not linked to the analytical methods (Mosier et al., 1996).

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[48] Concentrated animal feeding operations.

Emission Factors

The 2006 IPCC guidelines to assess N_2O emissions from managed soils (IPCC, 2006) consider all identified direct N_2O sources, except direct emission from biological fixation due to the lack of experimental evidence, and the two above-mentioned indirect pathways. The Tier 1, 2 and 3 methods consist first of a comprehensive accounting of all N input into the fields, including inorganic and organic fertilisers, as well as mineralised N from soil organic carbon due to land-use change or crop residues that indicate background emission levels linked to recovery from past managements. These Nr amounts are then multiplied by default emission factors (Tier 1), emission factors related to country-specific data when available (Tier 2), or the emissions are estimated with process-based models (Tier 3). Following the same order, Tier 1, 2 and 3 methods guide the estimation of input fractions to be multiplied by indirect emission factors with less to more country specificity, respectively.

Following the Tier 1 method and the given default emission factors, the 100 kg of Nr input on a US field (assuming maize for crop residues) as characterised by Duxbury and Mosier (1993) (Fig. 14) would emit 1.86 kg N-N_2O with a wide uncertainty range between 0.47 kg and 12.4 kg. These estimates include emissions from crop residues, without time lag, and emissions due to a secondary cycle through manure recycling and further run-off and leaching due to its application. Using the $1.25\% \pm 1\%$ of N input lost as N_2O (Bouwman, 1994) and further 0.75% of this input lost through indirect emissions, the same scenario would be expected to lead to 2 kg N-N_2O ± 1 kg emissions (Mosier et al., 1996). The rough 2.5 kg N-N_2O estimated by Duxbury and Mosier (1993) did not include all indirect contributions.

Statistical models aim at finding reproducible correlations, i.e. relationships representative of most data sets, between controlling factors and emissions, e.g. emission factors depending on the type and amount of applied fertiliser. In this sense, the more data collected on the different direct and indirect emissions, the more informative emission factors will be. The multiplication of data sets and the development of complementary techniques on various scales (aircraft measurements, micrometeorological techniques, chambers (see picture below), ^{15}N balance, C_2H_2 inhibition and lab work), have already made it possible to improve the understanding of controlling factors beyond a coarse linearity with N fertiliser. It has also been proven that measurements should be carried on throughout the year, because maximum fluxes were observed at different times for different treatments, and with a high frequency to capture temporal emission patterns (Mosier et al., 1996; Laville et al., 1997; Beheydt et al., 2007; Pattey et al., 2007). Frequent measurements appeared to yield lower total emissions (FAO, 2001; Pattey et al., 2007) due probably to less error with interpolation of punctual high emissions (Conen et al., 2000). Still, increased numbers of measurements that represent a wider range of agricultural systems are needed in order to improve statistical models (Mosier et al., 1996; Stehfest and Bouwman, 2006), as well as process-based ones.

Measurement of N_2O and CO_2 fluxes with automatic chambers in a sugar beet plot,
Estrees-Mons, June 2008 bessou©INRA

Given the complexity of N_2O emission control (Fig. 15), it is almost impossible to determine a quantitative relationship between the cause (a change in any ecological driver or environmental factors) and the consequence (N_2O fluxes) through simple correlation or regression analysis (Li, 2007). The extreme spatial and temporal heterogeneity of many primary drivers actually obscures the relationships between cause and effects for many of the biogeochemical processes, so that correlations between a change in primary drivers and linked changes in biogeochemical cycles are inherently non-linear (Li, 2007; Conrad, 1996). As regression models neglect several variables, because datasets used for developing the model did not distinguish these variables, for instance, emission factors cannot in essence lead to significant reduction

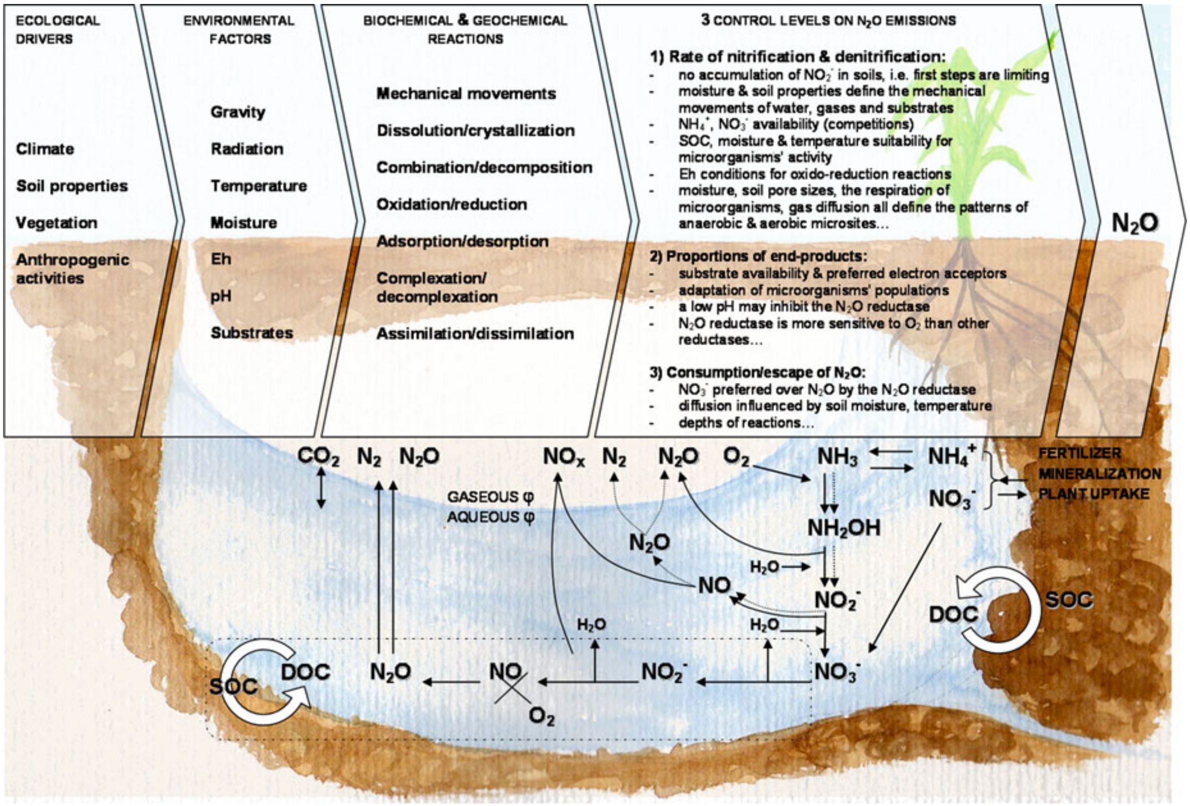

Fig. 15 Scheme of the determinism of N_2O emissions by soils adapted from Li (2007); Wrage et al. (2005); Farquharson and Baldock (2008). Soil particles, gaseous and aqueous phases (ϕ) are artificially "well distinguished". The two extremities of the symbolised pore represent the pore continuum throughout the soil matrix. Reactions take place at the interface of soil particles and aqueous phase where microorganisms and substrates are. Denitrification takes place in the "anaerobic dashed-line rectangle". Dashed arrows lead to N_2O emissions by nitrifier denitrification occurring in low O_2 conditions, which implies that some NH_3 oxiders process the whole reaction chain (Wrage et al., 2001)

of estimation uncertainty and cannot always be used to test different management or mitigation scenarios (Beheydt et al., 2007).

Process-based Models

Process-based models make it possible to assess emissions with more accuracy, because of a better accounting for all involved processes and local conditions. Numerous models can nowadays simulate cropping systems and the associated fluxes between the soil-plant-atmosphere compartments. By simulating plant uptake, biomass growth and residues, nitrate leaching or volatilisation, for instance, models can provide insight into the amounts of Nr that might be directly or indirectly emitted as N_2O. Specific sub-models then simulate the part of these amounts that is expected to be emitted as N_2O. Dynamic models for N_2O emission in relation to soil processes have been available for a dozen years and in more recent years developed for different ecosystems and N species (Sutton et al., 2007).

N cycling models can be classified following three approaches: (1) simplified empirical process models in which N cycling processes are assumed to be determined by easily measurable parameters; (2) microbial growth models, where N dynamics is simulated by explicitly representing the dynamics of involved microorganisms; and (3) soil structural models simulating physical processes such as diffusion into and out of soil aggregates where occurring anoxia leads to denitrification (Parton et al., 1996). Heinen (2006a) compared some 50 process-based denitrification models. Most simplified models are comparably based on a potential denitrification (D_p) weighted by a product of reduction functions due to nitrate content, degree of saturation, soil temperature and soil pH. The potential denitrification represents the soil microorganisms' capacity to reduce nitrates under non-limiting conditions, i.e. depending on the soil organic carbon content and microorganism populations. It can be measured by reproducing these optimal conditions on intact soil cores, for instance (Hénault and Germon, 2000), or deduced from CO_2 measurements that show the microorganisms' activity.

There is no consensus on the diverse reduction functions that are empirical and were calibrated from site-specific studies. Hence a universal simplified denitrification model is unlikely to exist and a chosen simplified model can only be used provided that parameters are calibrated for each location, with particular attention paid to determining the parameters of the saturation function, to which the model is the most sensitive (Heinen, 2006a).

$$\begin{aligned} D_{\mathrm{a}} &= D_{\mathrm{p}}\, f_{\mathrm{N}}\, f_{\mathrm{S}}\, f_{\mathrm{T}} \\ &= D_{\mathrm{p}} \underbrace{\frac{N}{K+N}}_{f_{\mathrm{N}}} \underbrace{\left(\frac{S-S_t}{S_m-S_t}\right)^{w}}_{f_{\mathrm{S}}} \underbrace{Q_{10}^{(T-T_r)/10}}_{f_{\mathrm{T}}} \end{aligned} \qquad (2)$$

D_{a} is the actual denitrification rate (mg N $\mathrm{kg^{-1}\ d^{-1}}$ or kg N $\mathrm{ha^{-1}\ d^{-1}}$),
D_{p} is the potential denitrification rate (mg N $\mathrm{kg^{-1}\ d^{-1}}$ or kg N $\mathrm{ha^{-1}\ d^{-1}}$),
f_{N} is a dimensionless reduction function for N, N is the nitrate content (mg N $\mathrm{kg^{-1}}$ or $\mathrm{L^{-1}}$), K is the nitrate content where $f_{\mathrm{N}} = 0.5$, f_{S} is the dimensionless reduction function for the dimensionless degree of saturation S, S_{m} is S above which $f_{\mathrm{S}} = 1$ (in the remainder of this paper $S_{\mathrm{m}} = 1$), St is a threshold value for S below which no denitrification occurs ($f_{\mathrm{S}} = D_{\mathrm{a}} = 0$), f_{T} is a dimensionless reduction function for soil temperature T, T_{r} is a reference T where D_{p} is determined at (mostly) $T_{\mathrm{r}} = 20°\mathrm{C}$, and Q_{10} is an increase factor for a 10°C increase in T. (Heinen, 2006b).

Testing a common simplified model (Eq. (2)) with eight Dutch data sets, the latter author showed that parameters differed across location and that no aggregation could be done based on soil type. The optimisation could not result in perfect prediction on the point scale, and was only good for cumulative denitrification for sand and loam soils, as under- and overestimations seemed to counteract in the long term (Heinen, 2006b). The model is very sensitive to errors in the estimates of the parameters. These errors (computed for 250 soil conditions and 25 parameter conditions) propagate in the prediction of denitrification, so that defining parameters with 10% accuracy would lead to a coefficient of variation in the relative denitrification rate of about 10% (Heinen, 2006a). A test on error propagation on parameter estimation with an artificial data set showed that w and Q_{10} were overestimated, while S_t and K were underestimated, with large coefficients of variation; the greatest uncertainty was found for K (Heinen, 2006b).

The NOE model (Hénault et al., 2005) uses similar equations to equation (2) to simulate denitrification and nitrification with Michaelis-Menten functions of nitrate and ammonium, respectively, two different saturation functions, and a common temperature function. The nitrification potential is not introduced explicitly. The NGAS model (Parton et al., 1996, 2001) is also an empirical denitrification and nitrification model that was developed using laboratory- and field-observed gas fluxes from different soils. N_2O fluxes are simulated using simple relationships controlled by soil saturation, texture, temperature, pH, respiration, and NO_3^- and NH_4^+ contents. Comparing these two sub-models, both coupled within the CERES crop model, NGAS appeared to be easier to operate as no site-specific data are needed; however, it was therefore also less accurate than NOE (Gabrielle et al., 2006).

Another comparison of four models simulating N cycling, CENTURY-NGAS (Del Grosso et al., 2001), DNDC (Li et al., 1992; Li, 2000), Nexpert (Engel and Priesack, 1993) and NASA-CASA (Potter et al., 1997), also showed discrepancies amongst results. Although all four models generally agreed on global N cycling rates, they presented a wide range of results concerning the different gas fluxes for both cumulated totals and temporal patterns; even when models agreed on N_2O emissions, then N_2, NO_x or NH_3 fluxes diverged (Frolking et al., 1998). An accurate modelling of soil moisture dynamics and the response of modelled denitrification to soil moisture appeared to be a key for reliable N_2O emissions (Frolking et al., 1998). Site-specific NOE parameters thus explained the performances better than NGAS. Nevertheless, site-specific parameters are not easily available, and pedotransfer functions would be needed to infer these parameters from basic soil characteristics (Gabrielle et al., 2006).

Up-scaling empirical models may come at the expense of prediction accuracy, whereas mechanistic models rely more on deterministic relationships based on fundamental knowledge on the interaction of predictor variables and modelled processes, which does not depend on the site or the study scale. Although mechanistic model structure is defined from the process knowledge, numerical fitting is also often used to parameterise such models, adding elements of empiricism (Farquharson and Baldock, 2008). DNDC is a microbial growth model that has been widely used to simulate N_2O emissions from diverse ecosystems; it has even been upgraded to deal with peak N_2O production due to freezing and thawing events (Pattey et al., 2007). This model tracks microbial activities in soils by computing the Nerst and Michaelis-Menten equations that describe interactions between microbial activities and the driving factors: soil redox potential, soil organic carbon and electron acceptors. At each time step, the concentration of oxygen and other electron acceptors determines the soil Eh and the consequent anaerobic volumetric fraction into which substrates are proportionally allocated. This defines conditions for microbial activities and the following substrate consumption that ends up with a change in soil Eh looping to the next time step (Li, 2007). Extensive testing of DNDC versus field measurements has demonstrated its ability to simulate N_2O and NO emissions but still with some significant uncertainty, which is at least partly due to the still limited knowledge about the ecosystem processes involved in C and N cycling (Sutton et al., 2007). This is confirmed by a test of DNDC with 22 long-term N_2O measurements. When N_2O simulations were in agreement with field measurements, the patterns of NH_4^+ and/or NO_3^- were not captured by the model or vice versa. In general, DNDC gave higher and more frequent N_2O peaks, leading to an average overestimation taking all measurements into consideration, and both (large) under- and overestimations when looking at individual results. Although statistics indicated that simulations were not optimal, the general agreement between simulated and measured N_2O total losses was better than with the three tested regression models, including Bouwman's emission factor (1.25% ± 1) used in the IPCC Tier 1 (1996). Improvement of the model would be necessary to use easily available data such as NO_3^- as a response variable and test mitigation scenarios, without having to measure N_2O to validate the model (Beheydt et al., 2007) Moreover, there are still recognised factors for modelling N_2O emissions that are not adequately understood (Farquharson and Baldock, 2008).

More precision on N_2O emissions is essential for compliance with the precise mandatory reduction targets. How to consistently reach a 10 or 20% greenhouse gas reduction target when emission estimate uncertainty is larger than 10 or 20%? Within the framework of local LCAs, the simulation of N_2O emissions with a process-based model may drastically reduce the uncertainty compared with emission factors, provided that the model performs correctly on the local scale, i.e. that parameters are well calibrated for the cultivation sites. Local LCAs might be the uniquely consistent scale to produce useful estimates of N_2O emissions, as long as the understanding of all controlling factors remains insufficient to improve process-based models, which would make it possible to produce robust estimates on various scales.

CO_2 Emissions and Land-use Change in Local LCA

Carbon may accumulate in soils, mainly in organic form. The removal of atmospheric C by plants and storage of fixed C in stable fractions of soil organic C (SOC) is termed "soil C sequestration" (Lal, 2004a). Hence, SOC comes from dead plant parts (leaves, roots, etc.), plant rhizodeposition and organic matter applications (animal waste, etc.). However, its storage is not definitive because dead organic matter undergoes a series of biogeochemical transformations, including decomposition, and is eventually mineralised by microorganisms and released as CO_2 or CH_4, through respiration or fermentation, respectively (Arrouays et al., 2002). Carbon stock in soil is the result of a dynamic balance between "inputs" of organic matter and "outputs" due to mineralisation, erosion, leaching or combustion. The soil may act either as a carbon source or as a carbon sink, according to the ratio between inputs and outputs. Carbon inputs depend on primary production (controlled by edaphic factors such as solar radiation, temperature, water and nutrient availability), and organic matter returned to the soils (e.g. crop residue management). Carbon outputs depend on biotransformation rates, controlled by the organic matter composition and local physico-chemical conditions (temperature, moisture, oxygen, etc.). Biotransformation is also slowed down when organic matter is associated with mineral particles (particularly clay) which provide "physical protection" against the activity of microorganisms (Balesdent et al., 2000).

Impact of Land-use Change on Soil Organic Carbon

On the global scale, the SOC pool is about 1550 GtC (+950 GtC inorganic carbon), exceeding by far the atmospheric (760 GtC) and biotic pools (560 GtC) (Lal, 2004a,b). Comparatively, geologic and oceanic pools represent 5000 GtC and 38 000 GtC, respectively (Lal, 2004a). Over the last 200 years or so, soils may have lost between 55 and 78 GtC because of land-use conversion and soil cultivation. On a global scale, the SOC pool to 1-metre depth has a predominant range of 50 to 150 tC ha^{-1}, but can reach 800 tC ha^{-1} in organic soils (Lal, 2004b). The SOC pool varies widely among ecological regions, being higher in cool and moist than warm and dry regions, and among ecosystems, SOC pools to 1-metre depth average for croplands 80–103 tC ha^{-1}, temperate grasslands 141–236 tC ha^{-1}, tropical, temperate and boreal forests, 122, 96–147, and 247–344 tC ha^{-1}, respectively (Lal, 2004a). SOC stocks are higher in cool temperate forests and wetlands where plant productivity is relatively high but the activity of soil microorganisms is slowed down by the temperature, whereas SOC stocks are lower in the wet tropics where organic matter turnover is rapid, and lowest in dry regions where plant growth is limited (Cowie et al., 2006).

Arrouays et al. (2001) provided estimates of average SOC stocks in France (to a depth of 30 cm), according to land use. Arable lands are characterised by relatively low stocks: 43 tC ha^{-1} on average, whereas permanent grasslands and forests (excluding litter) exhibit average stocks of nearly 70 tC ha^{-1}. These differences can be explained partly by a greater supply of carbon to the soil under grassland and forest (mainly from the roots but also from shoot litter), and partly by a shorter residence time of carbon under arable land (Soussana et al., 2004). Increased biodegradation rates in arable land may be due to multiple factors, e.g. changes in soil climate, nutrient availability and pH. A major factor would be the double impact of soil tillage that directly enhances mineralisation through increased oxygenation and de-protection of the organic matter by soil tillage (Balesdent et al., 2000; Germon et al., 2007). Indeed, a fraction of the organic matter included in micro-aggregates is physically protected from biodegradation, and inversely contributes to the aggregate cohesion through the binding of mineral particles by organic polymers and to the water stability of aggregates due to increased hydrophobicity (Chenu et al., 2000). Disruption of soil structure by tools and subsequent disruption of micro-aggregates by the action of rain expose the hitherto encapsulated C to biodegradation (Balesdent et al., 2000; Lal, 2004a). As organic matter is removed and dissolved from top soils, aggregates also become less stable, which could lead to synergetic losses of organic matter (Germon et al., 2007). A great part of carbon supply under grasslands is also due to larger root turnover and rhizodeposition than under arable crops. This process favours carbon storage because direct incorporation into the soil matrix leads to a higher stabilisation by physical protection and root litter is also chemically more stable (Soussana et al., 2004). In particular, grasses whose roots reach the deep part of the soil profile well below the plough layer make it possible to sequester C that is less prone to oxidation and loss (Fisher et al., 1994).

Kinetics of SOC accumulation or release following land-use change is non-linear and asymmetrical (Arrouays et al., 2002; Seguin et al., 2007). Variations are more rapid during the first years after land-use or land-management change and reach a plateau after several decades. The time taken to reach this new equilibrium (sink saturation) is highly variable, around 100 years in a temperate location, up to several centuries in boreal regions. Moreover, if the land-use change is reversed, the accumulated SOC will be lost, usually more rapidly than it was accumulated (Smith, 2004). This could be partly explained by the synergetic effect mentioned above. Arrouays et al. (2002) provided an estimation of mean carbon changes due to land-use change in France (Fig. 16). This estimate is based on the use of an exponential function, fitted with data available in the literature and French average soil C stocks at equilibrium for the main types of land use. According to this study, the mean carbon change implied each year over a 20-year period by converting forest to annual crop is about −0.75 tC ha^{-1}, and -0.95 ± 0.3 tC ha^{-1} by converting permanent pasture to annual crop. On the contrary, conversion of arable land to forest or grassland leads to a mean annual soil carbon storage of 0.45 ± 0.25 tC ha^{-1} for forest and 0.5 ± 0.25 tC ha^{-1} for grassland, over a 20-year period.

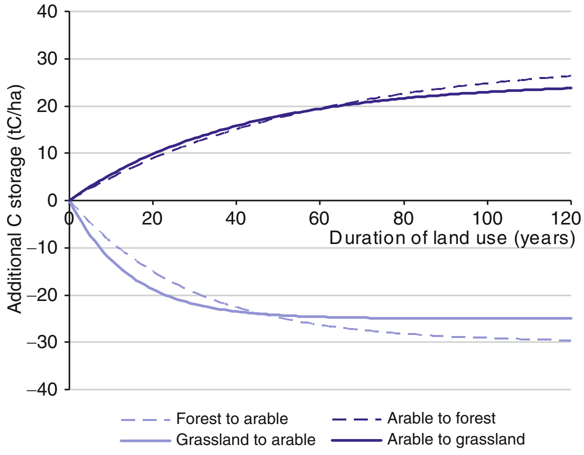

Fig. 16 Change in soil carbon stocks associated with land use change. These are modal values for mainland France. The 95% confidence interval of these values is about ± 40% (Arrouays et al., 2002)

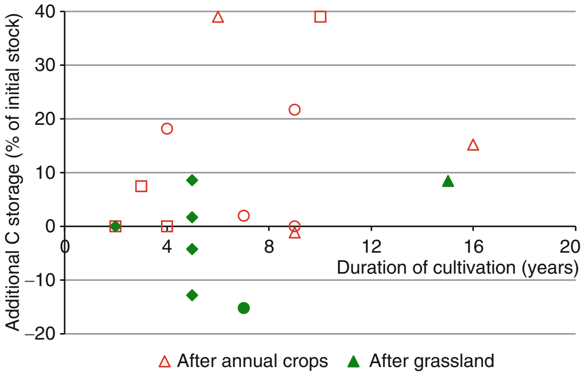

Fig. 17 Observed changes in soil carbon stocks associated with the introduction of perennial energy crops (SRC poplar or willow: circles, miscanthus: triangles and switchgrass: squares/diamonds) after annual crops or grassland. Drawn from data in Garten and Wullschleger, 1999; Jug et al., 1999; Ma et al., 2000a,b; Kahle et al., 2001; Zan et al., 2001; Frank et al., 2004; Hansen et al., 2004; Clifton-Brown et al., 2007

The variability of estimates is due mainly to the diversity in climatic conditions and soil characteristics (Seguin et al., 2007; Soussana et al., 2004). In a meta-analysis of 74 international publications, Guo and Gifford (2002) confirmed the high impact on SOC of grassland or forest conversion to cropland. According to this study, soil carbon stocks decrease on average by 42% after conversion of forest to crop and 59% after conversion of grassland to crop.

Introduction of perennial energy crops in current annual crop systems may increase carbon sequestration, due to the lack of soil tillage during the crops' growing cycle (typically 15–20 years), their high biomass production and pre-harvest losses, and their extensive root system (Lemus and Lal, 2005). In a field experiment in southern Quebec, Zan et al. (2001) measured a total root carbon content 4 to 5 times greater for 3-year-old SRC willow and switchgrass than for corn. High below-ground biomass (rhizomes and roots) was also measured for miscanthus. Below-ground biomass ranged, for example, from 15 to 25 tDM ha^{-1} for 4–9-year-old miscanthus in Germany, corresponding to 7.6—10.2 tC ha^{-1} (Kahle et al., 2001). It is thus expected that, as observed under grassland, root turnover and rhizodeposition should be a major carbon input under perennial energy crops. Perennial energy crops are usually harvested in late winter or early spring with high dry matter content. This practice causes pre-harvest losses, mainly by leaf senescence. Mean pre-harvest losses during 3 years of miscanthus cultivation were 4.5 tDM ha^{-1} yr^{-1}, corresponding to about 2 tC ha^{-1} yr^{-1}, in the same field experiment in Germany (Kahle et al., 2001). Several authors have evaluated impacts of perennial energy crops (short rotation coppice (SRC), miscanthus, switchgrass) on SOC, using field measurements in long-term experiments (Fig. 17).

The results of these experiments are highly heterogeneous, which is probably due partly to the diversity of climatic, pedological and agricultural conditions, and partly to differences in measurement methodology (e.g. soil sampling depth). However, in general terms, conversion of annual crops to perennial energy crops seems to increase carbon sequestration, which may not be the case when perennial energy crops are introduced after grassland. Also, there is no clear difference between perennial energy crops (short rotation coppice, miscanthus, switchgrass).

Land clearing (i.e. conversion of forest or grassland to arable crops) can lead to a large release of CO_2. To evaluate this effect, it is necessary to take into account the different carbon pools, i.e. not only the soil carbon pool but also the above-ground and below-ground biomass carbon pools. According to Fargione et al. (2008), the amount of CO_2 released during the 50 years following land conversion would be 737 tCO_2 ha^{-1} in the case of a tropical forest converted to soybean in Brazil, and 134 tCO_2 ha^{-1} in the case of natural grassland converted to corn in the US. It represents a "carbon debt" that should be included in LCA when biofuels are introduced after land clearing. The additional carbon sequestration by perennial energy crops compared with annual crops should also be taken into account in LCA. However, it is important to keep in mind that any carbon sequestration in soil is finite and reversible (Powlson et al., 2005).

Impact of Agricultural Practices on Soil Organic Carbon

Agricultural practices can modify SOC levels in arable lands, by changing the amount of carbon supply to the soil or by changing the residence time of carbon in the soil. Effects of soil management practices on carbon sequestration have been widely studied. No-tillage generally implies an increase in soil carbon levels, compared with tillage (see review by Arrouays et al., 2002; Germon et al., 2007). The mean increase in carbon sequestration due to no-tillage or reduced tillage was estimated at 0.2 ± 0.13 tC ha^{-1} yr^{-1}, over a 20-year period. However, there is no consensus about the magnitude of the differences between conventional tillage and no-tillage: some authors reported negligible differences; others found considerable differences (Arrouays et al., 2002). Origins of this variability are not well known.

According to Balesdent et al. (2000) comparing carbon stocks between tillage and no-tillage treatments causes some difficulties, due to changes in bulk densities and carbon repartition along the soil profile. Furthermore, no-tillage may refer to different degrees of "conservation tillage", such as direct sowing or non-inverting ploughing. Conservation tillage also encompasses intermediary techniques referred to as "reduced tillage", etc. Due to the complexity of soil organic matter (SOM) dynamics (Fig. 18), the varying depths and degrees of soil disturbance and the varying duration of the treatments across studies can also imply discrepancies amongst conclusions. The impact of conservation tillage on CO_2 emissions also varies amongst studies.

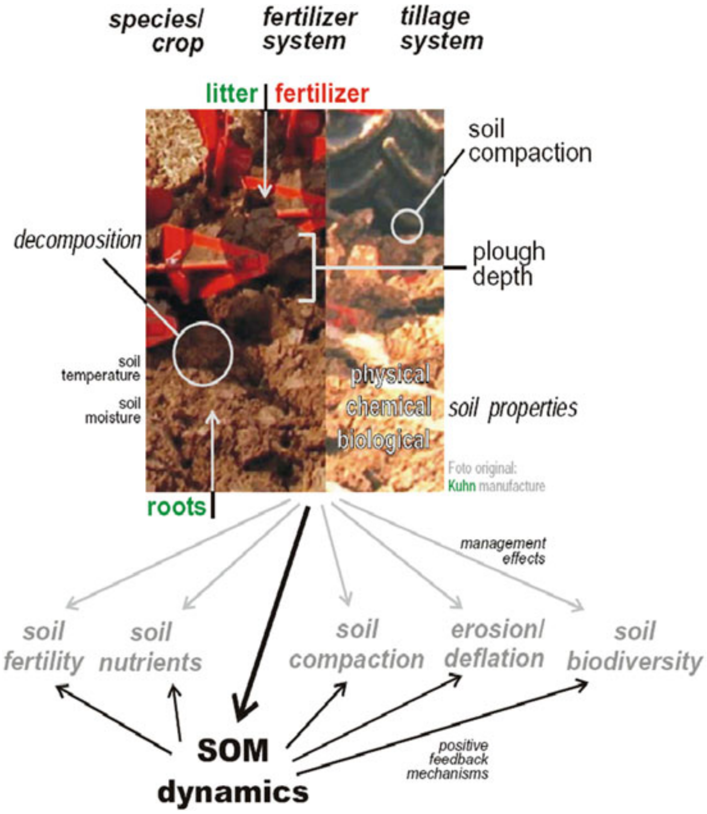

Fig. 18 Soil organic matter is the key indicator in sustainable soil management (in Van Camp et al., 2004)

Although CO_2 measurements directly after soil tillage mostly showed higher emissions than under direct sowing, impacts in the long term are less clear. CO_2 long-term measurements under conservation tillage are still lacking and little is known about CO_2 emissions when the new SOC equilibrium is reached under conservation tillage (Germon et al., 2007).

The use of catch crops over intercropping periods can represent an interesting option in terms of carbon sequestration. Arrouays et al. (2002) modelled a potential increase of 0.15 ± 0.08 tC ha^{-1} yr^{-1} over a 20-year period, for an annual incorporation of catch crops. In a field experiment with spring barley in Askov (Denmark), the mean difference in SOC between no catch crop and catch crop treatments was 1 tC ha^{-1} after 10 years[49], corresponding to an annual increase of 0.1 tC ha^{-1} yr^{-1} due to annual catch crop incorporation in soil (Thomsen and Christensen, 2004). Crop residue management can also impact soil organic carbon. Saffih-Hdadi and Mary (2008) compiled nine well-documented long-term field experiments, which compare effects of systematic removal or incorporation of cereal straws on SOC evolution. They differed in climate, soil type, carbon input and duration (from 12 to 35 years). The measured SOC increase due to straw return (as compared with straw removal) varied from 0.078 tC ha^{-1} yr^{-1} to 0.385 tC ha^{-1} yr^{-1}, corresponding to 4.2–19.1% of added straw carbon. Climate influenced the efficiency of straw incorporation in SOC. This incorporation is much more efficient under cold climates, where it can reach up to 0.90% of the initial SOC content compared with 0.53% under warm climates. Systematic removal of straw for bioenergy purposes will then lead to a decrease in SOC content. Using a simple carbon dynamics model called AMG, Saffih-Hdadi and Mary (2008) simulated the impact of straw removal one year out of two in nine experimental sites. After 50 years, it would reduce carbon stocks by 2.5–10.9% of the initial SOC, depending principally on the experiment (soil, climate, productivity).

As SOC sequestration is provisional, it can only play a minor role in climate change mitigation. The maximum global SOC sequestration potential of 0.9 ± 0.3 GtC yr^{-1} over 50 years (Lal, 2004a) could contribute to a maximum of 2–5% towards reducing the carbon emission gap under the highest emission scenarios (Smith, 2004). However, given the drastic CO_2 reduction needs to meet targets, it is already crucial that agricultural practices should aim to prevent carbon losses as much as possible, notably net CO_2 emissions due to land-use change, then to implement practices that enhance SOC sequestration. A better understanding of the SOC stabilisation in deeper soil layers could also open up new options in order to increase C sequestration.

Land-use Impacts in LCA

"Land-use impacts are the 'amount' of land quality not present in a certain area due to the studied system, compared to a situation where the studied system had not been established" (Milà I Canals et al., 2007).

The major environmental importance of land-use impacts contrasts with the lack of consensus on this area within the field of LCA. As a result, the issue is seldom included in LCA and the credibility of LCA results is insufficient for many stakeholders. Lack of consensus comes at least partly from the failure to recognise the value judgements behind the assessment methodology. These value judgements include the following: what are the functions of land that need protection, which are the thresholds? What are the time perspective and reversible impacts? What are the future or alternative land uses? Which indicators represent the impact pathways? (Milà I Canals et al., 2007). Focusing on bioenergy chain LCAs, many studies have lately put emphasis on the necessity to develop a methodology within the LCA tool to take into account the impacts of land-use change on the ecological functions of land. Among others, the impact of deforestation on biodiversity and CO_2 emissions, the impact of straw removal on soil fertility, the impact of perennial crops for future land uses, etc., are examples that show the importance of such factors as part of sustainability criteria.

Nevertheless, potential impacts of land-use change are difficult to assess for mainly two reasons. First, impacts due to land-use change have to be characterised in comparison with unchanged land use. When considering agricultural land use, in principle only degradation caused by the management practice during the cultivation period should be allocated to the crop harvested (Mattsson et al., 2000). This implies defining a reference scenario and a time frame for

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[49] Mean value of the 4 straw restitution treatments (Thomsen and Christensen, 2004).

the occupation of land or recovery period. But one reference scenario is sometimes not sufficient to cover the range of possibilities when it comes to dealing with the use of new land areas or longer time frames such as in the cases of crop rotations or the cultivation of perennial crops. Second, LCA methodology based on equivalency factors is hardly adaptable for land-use change. Indeed, aggregation of parameters such as soil organic matter and landscape values, for example, is difficult so that the land-use impact category should be less aggregated than other impact categories in LCA (Mattsson et al., 2000), leading to the complexity of dealing with two approaches and a resulting mix of quantitative and qualitative information. Other approaches and tools, such as Environmental Impact Assessment, may provide more detailed information than LCA on effects of different land managements. "However, LCA is the appropriate tool to bring a life cycle perspective to support complex decisions involving different land uses, and, consequently, it should incorporate a measure of the different impact pathways affected by land use" (Milà I Canals et al., 2007).

Many references focus on suggesting indicators to include the effects of land use on productivity and biodiversity, although the practical implementation of such sets of indicators is seldom checked with a consistent framework (Milà I Canals et al., 2007). Most proposed methodologies use a number of indicators that are largely submitted by the availability of data (Anton et al., 2007). Mattsson et al. proposed to divide "land-use change" into three sub-categories: (1) soil fertility, with a set of 7 indicators, (2) biodiversity, and (3) landscape values Mattsson et al. (2000). Schenck and Vickerman (2001) also gave a list of indicators for the assessment of impacts on biodiversity. Impacts on biodiversity, for instance, are currently considered in LCA through damage to the biotic environment, or concurrence of species (Jolliet et al., 2004); the effects considered have been traditionally limited to those caused by changes in the chemical composition of the environment (toxicity, eutrophication, etc.) (Milà I Canals et al., 2007). The World Resource Institute[50] showed that the greatest biodiversity losses are derived from changes in land use, rather than to any chemical impacts (Schenck et al., 2001). Some of the latest methods for LCA thoroughly address land-use impacts, but fail to include effects of occupation or transformation on the resource aspect of land (Milà I Canals et al., 2007). Despite the availability of indicators, there is still a lack of consensus on which is the most ideal indicator for evaluation (Anton et al., 2007). Comparing indicators from Köllner (2001) and Weidema and Lindeijer (2001), it appears that further research is still needed to refine them so that they could deal with more specific ecosystems and geographical areas (in Anton et al., 2007).

CO_2 emissions due to biofuel combustion are commonly not included within the system boundary since the fuel is considered as carbon-neutral; indeed, the released carbon during combustion (CO_2) had been fixed from the atmosphere in the first place. This exclusion is hence justified when comparing a biofuel chain starting from the biomass production with a fossil fuel chain, because the actual carbon cycle is fully considered. However, when the delay between carbon capture and sequestration by the plants and re-emission is longer (land-use change, waste treatment, woody biomass, etc.) this assumption may lead to wrong conclusions. In this sense, Rabl et al. (2007) recommended that emission and removal of CO_2 ought to be counted explicitly at each stage of the life cycle. In this way, the LCA is furthermore consistent with the "polluter pays" principle, which implies that each greenhouse gas contribution should be allocated to the causing agent. For example, CO_2 from woodfuel for heating should be taxed as CO_2 from oil heating is, and a credit for CO_2 removal then only paid when and where the wood is replaced by new growth (Rabl et al., 2007). Furthermore, this stage-CO_2 accounting would be useful in the framework of an implementation of sustainability criteria for biofuel chains at an international level, including the accounting of a carbon debt or credit in the case of a change in SOC due to land-use or land-management change.

Beyond uncertainties linked to estimates of carbon stock and its changes due to land-use or management changes, the main difficulties arise from the definition of prospective land-use scenarios for comparative LCA. Areas impacted by land-use or management changes can be part of a crop rotation or more complex combined land-use patterns including indirect impacts through crop displacements. Soya in Brazil, for instance, is established on grasslands, pushing cattle

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[50] WRI: Guide to World Resources 2000–2001: People and Ecosystems: The Fraying Web of Life, Elsevier, New York, 2002.

pastures further into forests[51]. Therefore, dependence between impacted areas must be considered within a matrix of land-use changes across a sphere of influence that must be defined. This is particularly relevant in the case of SOC storage in order to take into account the fact that dynamics are long-lasting and often reflect transition states from past changes, and that kinetics are reversible and asymmetric. Methods to estimate the impacts must be adapted to these temporal scales, otherwise results could be biased by the approximation of impact differences between two instantaneous pictures of land-use patterns (Arrouays et al., 2002). A better accounting of the impact of land-use change on the soil quality and direct CO_2 emissions is necessary for biofuel LCA. Emphasis should be put on defining a harmonised methodology to include some indicators on soil quality. However, accurate estimates may be limited to local LCA given the complexity to encompass the temporal and spatial dimensions of the impact of land-use changes and the data needs. In order to simulate soil C change in bioenergy projects, it would be recommended to establish the baseline labile and recalcitrant C stocks through measurements and to model C dynamics over the land-use duration (Cowie et al., 2006). Furthermore, more research would be necessary to also introduce within LCA quantified impacts of land-use change on the albedo, surface energy balance and water cycle, and their consequences on climate change. Indeed, agriculture significantly affects climate through greenhouse gas emission and absorption, and modifications of surface properties, which act directly on different spatial scales. To date, however, the complete evaluation of the net impact of agriculture on climate through the modification of the natural environment is still not feasible (Seguin et al., 2007).

5.3 Biofuel Greenhouse Gas Balances

Most studies have found that the use of 1st generation biofuels results in emission reductions of 20 to 60% of CO_{2eq} relative to fossil fuels. Expected reductions for future commercialised 2nd generation biofuels are in the range of 70 to 90% of CO_{2eq} relative to fossil fuels (FAO, 2008a). The large range of emission reductions for the 1st generation biofuels is due to various types of feedstock and conversion processes, and to the different sites of production and consumption. Varying LCA assumptions also explain that greenhouse gas balances of a given biofuel chain in one region may be variable (see part 5.1). Finally, field emissions in particular are complex to assess and imply further disparities amongst studies (see part 5.2). Greenhouse gas savings are therefore often presented as ranges; it does not make much sense to give a list of mean values for each biofuel chain. However, Table 4 presents some of the main published studies to put in contrast varying results due to changes in co-product handling, within a study and amongst studies. This overview completes the data in Fig. 11 and Table 2.

A sensitive analysis of the N_2O emission factors showed that these assumptions critically impact the balance. Greenhouse gas emissions rise from 40 to 50% for methyl esters and pure vegetable oils by using Bouwman's IPCC (1995 Guidelines) emission factors instead of those from Skiba et al. (1996). The resulting greenhouse gas savings fall, for instance, to −55.5% and −66% for the rapeseed pure oil and methyl ester, respectively. Although Bouwman's factors may be more accurate because the regression was based on more data sets on a wider range of soil diversity, whereas Skiba's factors were established for the UK's soils, extrapolation from any linear model implies a high uncertainty on the results due to the site- and time-dependence of field emissions. N_2O emissions in the JRC/EUCAR/CONCAWE study are likely to be more accurate as they were simulated with the DNDC model (version 82N) combined with the LUCAS land-cover survey model. The resulting emission factors, moreover, include N_2O indirect emissions from leached N. However, as the study assessed biofuel chains at the European level, the simulations were used to determine new emission factors through regression models. The averaged crop emission factors finally hardly give an approximate of total N_2O emissions at a national level, while emissions are too variable on such a scale to help distinguish between biofuel chains and co-product options at the local level. Nevertheless, biofuel chains with a valued co-product make it possible to save a lot of greenhouse gases, especially if biomass production is optimised to reduce field emissions as much as possible.

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[51] Dr. Emily Boyd, 25/11/2005, http://www.scidev.net/en/opinions/emissions-trading-cannot-solve-amazon-deforestatio.html.

Table 4 Biofuel chains and greenhouse gas savings. Ethanol is compared with gasoline, and biodiesel with fossil diesel. The results are given as they were published and without any harmonisation in background assumptions, except for units, e.g. emissions for the fossil fuels per MJ vary across studies

			Co-product handling				
Biofuels	Regions	Feedstock	Mass allocation (% applied to biofuels)	System expansion (substitutes)	Energy allocation (% applied to biofuels)	GHG/MJ compared with fossil fuels	References
Ethanol	Brazil + shipped to EU	Sugar cane		–		−66%	JRC/EUCAR/ CONCAWE, 2008*
				Excess bagasse for heat (diesel)		−81.6%	
	France	Wheat	DDGS (43) Straw 14% (96)	Straw 86% (IF)		−60%	ADEME/DIREM, 2002
		Sugar beet	Sugar, pulps (80)	Crop residues, molasses, slop (IF)		−52%	
	EU	Wheat		DDGS as animal feed (feed wheat and soya meal) + straw (IF)		−14.3%	JRC/EUCAR/ CONCAWE, 2008
				DDGS as fuel + straw (IF)		−28.6%	
				DDGS as feed (idem) + straw CHP		−62.7%	
				DDGS as fuel + straw CHP		−77%	
				DDGS to biogas (fuel)		−60%	
		Sugar beet		Pulps to animal feed (soya meal)		−47%	
				Pulps to animal feed (idem) + slops to biogas (fuel)		−65%	
				Pulps + slops to biogas/heat (idem)		−80.5%	

(continued)

Table 4 (continued)

Biofuels	Regions	Feedstock	Co-product handling Mass allocation (% applied to biofuels)	System expansion (substitutes)	Energy allocation (% applied to biofuels)	GHG/MJ compared with fossil fuels	References
	USA	Maize		DDGS (soybean meal, corn for cattle feed) Corn gluten, meal and feed, corn oil (whole corn, nitrogen-in-urea, soy oil)	15% of net energy allocated to fossil fuel co-products	−14%	Farrell et al., 2006
Biodiesel	France	Rapeseed RME	Meal (46), acid oils (97), glycerol (88)	Crop residues (IF)		−70%	ADEME/DIREM, 2002
		Sunflower SME	Meal (49), acid oils (97), glycerol (88)	Crop residues (IF)		−75%	
Biodiesel	EU	Rapeseed RME		Glycerine to chemicals (propylene glycol) + meal to animal feed (soya meal from imported soybeans)		−45.5%	JRC/EUCAR/ CONCAWE (2008)
				Glycerine and meal to animal feed (soya meal from imported soybeans)		−38.8%	
				Glycerine and cake to biogas (fuel)		−63%	

				Glycerine to chemicals (propylene glycol) + meal to animal feed (soya meal from imported soybeans)		−66%	
		Sunflower SME		Glycerine and meal to animal feed (soya meal from imported soybeans)		−59%	
				Glycerine and cake to biogas (fuel)		−80%	
Pure vegetable oil	France	Rapeseed	Meal (46), acid oils (97), glycerol (88)	Crop residues (IF)		−77.5%	ADEME/DIREM, 2002
		Sunflower	Meal (49), acid oils (97), glycerol (88)	Crop residues (IF)		−83%	
Cellulosic ethanol (pilots)	USA 2030	Maize stover			Electricity (79.6–91.2)	−86%	Wu et al., 2006
						−89%	
		Forest residues			Electricity + chemicals (91.45)	−85%	
	USA	Switchgrass		Electricity (local grid electricity)		−88%	Farrell et al., 2006

Notes: IF = industrial fertiliser

* Horizon 2020–2030 + N_2O emission factors (kgN-N_2O ha^{-1}): sunflower: 1.11; wheat: 2.23; sugar beet: 2.79; rapeseed: 3.12.

** Horizon 2005 + Skiba's N_2O emission factors (%kg N fertiliser ha^{-1}) sunflower: 0.8; wheat: 0.5; sugar beet: 1.60; rapeseed: 0.5. Greenhouse balances consider complete combustion of the fuels and zero CO_2 emissions from biofuels linked to this combustion: so-called credit for renewable combustion CO_2.

5.3.1 Prospects for Reducing Greenhouse Gas Emissions from Biomass Production

Agriculture greenhouse gas emissions increased by 10% between 1990 and 2000 (Stern, 2006); CH_4 and N_2O emissions alone increased by 17% between 1990 and 2005; 88% of these emissions are explained by three sources: biomass burning, enteric fermentation and soil N_2O emissions (Smith et al., 2007). Considering the increase in demand for agricultural feedstock, global agricultural greenhouse gas emissions are expected to rise by almost 30% in the period to 2020, with almost two-thirds of this increase coming from Africa, Latin America and China, half of it due to the use of fertiliser on agricultural soils (Stern, 2006). World nitrogen fertiliser demand is forecast to increase at an annual rate of about 2.6% until 2012, East Europe and Asia contributing to 81.9% of this increase (FAO, 2008b). N_2O emissions alone are projected to increase by 35–60% by 2030 due to increased use of fertilisers and animal manure production (FAO, 2002).

Drastic savings in agricultural greenhouse gas emissions are needed, and agricultural practices are the key to significantly reducing agricultural greenhouse gas emissions. Options for mitigating agricultural greenhouse gas emissions fall into three categories based on the underlying mechanism: (1) reducing emissions, (2) enhancing removals from the atmosphere, and (3) avoiding (or displacing) emissions (Smith et al., 2008). The global technical mitigation potential[52], including all gases for the two first categories, by 2030 is estimated to be some 5.5–6 $GtCO_{2eq}\ yr^{-1}$ ([53]), mainly through reduction of CO_2 emissions[54] (89%). The economic potential would vary between 1.5 and 4.3 $GtCO_{2eq}\ yr^{-1}$ at carbon prices from 20 up to 100 $US\$.tCO_{2eq}^{-1}$, respectively. At the same carbon price levels, some more 0.6–16 $GtCO_{2eq}\ yr^{-1}$ could be avoided, by substituting fossil fuels with bioenergy generating electricity, i.e. the above-mentioned third category (Smith et al., 2008).

The uncertainty on the savings due to bioenergy is especially large because the net benefit in CO_2 reduction from fossil CO_2 displacement will depend on the greenhouse gas balance over the whole bioenergy life cycle, including direct emissions during the biomass production. Therefore, production systems of biomass for energy should necessarily contribute to the two first above-mentioned categories. Table 5 presents the main measures for mitigating greenhouse gas emissions from agroecosystems. It appears that the mitigative effects of these measures on N_2O emissions are mostly uncertain, due to the lack of knowledge related previously in this review. However, since N availability is the bottom line for N_2O emissions, management practices that will improve the fertilisation efficiency can help reduce N_2O emissions as detailed below. Other practices could further reduce the total agricultural greenhouse gases, but the trade-off between the different gases is still unclear.

5.3.2 Improving Fertilisation Efficiency

Direct field emissions can be reduced by improving fertilisation efficiency, i.e. combining reduced input and increased uptake and production. The nutrient balance expresses this difference between the total quantity of nutrient inputs entering an agricultural system, and the quantity of nutrient outputs leaving the system, in terms of kilograms of nutrient surplus (deficit) per hectare of agricultural land per year. Any surplus represents potential losses of nutrients into the environment and the correlated risk of polluting soil, water and air, whereas a deficit can reveal environmental pressures such as declining soil fertility (OECD, 2008).

Focusing on nitrogen balance, the situation is quite contrasted between OECD countries and developing countries where fertilisation inputs are much lower. Nitrogen balance is in surplus in all OECD countries, whereas in Sub-Saharan Africa notably it is in deficit, as in Kenya, Mali and Ghana, for instance (OECD, 2008; Roy et al., 2003). In two-thirds of OECD countries the nitrogen surpluses decreased between the 1990s and 2000s, whereas in a few countries such as Canada, New Zealand, Portugal and the USA it increased, notably due to the rise in fertiliser use and livestock numbers. The higher use of fertiliser is in part explained by the expansion in crop production together with a shift in cropping patterns to crops requiring

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[52] Mitigation potentials for CO_2 represent the net change in soil carbon pools which were derived from about 200 studies; the emission ranges for CH_4 and N_2O were derived using the DAYCENT and DNDC simulation models. All estimated potentials are followed by the mention *medium agreement, low evidence.*

[53] About 20% of 1990s global greenhouse gas emissions, or 5%, 9% and 14% for the three different economic potentials.

[54] Notably from SOC sequestration due to restoration of organic soils; 9% CH_4, 2% N_2O.

Table 5 A list of proposed measures (also referred to in literature as "Recommended Management Practices" RMPs) for mitigating GHG emissions from agricultural ecosystems, their apparent effects on reducing emissions of individual gases (mitigative effect) and an estimate of scientific confidence that the proposed practice can reduce overall net emissions (Smith et al., 2008)

		Mitigative effects[a]			Net mitigation[b] (confidence)	
Measure	Examples	CO_2	CH_4	N_2O	Agreement	Evidence
Cropland management	Agronomy	+		±	***	**
	Nutrient management	+		+	***	**
	Tillage/residue management	+		±	**	**
	Water management (irrigation, drainage)	±		+	*	*
	Rice management		+	±	**	**
	Agroforestry	+		±	***	*
	Set aside, land-use change (LUC)	+	+	+	***	***
Grazing land management/pasture improvement	Grazing intensity	±		±	*	*
	Increased productivity (e.g. fertilisation)	+		±	**	*
	Nutrient management	+		±	**	**
	Fire management	+		±	*	*
	Species introduction (including legumes)	+		±	*	**
Management of organic soils restoration of degraded lands	Avoid drainage of wetlands	+	−	±	**	**
	Erosion control, organic amendments, nutrient amendments	+		±	***	**
Livestock management	Improved feeding practices		+		***	***
	Specific agents and dietary additives		+		**	***
	Longer term structural and management changes and animal breeding		+		**	*
Manure/biosolid management	Improved storage and handling		+	±	***	**
	anaerobic digestion		+	±	***	*
	More efficient use as nutrient source	+		+	***	**
Bioenergy	Energy crops, solid, liquid, biogas, residues	+		±	***	**

[a] '+' denotes reduced emissions or enhanced removal (positive mitigative effect); '–' denotes increased emissions or suppressed removal (negative mitigative effect); '±' denotes uncertain or variable response.

[b] A qualitative estimate of the confidence in describing the proposed practice as a measure for reducing *net* emissions of GHGs, expressed as CO_2 equivalence. 'Agreement' refers to the relative degree of agreement or consensus in the literature (the more asterisks, the higher the agreement); 'Evidence' refers to the relative amount of data in support of the proposed effect (the more asterisks, the greater the amount of evidence).

higher inputs per kg of output, such as from wheat to maize in Australia and the United States. Some countries, whose surpluses diminished, still have amongst the highest surpluses. This is the case for Korea, Japan, Belgium, Denmark and the Netherlands, for instance, which have rather low nitrogen efficiency[55], between 30 and 50%, below the averages in the OECD and the EU-15, respectively, 55%–60% (OECD, 2008). Reduced nitrogen surpluses were notably correlated with the adoption of "nutrient management and environmental farm plans", and the improvement of N-use efficiency linked to reduced inorganic fertiliser input per unit of crop output, a closed storage system, and an optimisation of the timing and spreading of manure (OECD, 2008). Indeed, nitrogen efficiency can be increased by optimising the crop's natural ability to compete with processes whereby plant available N is lost, i.e. by matching the N supply with crop demand better [e.g. optimised split application schemes and doses, foliar application, application during stem elongation or later (Recous, 2001)] and adapt it to specific risks; e.g. avoiding nitrate application in case of leaching risk (Recous, 2001), applying fertiliser below the soil surface in case of possible volatilisation, choosing to apply nitrates when nitrification is more likely to happen, or ammonium-based fertilisers when it is denitrification; for instance, during seasonal precipitations (Mosier, 1996).

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[55] Nitrogen efficiency measured as the percentage ratio of total nitrogen uptake by plants and forage (tonnes) over the total nitrogen available from fertiliser, livestock manure and other nitrogen inputs (tonnes).

Integrated fertilisation management, including the introduction of catch crops or legumes in the crop rotation to uptake or fix nitrogen, respectively, and the incorporation of crop residues or manure spreading will also influence N_2O emissions. Indeed, fertilisation efficiency strategies must be developed considering both the cropping and intercropping cycles. Whereas high N inputs may be well correlated with high N_2O emissions during the vegetation period, over the year unfertilised plots can also emit high quantities of N_2O, notably depending on the amounts and the C/N ratio of crop residues in the soil (Kaiser et al., 1998). Organic amendments can influence N_2O emissions in three ways: (1) the amount and recalcitrance of the N supply, (2) those of the C supply, and (3) local increases in the oxygen consumption (Velthof et al., 2002). Through the crop residue C/N ratio, it is possible to influence the nitrogen mineralisation-immobilisation turnover[56] by microorganisms (Recous, 2001; Velthof et al., 2002), which determines the evolvement of the soil N pool, including the competition for the N substrates between plants and microorganisms, and potential N_2O emissions. A narrow C/N ratio and high contents of easily mineralisable N[57] in crop residues would favour N_2O emissions (Velthof et al., 2002). While organic amendments with high N content may accentuate N_2O, NH_3 and CH_4 emissions, they may also contribute to the rise in soil organic carbon, especially in the form of stabilised manure and recycled organic compost, that contain a greater fraction of recalcitrant carbon than fresh green manure, i.e. fresh crop residues (Larsson et al., 1998; Lal, 2004c; Cowie et al., 2006). Moreover, there could be possible reductions of N_2O and NH_3 emissions in the field, depending on the soil type, through digestion of the fresh green manure and slurries before application (Oenema et al., 2005). The initial N content of composts may be more determining for leaching risk and fertilising value than amendments' stability but this latter could play an interesting role in optimising fertiliser application timing and crop N recovery (Gabrielle et al., 2005).

Although nitrogen efficiency is not identically defined across the literature, authors agree that it could and should be widely improved in future (Crutzen et al., 2008; OECD, 2008; Galloway et al., 2008). In 2002–2004, nitrogen efficiency reached 70–78%, for instance, in Italy and Greece, respectively (OECD, 2008). This issue is especially important as N-intensive biofuels could cancel out any CO_2 savings due to N_2O and NO_x emissions. As critical examples, US corn and Brazilian sugar cane production have low N efficiency; only 30% of N input ends up in sugar cane tissues (Galloway et al., 2008). An increase in the nitrogen efficiency from 40% to 60%, resulting in the assumption that 3 instead of 5% of N input would be lost as N_2O over the whole nitrogen cascade, makes the maize ethanol and rapeseed biodiesel become carbon-neutral and beneficial, respectively (Crutzen et al., 2008).

A more efficient use of fertiliser would lead to direct reduction of field emissions, while at the same time it would also imply reduction of upstream industrial emissions during the fertiliser production and spreading in the fields. It could lead to a decrease in industrial Nr creation of about 15 MtN per year, i.e. 8% of total Nr created in 2005. The same amount could also be saved through improved animal management strategies (Galloway et al., 2008).

5.3.3 Other Cultural Practices

Basically, recommended management practices (RMPs) aim to improve the agroecosystem productivity while maintaining or reducing the input levels. In general, the choice of resistant varieties and an optimal adaptation of crop rotations to site-specific conditions will make it possible to reduce the greenhouse gases by combining high yields and low inputs. Farming operations should be limited as far as possible, since all inputs also imply an environmental cost starting with fossil fuel CO_2. In some cases though, the final greenhouse gas benefits will depend on the balanced gain in CO_2 sequestration due to enhanced biomass productivity over the cost in CO_2 emitted by the operations of irrigation, drainage or tillage, etc. and in other greenhouse gases. Reducing fallow will, for instance, imply higher energy, but this cost appears to be globally offset by greater benefits (Grant et al., 2004; Lal, 2004a). While some energy inputs are unavoidable, improved energy efficiency in agriculture could deliver an additional 0.77 $GtCO_{2eq}\ yr^{-1}$ mitigation potential by 2030 (Smith et al., 2008).

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[56] Through mineralisation N is made available for the plants, through immobilisation/organisation N is consumed for the development of the microorganisms.

[57] Easily mineralisable N is usually more abundant in fresh green material than in straw (Velthof et al., 2002).

The opportunity to save energy input by reducing soil tillage is the major factor that has first fostered the change from conventional tillage to reduced/conservation tillage or no-tillage. In 1999, the worldwide area under no-tillage was approximately 50 Mha, representing 3.5% of total arable land (Smith et al., 2007). Conversion from conventional tillage to no-tillage can reduce emission of farming operations by 110 to 130 kg CO_2 ha^{-1} per season (Lal, 2004b). Since soil disturbance caused by soil tillage enhances SOC losses through decomposition and erosion (see part 5.2), reduced or no-tillage often also results in SOC gain besides fossil CO_2 savings (Robertson et al., 2000; Lal, 2004a; Seguin et al., 2007; Smith et al., 2008). Such practices are, however, frequently combined with periodical tillage, which reverses the SOC storage trend, thus making the assessment of the greenhouse gas uncertain (Smith et al., 2007). SOC sequestration through reduced soil tillage is an explicit illustration of greenhouse gas trade-offs, or hidden costs (Lal, 2004c) that are likely to obscure the real impact of a mitigation measure. Indeed, while reduced tillage may imply SOC sequestration and globally less CO_2 emissions, it adversely can lead to higher emissions of N_2O and CH_4, though not always (Robertson et al., 2000; Six et al., 2004; Lal, 2004b; Grant et al., 2004; Chatskikh and Olesen, 2007; Oorts et al., 2007). Enhanced CH_4 uptake in no-tillage systems has also been reported due to higher SOC stock and the presence of ecological niches for methanotrophic bacteria (Six et al., 2004; Lal, 2004b). No-tillage can increase N_2O and CH_4 fluxes because of higher bulk density and reduced porosity that diminish gas diffusion and increase water conservation at the surface, thereby increasing the likelihood of anaerobic conditions (Gregorich et al., 2006; Germon et al., 2007; Ball et al., 2008). Accumulation of organic matter and residue mulching at the surfaces of no-tilled fields can also favour N_2O emissions (Jørgensen et al., 1997; Ball et al., 2008). However, residue mulch can also limit N_2O emissions during freezing-thawing cycles by maintaining a warmer temperature that decreases the frequency or intensity of freezing events (Wagner-Riddle et al., 2007). Moreover, when N is a limiting factor, N_2O emissions may be enhanced by soil tillage due to an easier diffusion through the soil matrix without being further reduced (Gregorich et al., 2006; Chatskikh and Olesen, 2007).

The determinism of N_2O being especially complex, all changes related to the soil tillage system may influence the N_2O emissions. The tillage timing, as well as the cumulative effect of a tillage system in the long term, will also be determinant. Furthermore, dry-wet or freezing-thawing cycles can create cracks and enhance the sensitivity of compacted zones to fragmentation during tillage. Thus, seedbed preparation in spring will be more efficient in reducing the proportion of compacted zones, whereas seed bed preparation in autumn will only depend on the initial state (Boizard et al., 2002). Impacts of these weather cycles may also explain how the difference in N_2O fluxes between no-tillage and conventional tillage could change over time. In a review of 44 data sets, the higher N_2O fluxes trend in no-tillage systems compared with conventional tillage systems was reversed after 20 years in humid climates and fluxes became similar between tillage systems in dry climates (Six et al., 2004).

Finally, no-tillage could lead to other “hidden costs” due to possibly increasing use of herbicides and pesticides (Lal, 2004b) and decreasing yields (Chatskikh and Olesen, 2007). Indeed, tilling the soil just before sowing increases soil temperature and can favour germination (Richard and Cellier, 1998). Lower N uptake in no-tilled fields could result in higher gaseous losses, and differences between greenhouse gas balances of conventional tillage and no-tillage systems eventually further shrink (Chatskikh and Olesen, 2007).

Management practices that will reduce agricultural greenhouse gases can hardly satisfy all criteria, especially as determinisms for the diverse greenhouse gas emissions can be opposed, e.g. aerobic or anaerobic conditions that enhance CO_2 or N_2O production, respectively; draining rice paddy fields in order to reduce the emissions of CH_4 enhances N_2O emissions, etc. (Duxbury and Mosier, 1993). Moreover, trying to reduce N_2O emissions by preventing the optimum conditions from occurring could lead to compensating for N_2O emissions by decreasing the rate of reduction into N_2, while complete denitrification would be the less polluting pathway to close the N cycle (Galloway et al., 2003). As long as our understanding of all involved processes remains too incomplete, the best options to reduce the agricultural greenhouse gases are to improve its overall efficiency and to reduce all inputs, especially fertilisers. Perennials are therefore particularly interesting.

6 The Quantitative Potential of Biofuels

To avoid CO_2 emissions, substituting coal is at present a very effective way of using biomass. In the future, though, using biomass for transport fuels will gradually become more attractive from a CO_2 mitigation perspective because of the lower greenhouse gas emissions for producing second generation biofuels and because electricity production on average is expected to become less carbon-intensive due to increased use of wind energy, photovoltaic and other solar-based power sources, carbon capture and storage technology, nuclear energy, and fuel shift from coal to natural gas (IEA Bioenergy, 2007). In this context, land and biomass availability will become the primary limiting factors and the ceiling for contribution to global primary energy can already be foreseen.

6.1 Biomass Availability: Bottom-up Approach

Biomass currently provides an annual amount of energy ranging from 40 EJ yr^{-1} (Parikka, 2004) to 45 ± 10 EJ yr^{-1} (IEA Bioenergy, 2007), of which roughly 7 EJ yr^{-1} are considered as modern biomass in opposition to traditional use of woodfuel (UNDP, 2000). In 2050, the total primary energy demand will vary between 800 EJ yr^{-1} and 1400 EJ yr^{-1} (IEA Bioenergy, 2007), and the share of biomass to meet that need is quite speculative. Due to the complexity of the numerous factors interacting to determine the potential and cost of bioenergy production, projecting future bioenergy consumption cannot be done by matching demand and supply. On the contrary, studies focus either on the supply-driven potential, i.e. resource assessment, or on demand-driven amounts required to meet exogenous targets without specifically defining the exploitable resources (Berndes et al., 2003).

Therefore, assumptions vary widely among studies and significantly impact resulting global potentials. Biomass supply could amount to 200–400 EJ yr^{-1} by 2050, i.e. 14% to 50% of total primary energy demand, without jeopardising the world's future food supply. Considering expected average conversion efficiencies, this primary bioenergy could correspond to 130–260 EJ yr^{-1} biofuels or 100–200 EJ yr^{-1} electricity (IEA Bioenergy, 2007). These future bioenergy potential estimates, which are rather larger than potentials for the current situation, are based on the assumptions of future higher yields, notably thanks to perennials and advanced conversion technologies, but also through an improvement in agricultural system efficiency and the use of marginal and degraded lands. They are average values of extreme supply scenarios in 2050, from a scenario with bioenergy exclusively from waste biomass (40 EJ yr^{-1}) up to a scenario with an intensive dedicated agriculture concentrated on the better quality soils (1100 EJ yr^{-1}). This wide range calls for scrutiny when examining potential assessments.

6.1.1 Bottom-up Models

Supply-driven studies have proven, though, that technical biomass potential could meet the amount levels of bioenergy use reported in demand-driven studies. Most studies also agree on the fact that energy crops represent the main potential biomass source compared with forest products or residues; land availability for energy crops and biomass yields hence appears to be the main key assumption (Berndes et al., 2003; Smeets et al., 2007). Diverging assumptions on the energy crop yields alone lead to a 40% difference in the maximum bioenergy potential produced from woody energy crops on about the same amount of surplus land when comparing the studies Hoogwijk et al. (2003) with Smeets et al. (2007).

Most complex approaches use models, such as IIASA's BLS model or IMAGE 2.2 model, etc., to simulate land uses and biomass availability on a geographical grid, taking into account geo-climatic conditions, the types of soils and crops, and agricultural practices. Still, the definitions of land-use patterns, geographical aggregation, and further assumptions on the evolution of both the crop yields and the efficiency of agricultural systems differ, inducing a wider range of bioenergy potentials. For instance, the management factor defined as the gap between theoretically feasible and actual crop yields, i.e. introducing yield limitation by less than optimal management practices and technologies, varies between 0.7 and 1.5 when comparing the two above-mentioned studies. A management factor above 1 expresses an increase in the harvest index, the development of irrigation and biotechnologies (Hoogwijk et al., 2005), reflecting a growing confidence in future biomass production systems up to very optimistic scenarios above current theoretical optimal systems.

Table 6 Global land uses (Gha) and annual conversions in 2000 (Gha yr^{-1}) (Holmgren, 2006)

Into: From:	Forest	Woodland/ Grassland	Cropland	Urban area*	Losses (% yr^{-1})	Gains (% yr^{-1})	Net change (% yr^{-1})
Forest	3.97	0.003	0.0098	0.0002	–0.33	0.14	–0.18
Woodland/ Grassland	0.0014	3.44	0.001	0.0002	–0.08	0.15	0.07
Cropland	0.0043	0.002	1.51	0.0016	–0.52	0.70	0.19
Urban area*	n.s.	n.s.	n.s.	0.038	–0.00	5	5

n.s. not significant; *urban area only encompasses here areas with more than 100 000 inhabitants, total area of 13.4 Gha including "other lands" for which no significant changes were identified.

Land availability for energy crop depends on the competitive uses of land for food and feed, biomaterials, forest, conservation areas, and build-up. The background assumption in most studies is that land demand for food and feed production has to be fulfilled before land is allocated to bioenergy production. Future land demand for food and feed is then assessed, taking into account expected population growth and diet evolvement; diets having a dual effect on land use. Indeed, diet in MJ day^{-1} per capita increases as a function of the income in absolute quantity terms as well as in qualitative ones, tending to an increasing share of livestock and oilseed products in the global average diet. Growth in meat and dairy product production and consumption is expected to continue, especially in developing countries, where people eat only about 30 kg of meat per capita a year, whereas this rate is above 80 kg yr^{-1} in the industrial world. Experts predict that by 2050, nearly twice as much meat will be produced as today. The impact on land use will be severe as animal husbandry is very land-consuming. In 2002, more than 70% of the agricultural lands worldwide were dedicated to the production of animal products, while these only accounted for some one-fifth of the total calorie intake (FAO, 2003). Therefore, an analysis of the sensitivity of land availability to the evolvement of animal production systems is also necessary.

Nowadays, of the 13.4 Gha of land area in the terrestrial biosphere (Holmgren, 2006):

- 5 Gha are used for agriculture

(roughly 1.3 Gha food crops + 0.2 Gha fodder crops + 3.5 Gha pastures)

- 4 Gha are under forest cover (56% subtropical and tropical forests, overall 95% are natural forests while the remaining 5% are plantation forests)
- 4.4 Gha of the rest of the land encompass semi-natural vegetation types such as savannas, etc., barren land and about 0.26 Gha of build-up area (FAO, 2002, 2003; Hoogwijk et al., 2005; Smeets et al., 2007).

Table 6 shows land-use change patterns in 2000 based on annual changes between 1990 and 2005.

Table 7 gives an overview of past and future trends of population, average calorie intakes and agricultural production. World population growth and demand for agricultural products have been slowing down since the late 1970s. Indeed, although the world average calorie intake has been rising, especially in developing countries where incomes have increased, high levels of food consumption have now been reached in many countries. In particular, China has already passed its phase of rapid growth. In the past four decades, rising yields accounted for about 70% of the increase in crop production in the developing countries and yield growth, even if not as rapid as in the past, will continue to play the same role for the next 30 years. The contribution of irrigation to this yield growth and intensification of livestock production is also expected to increase. Hence, the expansion of agricultural land at the expense of forest is expected to be concentrated in the developing countries and limited to an overall 12.5% increase, i.e. half of the increase between the early 1960s and late 1990s. However, more than half the land that could be opened up is in just seven countries of tropical Latin America and Sub-Saharan Africa, where 80% of land expansion is expected to take place, whereas other regions face a shortage of suitable land, e.g. in South Asia more than 94% of suitable land is already farmed.

Agricultural production can grow in line with demand, provided that regional shortages, notably in livestock products in developing countries, are reduced through international trade efforts. Net cereal imports

Table 7 World population and agricultural production projections at a glance (FAO, 2002)

	Time frame	1979–1981	1997–1999	2015	2030	2050
Population in billions (annual growth rate %)	World	4.430	5.900	7.207	8.270	9.322
		(1.6)	(1.5)	(1.2)	(0.9)	(0.6)
	Developing countries:	3.259	4.595	5.858	6.910	7.987
Remaining countries		(1.9)	(1.7)	(1.4)	(1.1)	(0.7)
	Industrial countries:	0.789	0.892	0.951	0.979	0.986
26 countries: Western Europe, USA, Canada, Australia, New Zealand, Japan, South Africa, Israel		(0.7)	(0.7)	(0.4)	(0.2)	(0.0)
	Transition countries:	0.382	0.413	0.398	0.381	0.349
28 countries: Eastern Europe, former Yugoslavia SFR, CIS, Baltic States		(0.5)	(0.1)	(−0.2)	(−0.3)	(−0.4)
Calorie consumption in kcal capita^{-1} day^{-1}/in MJ.capita^{-1} day^{-1} (1 kcal = 4.186×10^{-3} MJ) (annual growth in demand for agricultural products in %) – [calories from animal products]						
	World average	2 552/107	2 803/117	2 940/123	3 050/128	3 302/138
		(2.1)	(2.0)	(1.6)	(1.4)	[550]
	Developing countries	2 312/9.7	2 681/112	2 850/119	2 980/125	3 236/135
		(3.7)	(4.0)	(2.2)	(1.7)	[549]
Calorie consumption in kcal capita^{-1} day^{-1}/in MJ.capita^{-1} day^{-1} (1 kcal = 4.186×10^{-3} MJ)						
	Industrial countries	3 135/131	3 380/141	3 440/144	3 500/147	3 448/144
		(1.0)	(1.0)	(0.7)	(0.6)	[1 054]
	Transition countries	3 389/142	2 906/122	3 060/128	3 180/133	3 629/152
		(−1.7)	(−4.4)	(0.5)	(0.4)	[941]
Total arable land in Gha (irrigated land in Gha)						
	World	1.608				
		(210)	(271)			
	Developing countries	–	0.956	1.017	1.076	–
			(202)	(221)	(242)	

	Industrial countries	–	0.387 (42)	–	–	
	Transition countries	–	0.265 (25)	–	–	
Cropland in Gha in developing countries (yields tons/ha)						
	Wheat	0.096 (1.6)	0.111 (2.5)	0.113 (3.1)	0.118 (3.5)	–
	Rice (paddy)	0.138 (2.7)	0.157 (3.6)	0.162 (4.2)	0.164 (4.7)	–
	Maize	0.076 (2.0)	0.097 (2.8)	0.118 (3.4)	0.136 (4.0)	–
	All cereals	0.408 (1.9)	0.465 (2.6)	0.497 (3.2)	0.528 (3.6)	–
	% of total harvested land	60	55	53	51	–
Forests in billion ha (annual change in Gha)						
	World	–	3.870 (−0.0094)	–	>2.940 (if <−0.0094)	–
	Tropical and sub-tropical	–	2.168 (−0.0123)	–		–
	Non-tropical	–	1.702 (+0.0029)	–		–
	Build–up land worldwide in Gha	–	0.26	–	0.39–0.52*	–

Calculations from Smeets et al. (2007) with the data from FAO, 2002 are in italics.

* Data from the Global Environment Outlook, UNEP 2002 in Hoogwijk et al. (2005).

by developing countries will almost triple over the next 30 years while their net meat imports might even increase by a factor of almost five. By 2030 about 440 million undernourished people may still remain. The FAO analysed that developing countries' farmers could gain a lot from lower trade barriers, provided domestic policies permit one to remove the domestic bias against agriculture and to improve productivity and product quality to the standards demanded abroad. Investments in transportation and communication facilities, upgraded production infrastructure, and improved marketing, storage and processing facilities could be particularly important. In resource-rich but otherwise poor countries, a more export-oriented agriculture could provide an effective means to fight rural poverty (FAO, 2002).

6.1.2 Availability of Agricultural Land

Estimation of land availability for energy crops finally essentially relies on the surplus land area that may be released in certain regions either because of higher yields and intensified production, or some agricultural lands being abandoned as they become no longer suitable for food and feed production.

First expectations are reinforced by the fact that in many countries average wheat yields, for instance, for the period 1996–2000 were below the agro-ecologically attainable levels; in India, Brazil, and even Australia and the US, they were calculated to be roughly half the maximum levels (FAO, 2002 in Smeets et al., 2007). Also, feed conversion efficiency[58] of bovine meat and dairy products was in 1998 three to four times higher in industrial countries than in developing countries, indicating that part of the land demand for livestock production could be outpaced by an increase in efficiency of livestock production systems. Moreover, the intensification of husbandry systems could permit one to spare grassland for other crops as some part of suitable croplands are currently used as pastures, especially in developing countries (Smeets et al., 2007).

Testing diverse scenarios of combined population growth, and change in diets and food and feed production systems (Table 8), recent studies show that considerable parts of agricultural lands could be allocated to bioenergy production without jeopardising the food and feed supply: 0.15 to 2.4 Gha (Hoogwijk et al., 2003), 0.6 to 1.3 Gha (Hoogwijk et al., 2005) and 0.7 to 3.6 Gha (Smeets et al., 2007). The highest estimates, notably in the systems 3 and 4 of Smeets et al. (2007), are rather too optimistic though, combining very intensive agricultural production with high yield increases and landless animal production, together with a management factor of 1.5, that indicates even further possible improvements; more irrigation, more fertilisers, while system 4 already corresponds to a 25% addition to the yield levels at a very high rain-fed/irrigated level of agricultural technology. The landless animal production system especially leads to consequent surplus pasture areas, up to 613–820 Mha in some developing countries, which can partly serve for bioenergy production (Smeets et al., 2007). However, such a system could hardly be implemented in these regions by 2050. On the contrary, a low productive agricultural system (Y1 in Table 8 = "Low External Input" in the study Hoogwijk et al., 2003), which could be roughly compared with an organic production system, does not permit one to grow any energy crop on agricultural land. This scenario suggests yields in 2050 that would be around the same as today's, once again emphasising that agricultural intensification is a prerequisite.

Considering medium population scenarios with affluent diets and relatively high production systems across the three studies, surplus agricultural land areas allocated to energy crops would vary between 0.45 and 1.3 Gha. Maximum bioenergy potential on these surplus agricultural land areas would vary between 135 EJ yr^{-1} and 409 EJ yr^{-1} (LHV = 15 GJ tDM^{-1}), i.e. roughly 25% to 50% of total primary energy demand in 2050, considering a medium energy demand scenario of 837 EJ yr^{-1}. Nevertheless, this maximum potential is simulated considering a unique type of biomass source which is short rotation coppices (SRC). These crops provide high yields and are convenient for assessing maximum primary bioenergy potential based on gross energy contents, whereas potentials expressed based on first generation biofuel crops would not be as relevant without calculating conversion factors. However, SRC are not representative of the portfolio of energy crops and present higher yields than other energy crops. Considering the cereal yields of the High External Input system and assuming that the

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[58] Feed conversion efficiency is defined as the amount of animal product produced per amount of animal feed input.

Table 8 Comparison of three selected assessments of surplus agricultural land areas available for energy crops

Scenarios for 2050	Land available pool (Gha)	World population (billion inhabitants)			Diet per person in MJ day^{-1} (grain $_{eq}$ kg^{-1} day^{-1})			Production systems: yields in tDM gr$_{eq}$ ha^{-1} yr^{-1} (irrigated)		Constraints	Available surplus agricultural area in Gha (EJ yr^{-1} of primary energy from dedicated crops)
Hoogwijk et al., 2003	5=	P1	P2	P3	D1	D2	D3	Y1	Y2	Total agricultural land is constant = 5 Gha	Yields weighted by MF = 20 tDM ha^{-1} yr^{-1} HHV* = 19 GJ t^{-1}
		8.7	9.4	11.3	10.1	10.1	11.5	2.2	5.9	No deforestation for bioenergy production	D1 “not likely”
	1.5 croplands (including 20% irrigated)				(1.3)	(2.4)	(4.2)	(4.0)	(14.3)	Area needed for food and feed is doubled to take into account losses, risks, uneven accessibility to the resources, etc.	Y1,D2–D3,P => 0 P1,D2,Y2 => 2.6 (988) P2,D2,Y2 => 2.4 (912) P3,D2,Y2 => 1.9 (722) P1,D3,Y2 => 0.8 (304)
	+3.5 grasslands									Management factor (MF) for energy cr ops: 0.7	P2,D3,Y2 => 0.45 (171) P3,D3,Y2 => 0.15 (57)
Hoogwijk et al., 2005 interpreting the IPCC SRES: A1, A2, B1, B2	13	A1 B1	B2	A2	A2	B2	A1 B1	A2 B2	A1 B1	Land-claim exclusion factors (% of global land area): agricultural land needed for food and feed production, forest areas, tundra, nature reserves: 5% (A1, A2), 15% (B1, B2), urbanisation (3–4%), extensive grassland areas, rest land areas sensitive to diverse ecological stresses such as scarce water resources: 50% (A1, A2) 90% (B1, B2)	*Yields weighted by MF = 6–34 tDM ha^{-1} yr^{-1} [*mean yields tDM ha^{-1} yr^{-1}] LHV = 19 GJ t^{-1}
	–excluded and allocated areas	8.7	9.4	11.3	12.5	12.8	13.2	Regional yields of 12 food crops weighted by MF			
								MF$^{\bullet}$ = 0.78 MF$^{\bullet}$ = 0.82			

(continued)

Table 8 (continued)

Scenarios for 2050	Land available pool (Gha)	World population (billion inhabitants)	Diet per person in MJ day^{-1} (grain $_{eq}$ kg^{-1} day^{-1})	Production systems: yields in tDM gr$_{eq}$ ha^{-1} yr^{-1} (irrigated)	Constraints				Available surplus agricultural area in Gha (EJ yr^{-1} of primary energy from dedicated crops)
					Impact of climate change is included				A1 => 1.3 (409) [20]
					Pressure on land quality is not included				B1 => *1.3 (398) [20]
					Management Factor (MF) for energy crops: A2, B2: 1.1; B1: 1.3; A1: 1.5				A2 => 0.6 (129) [16] B2 => *1 (279) [18]
Smeets et al., 2007	13	8.8	13	Systems	S_1	S_2	S_3	S_4	Yields weighted by MF(=1.5) = 4–39 tDM ha^{-1} yr^{-1}
				Animal production	mixed	mixed	landless	landless	[mean yields tDM ha^{-1} yr^{-1}]
	–excluded and allocated areas		details by regions and animal products contribution given by the authors	Feed conversion efficiency	high	high	high	high	HHV = 19 GJ t^{-1}
				Technology for crop production	very high	very high	very high	super high	
				Irrigation	none	partly	partly	partly	S1 => 0.7 (215) [16]
				Average yield increases (19 crops) between 1998–2050	×2.9	×3.6	×3.6	×4.6	S2 => 1.15 (455) [21] S3 => 3.3 (1,101) [17] S4 => 3.6 (1,272) [20]

The yields of the 19 crops are calculated with IIASA model and weighted (0–100%) in function of the suitability (5 levels) of the land area allocated. Demand for feed crops, including increase in demand for feed from grasses and fodder compared with 1998, is added to total demand for food crops. Allocated land areas excluded from available pool for bioenergy crops: other land including uncultivated land, barren land, etc., build-up, plantations and natural forests, permanent crops, crops not in the model (13% of total harvested area), agricultural land needed for food and feed production including trade at regional levels to ensure food and feed security.
No deforestation for bioenergy production
Water is excluded as a limiting factor except in arid and semi-arid regions
Irrigation is limited to areas in which climate, soil and terrain permit it
Impact of climate change is excluded
Management Factor (MF) for energy crops: 1.5

Figures in italics are the closest to FAO simulations of business-as-usual scenario for 2050. "not likely" expresses the opinion of this article's authors and not any mentioned comment of the assessments' authors; *H/LHV: high/low heating value i.e. including/or not the energy recovered from vapour condensation; •Average management factors that affect the yields of the 12 food crops calculated with IMAGE 2.2 model at a geographical grid cell level of 0.5° × 0.5°; *read on the graphs.

whole cereal crop is harvested for energy purposes, the 0.45–1.3 Gha would only provide 40–279 EJ yr^{-1} (LHV = 15 GJ tDM^{-1}) (Hoogwijk et al., 2003). Yields of energy crops assumed in other studies range from 7 to 49 tDM ha^{-1} yr^{-1} (Smeets et al., 2007). Moreover, SRC production systems require specific machines and overall high investments that are unlikely to be widely affordable in order to reach optimum yields all over the world by 2050.

Theses studies are often quoted throughout the literature, because of the scarcity of such global assessments. A deep insight into these studies' background assumptions would make it possible to apprehend the relevance of the results better. However, other assessments based on different models and hypotheses would be needed to test the robustness of the results.

6.1.3 Biomass from Forest and Residues

Studies also differ fundamentally in their conclusions about the availability of forest biomass for bioenergy purposes (Berndes et al., 2003). Assumptions diverge both concerning the projected forest plantations and forest growth rates, and the volume restrictions due to competitive wood industrial demand. According to Smeets et al., 2007, energy potential from surplus forest growth in 2050 ranges between 59 EJ yr^{-1} in the case of a low plantation scenario and a high forest product demand, and 103 EJ yr^{-1} in the case of a high plantation scenario and a low demand; meanwhile, it could reach 74 EJ yr^{-1} in a medium scenario. Woodfuel is one of the main forest products; about 60% of the world's total wood removals from forests and trees outside forests are used for energy purposes. Demand for woodfuel will remain strong for many years to come, although its share in total energy demand is expected to decrease, as most developing countries have adopted energy policies aimed to promote the use of other options by households, such as liquefied petroleum gas (LPG), bottled gas and kerosene. This decrease is being largely compensated for, though, by the increased woodfuel use for industrial energy in developed countries (Trossero, 2002). The overall demand for forest products will continue to grow as world population and income grow, but improvement in forest exploitation efficiency, increases in plantation and an expansion of the role of trees outside forests should ensure sufficient wood supply.

The key questions now are where it should come from and how it should be produced (FAO, 2002). Ensuring a sustainable woodfuel supply for the poorest people of developing countries remains a serious problem. In places with intensive woodfuel use, for example around large urban centres and in zones with a high concentration of commercial activities such as brickmaking, the pressure on woodfuel supply sources can be heavy, with consequent deforestation. Therefore, generalisation on sustainability of woodfuel use at the local level cannot be done without careful analyses in the field (Trossero, 2002). Because of the decentralised nature of wood energy systems, energy and forestry statistics seldom include the same level of detail about woodfuel consumption as for other conventional energy sources or forest products (Trossero, 2002). Some studies hence only mention forest biomass potential from residues.

Indeed, forest exploitation produces high amounts of residues, although the production of wood-based materials is continually increasing in efficiency, i.e. the ratio of residues over final products is decreasing (FAO, 2002). About 60% of the total harvested tree is left in the forest and the non-commercial species are often felled and left on site to ease the logging, providing a valuable source of bioenergy (Parikka, 2004), especially as industrial round wood production is expected to rise by 60% by 2030, from current levels to around 2400 million m^3, with one-third from plantations, about twice as much as today's plantation production of 400 million m^3 (FAO, 2002). After processing, about half of the log input becomes wastes that can have various alternative uses such as chips for pulp or chipboard, etc., and fuel for internal energy use or as compacted commercialised fuels, depending on the sale values on the diverse markets (Parikka, 2004).

Overall, biomass residues may be classified as follows: primary ones from agriculture (crop residues) or forest (logging residues), secondary ones from agriculture (from food processing, animal manure) and forest (mill and manufacture residues), and tertiary ones including all kind of final biomass waste. Although residues can provide a substantial source of biomass in a global energy context (Berndes et al., 2003), potential assessments are highly uncertain. First, residue generation is a multiplier factor of assumed total food and feed production. Fischer and Schrattenholzer (2001) thus stressed that this factor will furthermore decrease while the harvest index of crops is expected to increase thanks to progress in agricultural technology (e.g. bioengineering, agricultural practices, etc.), which implies another degree of uncertainty. Second, only a fraction of total residues is recoverable in practice. Third, residue amounts available for energy production also depend on possible alternative uses of residues such as demand for feed, fertiliser (including the need to maintain soil quality), and for wood products, although these last may also partly become an eventual energy source. Most studies assume a recoverability fraction of 25% for primary residues, and higher fractions for secondary and tertiary residues between 75 and 100%. Estimates, from the literature, of potential contribution from biomass residues in 2050 vary between 38 and 245 EJ yr^{-1} (Hoogwijk et al., 2005). In Smeets et al. (2007), this range is reduced to 76 to 96 EJ yr^{-1} as residues needed for feed are excluded. These amounts should be further reduced though when compiling all residue demands at regional levels. Considering recoverability limits and some competing uses, the total maximum available residues for bioenergy accounts for roughly 6% of total bioenergy potential (Hoogwijk et al., 2003; Smeets et al., 2007).

6.1.4 Geographical Distribution

Studies mostly agree on the fact that maximum bioenergy potentials, as a function of surplus non-food competitive land areas, are likely to be concentrated in a few regions. In Sub-Saharan Africa, Oceania, the Caribbean and Latin America, large areas suitable for crop production are currently used as pastures (Smeets et al., 2007). Intensification of animal production systems and overall yield increase in these regions would lead to the release of land areas for energy crops. Together with the CIS and Baltic States, these regions have the highest bioenergy potentials from energy crops on surplus agricultural lands (Hoogwijk et al., 2005; Smeets et al., 2007). The largest energy potential from surplus forest growth is found in the CIS and the Baltic States, the Caribbean and Latin America, and partially North America and Western Europe Smeets et al., 2007. Figure 19 shows regional bioenergy future potentials of the four scenarios in Smeets et al. (2007). Figure 20 illustrates that considering current technologies and land and water availability as well as food insecurity, biofuel potential is

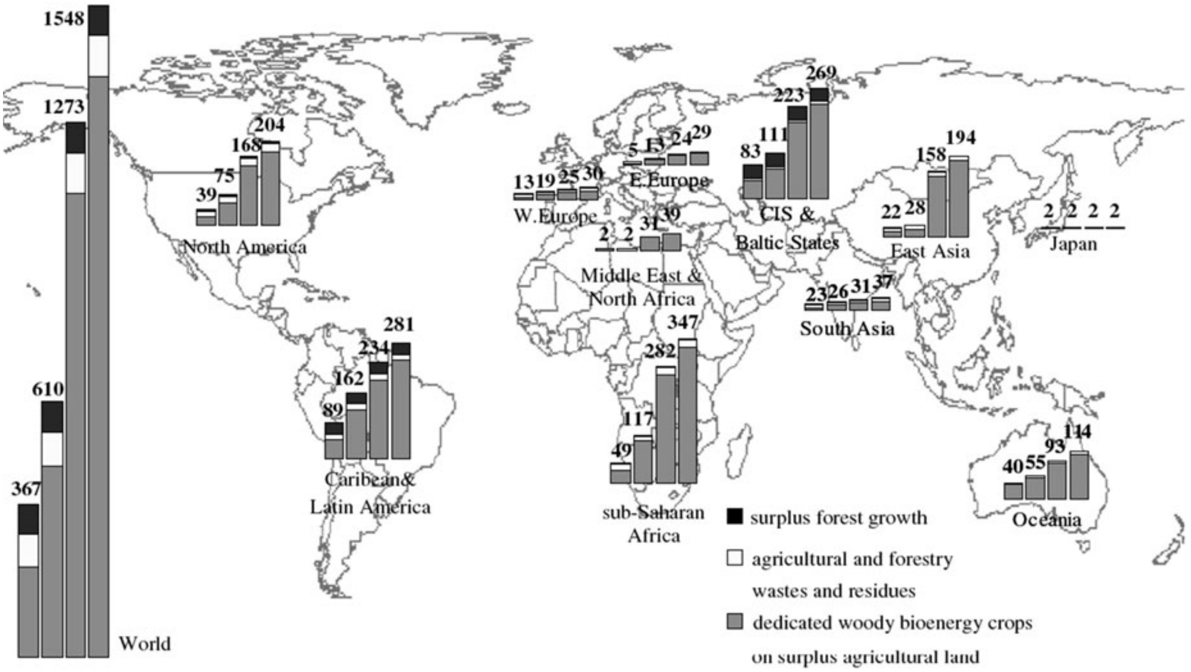

Fig. 19 Total technical bioenergy production potential in 2050 based on systems 1–4 (EJ yr^{-1}; the left bar is system 1, the right bar is system 4) (Smeets et al., 2007)

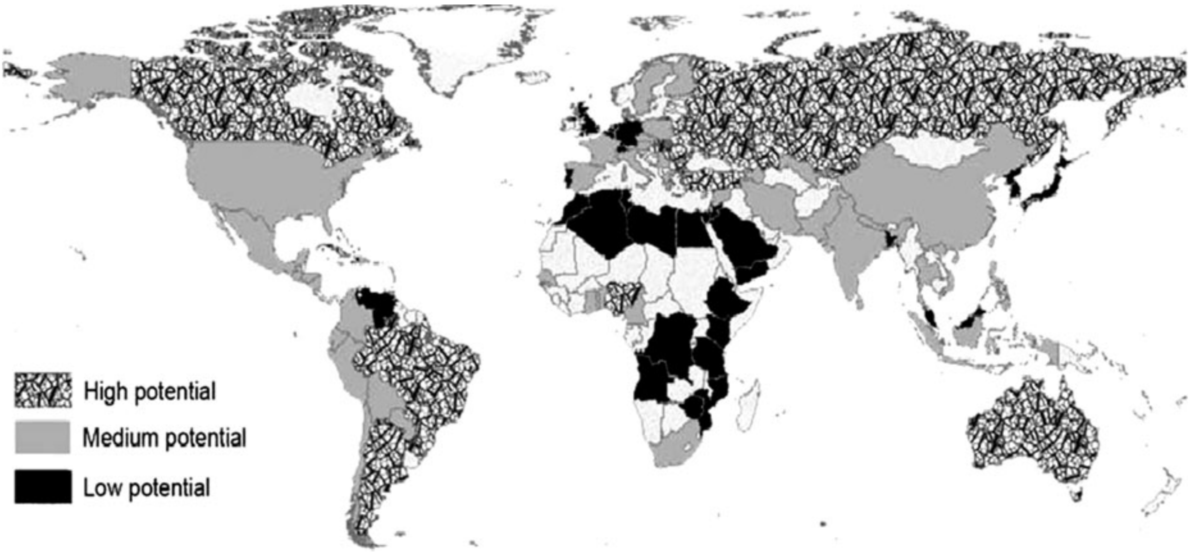

Fig. 20 Biofuel production potential (Von Braun, 2007). Sources: data on food insecurity are from FAO (2006). The land availability index is derived by the author based on data from FAO (2007); IEA (2007a,b); and USDA (2006). Data on water availability are from WRI (2007)

nowadays still limited in Africa and Asia notably (Von Braun, 2007).

Oceania is the least land-stressed region, whereas Japan is the most land-stressed country. The Middle East and North Africa, and South and East Asia have relatively scarce agricultural land (Smeets et al., 2007). High land requirements for urbanisation were estimated in South and East Asia, mainly India and China, whose abandoned agricultural land areas should increase at the end of the century with the decrease in population growth (Hoogwijk et al., 2005). In industrial or transition countries, bioenergy production requires less drastic changes than in developing countries. Furthermore, in Eastern Europe, the CIS and Baltic States, food consumption and population are projected to decrease, which makes bioenergy potential in these regions more robust than in others. Regions with the highest potentials could turn into bioenergy exporters. In developing countries, bioenergy may provide new incentives for investments in agricultural research and, by providing new income, carry out a modernisation of the agricultural production systems, with a positive feedback on yields possible (Smeets et al., 2007). This is especially crucial for regions that are expected to have high bioenergy potential, such as Sub-Saharan Africa and Latin America, but which also suffer from soil erosion or nutrient depletion. In Sub-Saharan Africa, some 95 Mha of land is threatened with irreversible degradation if soil nutrient depletion continues (Henao and Baanante, 2006) in (Agard et al., 2007). Furthermore, these regions are expected to be inversely impacted by climate change. According to Tubiello and Fischer (2007), cereal production would decrease by 3.9–7.5% between 1990 and 2080 due to climatic risks, whereas it would increase by 5.2–12.5% in Latin America (Table 9).

Table 9 Expected impacts of climate change on global cereal production. Adapted from Tubiello and Fischer (2007) in Von Braun (2007)

Localisation	1990–2080 (interval change %)
World	−0.6 to −0.9
Developed countries	2.7 to 9.0
Developing countries	−3.3 to −7.2
Southeast Asia	−2.5 to −7.82
South Asia	−18.2 to −22.1
Sub-Saharan Africa	−3.9 to −7.5
Latin America	5.2 to 12.5

As shown in Fig. 21, many areas are affected by degradation; Tropical Africa, Southeast Asia, North-Central Australia, Central America, the Caribbean, southeast Brazil, and boreal forests in Alaska, Canada and eastern Siberia are the most severely touched. Here, land degraded areas are defined as areas with a combined declining trend of Net Primary Production and declining Rain-Use Efficiency over the past 22 years, excluding the simple effects of drought.

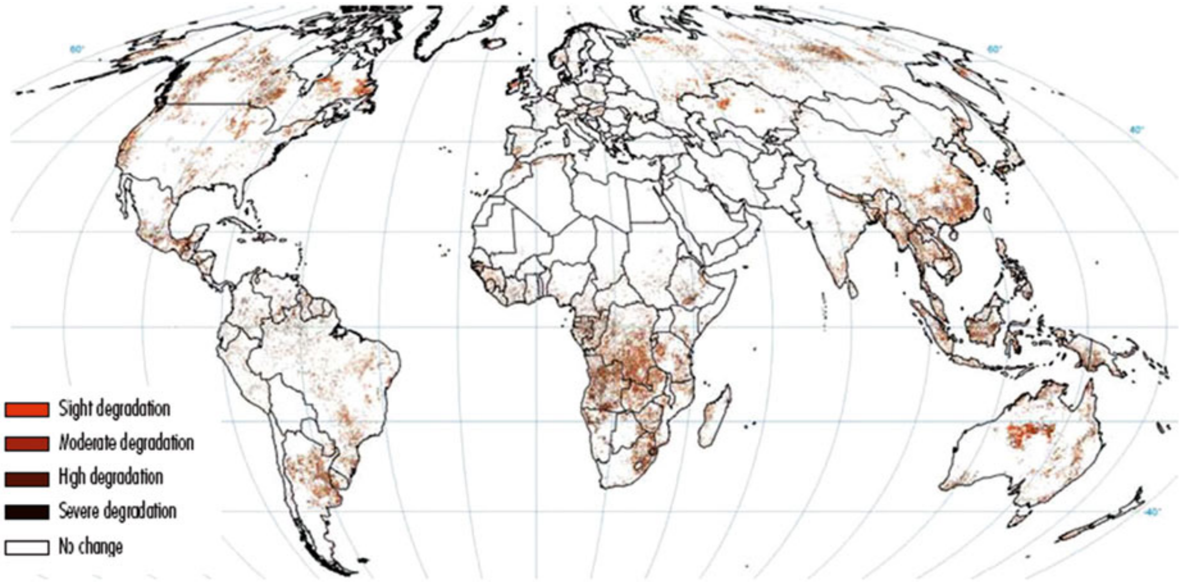

Fig. 21 Global land degradation using biomass production and rain-use efficiency trends between 1981–2003 (Bai et al., 2007, online: http://www.fao.org/nr/lada/images/maps/ndvi_preliminar.jpg)

Table 10 Tropical degraded lands with potential for plantation establishment (Mha) in Grainger (1991). Total degraded land in the tropics could average 2 Gha (Grainger, 1988)

Degraded land areas	Forest fallows	Deforested watersheds	Degraded drylands	Total
Africa	59	3	110	172
Asia	59	57	110	226
Latin America	85	27	10	222
Total	203	87	330	620

23.5% of the world land area is in a state of more or less severe degradation (Bai et al., 2008). The most degraded areas are mainly associated with forest degradation, although the precise history of land degradation processes has to be investigated on a regional scale. The degraded areas represent a loss of NPP of about 800 million tons of carbon that were not fixed during this period, added to CO_2 emissions into the atmosphere of one or two orders of magnitude more than this amount from the loss of soil organic carbon and standing biomass (Bai et al. 2007 in Agard et al. 2007).

From about 2 Gha of degraded areas in the tropics solely, some 420 to 620 million ha, could be suitable for afforestation or vegetation enhancement, respectively, subtracting or not the forest fallows that are part of shifting cultivation systems (Table 10, Grainger, 1991). Houghton et al. (1991), also assessed that around 580 million ha of degraded land, formerly covered with forests or woodlands, may be available to be planted or managed as plantations. Parts of these areas could be used to produce energy crops and SRC could be a means of recovering parts of the lost former sequestered carbon. Although yields on these lands will not be optimum, some species with low needs, such as *Jatropha curcas*, could be used in agroforestry systems (see picture below) in order to limit further degradation of these areas and CO_2 emissions. On 420–580 million ha of degraded land, also referred to as "low-productive land", some 8–11, and 24–33 up to 80−110 EJ yr^{-1} could be produced considering yields of 1, 3 and 10 tDM ha^{-1} yr^{-1}, respectively (Hoogwijk et al., 2003; Smeets et al., 2007). Finally, Smeets et al. (2007) also mentioned that some further 247 EJ yr^{-1} could be produced on the 3.6 Gha classified as other land. This is an absolute finite potential, assuming that all other land could be allocated to energy crops. "Other land" or "rest land" areas include several kinds of natural vegetation and other sites remaining once agricultural, build-up and forest land areas are allocated that are not particularly suitable for production. They are therefore also classified as "low-productive lands". More specific studies would be necessary to evaluate on a regional scale the effective availability of rest land areas to be cultivated and the overlapping between these areas and degraded land areas.

Agro-forestry system: *Jatropha curcas*/chili
Belize, February 2003 bessou©INRA

To summarise, biomass availability for bioenergy can be assessed through five steps, each corresponding to a gradually decreasing potential (Hoogwijk et al., 2005). The first theoretical potential in 2050, some 3500 EJ yr^{-1} (Hoogwijk et al., 2005); 4435 EJ yr^{-1} (Smeets et al., 2007) takes into account the conversion of solar energy by vegetation (Net Primary Productivity), giving an upper limit of primary biomass energy potential on the total terrestrial surface. This indicative potential is severely reduced at a regional level by the multiple uses of land, which leads to a smaller geographical potential, also diminished due to losses through conversion processes of primary biomass to secondary energy carriers corresponding to a technical potential. Finally, economic and other socio-technical constraints may also drastically limit final economic and implementation bioenergy potentials.

Economic feasibility notably depends on raw material costs, conversion efficiency and incentives that will translate into political orientation choices. In the case study of woody biomass for energy production as a means of greenhouse gas reduction, Dornburg et al. (2007) showed that, in Poland, biomass potential is larger than the amount that could make possible cost-effective greenhouse gas savings at low costs. Biomass cost is notably strongly affected by land price elasticity; so that electricity and methanol from woody biomass remain interesting ways of cost-effective reductions in greenhouse gas emissions only as long as markets are large enough to absorb the supply without lowering market prices. In some cases, theoretical and technical potentials might be well above these thresholds. For biomaterials, market volumes are an even more critical issue. Hence economic potential highly varies depending on the supply curves of bioenergy and the internalised environmental costs, which also highly depend on market sizes.

Implementation potential, that actually defines the overall final bioenergy potential, is further limited by diverse constraints that can barely all be embraced in any assessment. For instance, the actual feasibility of the implementation of energy crops and the impacts on degraded lands are merely mentioned; studies refer to exogenous assessments of the actual extent of degraded land that could be suitable for plantation establishment (Berndes et al., 2003). Also, many environmental aspects are not taken into account, such as impacts on biodiversity, erosion, water and nutrient uses, etc. The issue of water and nutrient uses is especially crucial in SRF with species such as willow or eucalyptus that take up lots of water and nutrients from the soils. Large-scale energy crop implementation would in some countries, such as Poland or South Africa, lead to further exacerbation of an already stressed water situation (Hoogwijk et al., 2005).

Therefore, more research is needed in modelling interactions between the competitive land uses and ecological issues (Berndes et al., 2003). Notably, land-use change from forest area to bioenergy area is excluded in most studies, but land-use change from forest area to agricultural land for food and feed production is not, meaning that the actual forest areas decrease/shrink. In the scenario with high demand for food and low technology development (A2) a significant amount of forest is cut down: 45% of South American forest area could disappear within 100 years (Hoogwijk et al., 2005). Part of the abandoned agricultural land areas should therefore be allocated to reforestation and not to bioenergy production, in order to compensate for the loss in forest areas due to land clearing for agriculture.

6.2 Focus on Europe

A report of the European Environment Agency (EEA, 2006b) focused on how much bioenergy could be produced without harming the environment, leading to the following strict assumptions: the use of energy crops with low environmental pressure, the preservation of current protected forests and extensively cultivated agricultural areas (almost 6 million ha of grassland, olive groves and dehesas), a minimum 30% share of used agricultural area dedicated to *environmentally-oriented farming EOF*[59], 3% of intensively cultivated agricultural land set-aside by 2030, ambitious waste minimisation strategies, and the further liberalisation of agricultural markets with a reduction of 40% below the greenhouse gas emission 1990 level that would make the price for carbon emission permits increase.

The results show that primary biomass potential could rise to 7.9 EJ yr^{-1} (190 Mtoe yr^{-1}) in 2010 (target 6.3 EJ yr^{-1} $\approx$ 150 Mtoe yr^{-1} by 2010) up to around 12.3 EJ yr^{-1} (295 Mtoe yr^{-1}) in 2030, i.e. 17% of European current global energy consumption (EEA, 2006b). This *conservative estimate*[60] concludes that the largest potential comes in the short term from the waste sector, especially in Germany and France (all kind of waste included: around 4.2 EJ $\text{yr}^{-1} \approx 100$ Mtoe yr^{-1}), whereas energy crops from agricultural land would overtake it in the long term (up to around 5.9 EJ $\text{yr}^{-1} \approx 140$ Mtoe yr^{-1}). *Environmentally-compatible* bioenergy potential[61] from forestry is estimated to be almost constant throughout the period analysed (around 1.7 EJ yr^{-1} $\approx$ 40 Mtoe yr^{-1}) (EEA, 2006b).

The modelling of the released and set-aside land area was based on the CAPSIM model (EuroCare, 2004). The available arable land within the EU-22 that could be used for dedicated bioenergy production increases from 13 million ha in 2010 (8% of the total UAA) to 19.3 million ha in 2030 (12% of the UAA). Assumptions include that current areas used for energy crop production remain available for bioenergy production, but other drivers interact to influence this land availability. Most of the land is made available through release of land from food and fodder production. As maintaining the current European food self-sufficiency level was set as a framework condition, the competition between food and bioenergy production was assumed to be relevant only for the part of agricultural production that corresponds to projected food exports. Thus, consideration of the competition effect between bioenergy and food production was restricted to France and Germany, the only member states which are projected to combine a very high export surplus for cereals with a large agricultural land area (EEA, 2006b).

The main "suppliers" of available land for bioenergy production are Poland, Spain, Italy, the United Kingdom, Lithuania and Hungary. These countries, plus Germany and France, will produce more than 85% of the *environmentally-compatible* bioenergy potential in Europe. Population size and the economic competitiveness of the agricultural systems in each member state are the main factors determining land potential. On the basis of the available land and an environmental ranking of energy crops, sustainable crop mixes were determined for different environmental zones in Europe. Europe was divided into 13 zones with homogeneous pedo-geo-climatic characters. The *environmentally-compatible* agricultural bioenergy potential is shown in Fig. 22, taking into account the LHV for the conversion of the harvested dry biomass into an energy potential (EEA, 2006b).

Figure 22 shows that high shares of bioenergy would be supported by a complete shift to second generation biofuels with notably a crop mix, not at all

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[59] EOF encompasses organic farming and high natural value farming (NHV).

[60] Quoted as written in the study: related to the overall value judgements in the study that limit the available potential including strict environmental assumptions.

[61] "*Environmentally-compatible*" bioenergy potential = the quantity of primary biomass that is technically available for energy generation based on the assumption that no additional pressures on biodiversity, soil and water resources are exerted compared with a development without increased bioenergy production (EEA, 2006b).

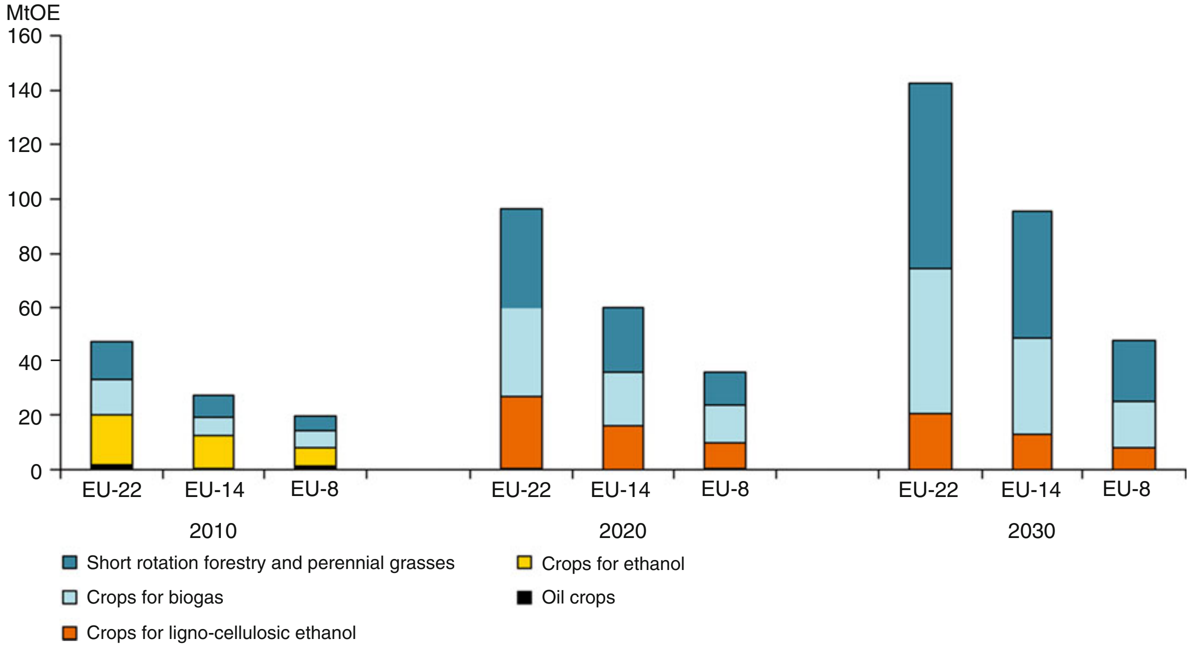

Fig. 22 Environmentally-compatible agricultural bioenergy potential (EEA, 2006b).
Note: No data available for Cyprus, Luxembourg and Malta. 'Oil crops' comprise rapeseed and sunflower. 'Crops for ethanol' include the potential of grains from maize, wheat, barley/triticale. 'Crops for ligno-cellulosic ethanol' cover the energy value of the whole plant (corn and straw) for wheat and barley/triticale. 'Crops for biogas' are maize (whole plant), double cropping systems, switch grass and the grass cuttings from permanent grassland. 'Short rotation forest and perennial grasses' include poplar, willow, miscanthus, reed canary grass, giant reed and sweet sorghum, which may often be used in whole-plant conversion systems like gasification, or biomass-to-liquid processes

relying on oil crops any more. Following the scenario presented in Annex 3 of the Biomass Action Plan, the distribution of biomass used in 2010 would be: 50% for heating, 37% to produce electricity and 13% in the transport sector. The main drivers in the increase in bioenergy potential are productivity increases[62], notably due to the introduction of advanced technologies, and the assumed liberalisation of the agricultural sector, notably linked to the CAP reform that should result in additional land area available for dedicated energy crops. The EEA study shows that there is a significant development potential for bioenergy within Europe, even considering strict environmental assumptions (EEA, 2006b). It is, though, in agreement with the previous detailed studies, showing that this potential will be limited by land availability issues, especially in Western Europe.

6.3 *Liquid Biofuel Potential: Top-Down Approach*

The top-down approach does not aim to assess maximum biomass potential but to study the feasibility of biofuel development plans, i.e. the land areas that would be necessary to produce certain amounts of biofuels (Fig. 23). Given the numerous types of feedstocks and processes that determine biofuel yields per unit of biomass input, there would be multiple scenarios for future global biofuel potentials and as many possible studies. Global assessments therefore extrapolate current biofuel trends and implemented target policies.

In 2004, a study estimated that 37% of total harvested grains, oilseeds and sugar crops area would have been needed to reach a 10% biofuel share (on an energy basis) in world transport fuel consumption within the major biofuel producing regions (Brazil, US, EU[63],

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[62] Assumed yield increases: 1% per year for conventional arable crops, 1–2.5% for dedicated energy crops. A lower yield increase of 1% for all crops would reduce the bioenergy potential by 2% in 2010, and by 13% in 2030.

[63] In both studies here (OECD, 2006) and (Fulton et al., 2004) data concern the years 2000–2004, hence projections for the

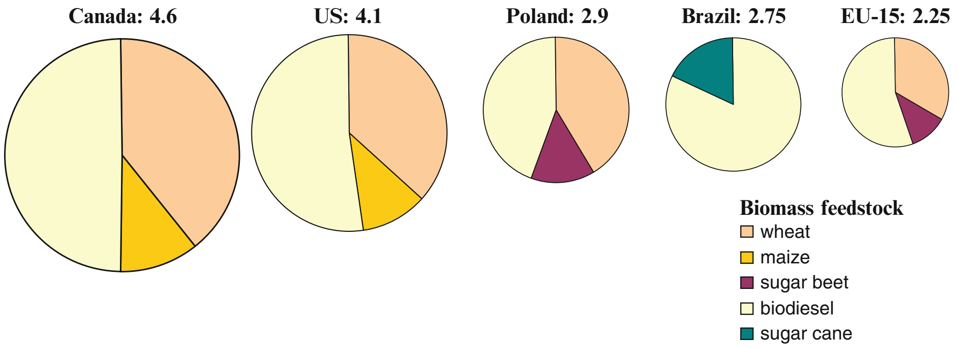

Fig. 23 Land areas (ha) needed to produce 3000 L of gasoline equivalent of biofuel energy; 1000 L per type of biomass feedstock except for Brazil 2000 L of ethanol from sugar cane. Drawn from data in OECD (2006)

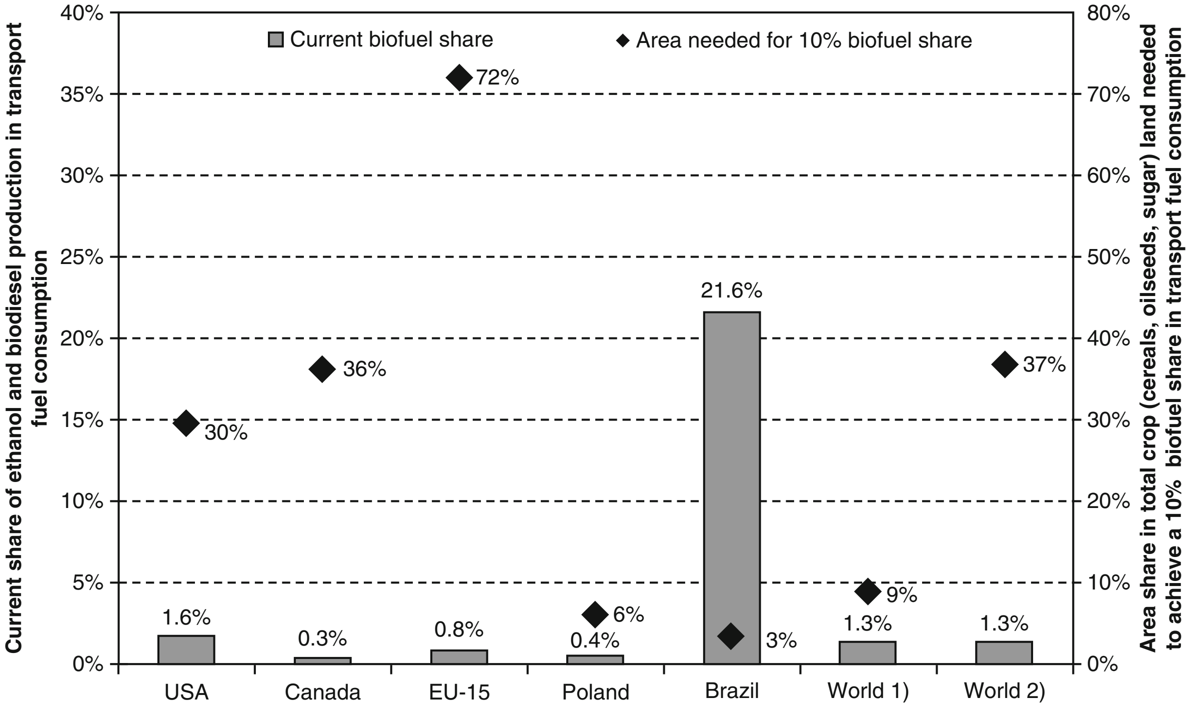

Fig. 24 Biofuel shares in transport fuel consumption and land requirements for 10% biofuel shares in major biofuel producing regions (OECD, 2006).
Notes: Current biofuel shares include ethanol and biodiesel only – shares are on an energy basis. World area shares are calculated relative to land used for cereals, oilseeds and sugar globally (World 1) and within the five major biofuel producing regions only (World 2). All areas requirements are calculated on the basis of average crop area and yield data for 2000–2004 and transport fuel consumption in 2004. For these calculations, the 2004 shares in the feedstock mix are assumed to remain unchanged. Note that calculations for the EU exclude ethanol transformed from wine which represented about 18% of EU ethanol production in 2004

Canada) (OECD, 2006). Except in Brazil and Poland, such a share would require excessive amounts of cropland areas (Fig. 24). In Brazil, the combination of high sugar cane yields and low transport fuel consumption per capita make it possible to go beyond 10% shares up to even more than the current 40%. In the EU, on the contrary, the situation is especially critical as land endowment per capita is particularly low. Moreover, biodiesel represents the largest share of

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European Union encompass only the 15 Member States before the entrance of the other 12 members if not mentioned.

biofuels produced in Europe and also consumes much land area, about five times more than ethanol from sugar beet (OECD, 2006).

As an extrapolation of land areas allocated to biofuel production in 2004 and the correspondent transport fuel share of biofuels, 72% of the total cropland area of relevant crops harvested in 2004, would have been needed to reach the same target in the EU (OECD, 2006). Fulton et al. (2004) assessed the potential impacts on cropland areas if the US and the EU were to expand 1st generation biofuel production to reach targets of 5% in 2010 and 10% in 2020 displacement of both road transport gasoline and diesel future consumption (on an energy basis). Considering constant total cropland areas, including set-aside lands in the EU, and annual increases in crop yields (1% for all crops) and conversion efficiencies (1% for ethanol and 0.3% for biodiesel), by 2010 some 20 and 21% of total cropland area would be needed to produce enough biofuels in the EU and the US, respectively, rising to 38% and 43% by 2020. Especially for biodiesel to displace 10% of consumed transport diesel much higher land allocations would be necessary, even over 100% of projected EU oil-seed croplands as soon as by 2010 or by 2020 in the US (Fulton et al., 2004).

Despite fundamental differences between the two estimates, i.e. the time frame and calculation method, total land areas, assumptions on crop yields and fuel consumption increases, the share of sugar beet ethanol in the EU and the biodiesel production projection in the US, both estimates show very high requirement of land areas to reach the 10% biofuel target. They also emphasise that it may make sense to focus more on ethanol blending rather than on biodiesel, since in the medium term substantial amounts of biofuels can only be achieved if the feedstock mix is adjusted in favour of commodities with a higher biofuel output per hectare. This may also include imported feedstock and biofuels (OECD, 2006).

Within the EU (27 Member States) and Ukraine, a 10% biofuel share could be reached by 2020 relying on low-cost 1st generation biofuels without endangering food security or nature conservation, assuming that demand for other bioenergy sectors remains confined to forest feedstock, i.e. 100% of the agricultural feedstock for bioenergy is dedicated to biofuels, and provided that 30% of the target is met by imports from outside Europe, without imports a 9% share could be reached by 2030. Compared with previous estimates, higher biofuel potential within the EU is notably due to higher potentials in new member states. However, if 10% is not the end term for biofuels, 2nd generation biofuels will become more competitive (REFUEL, 2008).

The development of 2nd generation biofuels produced from residues and dedicated crops appears crucial to lower the pressure on cropland areas. Compared with 1st generation biofuels, 2nd generation biofuels can be produced from a wider range of feedstock, including agricultural and wood-related residues without direct use of land, or dedicated crops that can be grown on a wider spectrum of soils. Second generation biofuels also make two- to four-fold higher land-use efficiencies possible due to higher crop yields, less agricultural management inputs and better conversion efficiency.

By 2030, a 15 to 25% biofuel share could be met by a mix of 1st and 2nd generation biofuels produced in Europe only (REFUEL, 2008). Considering the estimated cellulosic feedstock from residues and bioenergy crops on marginal lands in the US ($\approx$388.5 million dry tons per year at US\$50.ton^{-1}) and a conversion efficiency in a post-2010 scenario of 400 L ethanol per dry cellulosic feedstock ton, second generation ethanol could provide up to 26% of US annual motor gasoline consumption by 2020 without using dedicated cropland area (in Fulton et al., 2004).

Furthermore, yields of dedicated lignocellulosic energy crops are expected to grow much faster than those of conventional crops as research in breeding new varieties or adapting crop farming are still in the early stages. Finally, cooking oils and other municipal wastes could contribute to a lower extent to biofuel production, poor economics of scale being compensated for by low feedstock prices. In the EU and the US, 1 and 1.9 billion litres of biodiesel, respectively, could be produced annually, about one percent of diesel consumption in the US in 2010 (Fulton et al., 2004).

6.4 Projected Worldwide Biofuel Production and Consumption

In the reference scenario of the IEA with 2004 as the baseline and a global primary energy demand increase of 1.6% per year (IEA, 2006), total world

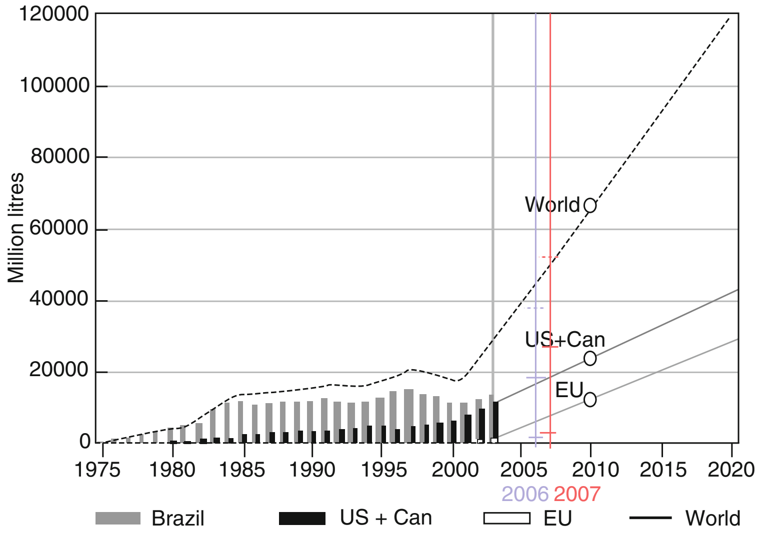

Fig. 25 Fuel ethanol, production and projections to 2020 (Fulton et al., 2004) added production data for 2006 and 2007 from F.O. Licht

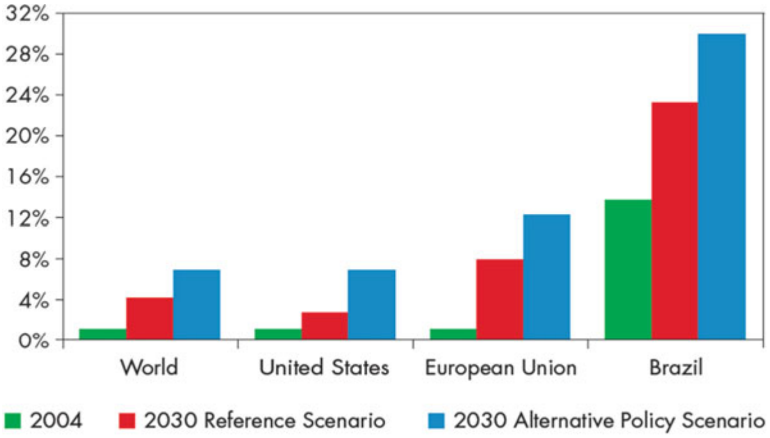

Fig. 26 Share of biofuels in road-transport fuel consumption in energy terms (IEA, 2006)

production of 1st generation biofuels is projected to climb up to 3.85 EJ yr^{-1} (92 Mtoe) by 2030, expressing an average annual growth rate of 6.3%. Figure 25 gives an overview of past and projected ethanol production, showing that global trends follow the predicted scenario, the EU still produces less than expected, compensated for by the US where it is the contrary. In 2008, world biofuel production reached 46 Mtoe, 65 Gl of ethanol (beyond the prediction in Fig. 25) and 16 Gl of biodiesel (ENERS, 2009).

The IEA Alternative Policy Scenario incorporates 1400 different policies and measures that aim at enhancing energy security and mitigating CO_2 emissions. Measures in the transport sector would produce close to 60% of all oil savings in this scenario, more than two-thirds linked to more efficient new vehicles, the rest being related to the increased use of biofuels. With a faster assumed growth of 8.3% per year, biofuel production could rise to 6.15 EJ yr^{-1} (147 Mtoe) by 2030. In the Reference or Alternative Scenarios, biofuels meet 4% or 7% of the world road-transport fuel in 2030, occupying 2.5% (34.5 Mha) or 3.8% (52.8 Mha) of world total agricultural land, respectively (Figs. 26, 27). If cellulosic ethanol were to be largely available by 2030, a larger share of biofuels (10%) could be possible with only a little extra land area (+ 5.7 Mha).

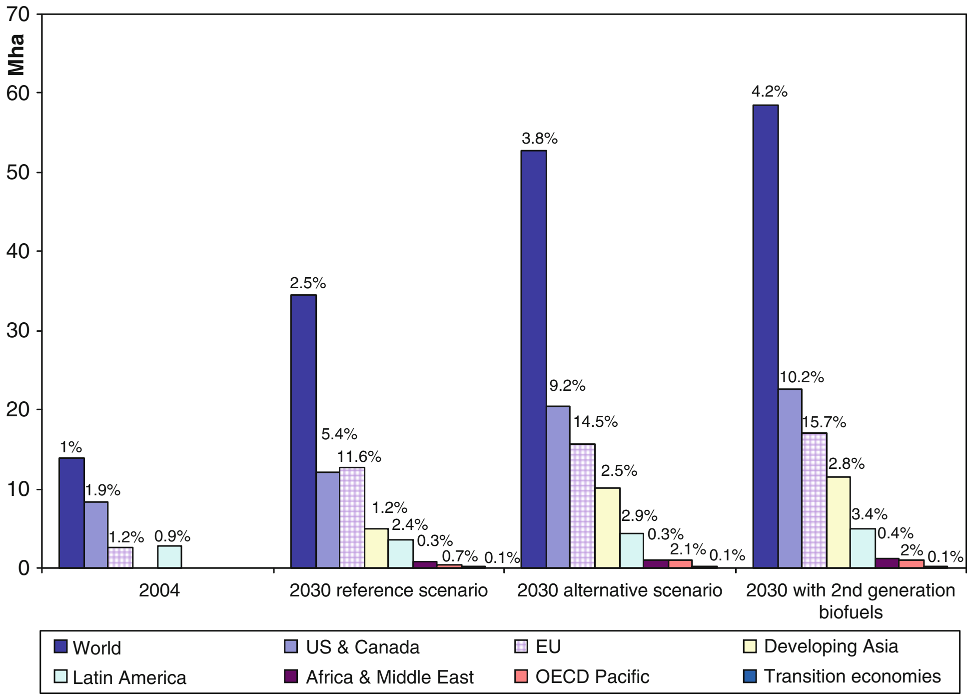

Fig. 27 Land requirements in Mha and percent of respective agricultural land areas for biofuels production in the different scenarios from the IEA. Drawn from data in IEA (2006)

Ethanol is expected to account for most of the biofuel increase worldwide as production costs are expected to fall faster than those of biodiesel and as it is likely to become a more attractive option for fuel suppliers in Europe. The global share of biodiesel nonetheless will grow in both scenarios because of production increase in the US and Brazil; it could reach up to 15% of total biofuel use in both countries. In both scenarios, the biggest increase in biofuel production and consumption occurs in Europe and the US may become a sizeable net importer of biofuels. Brazil remains the biggest ethanol exporter but other countries such as Malaysia or Indonesia could also become biofuel exporters. However, the assumed development of today's still limited international trade in biofuels will depend on whether trade barriers are removed or not (IEA, 2006). Biofuel trade liberalisation would lower the prices of blended fuel and enhance total biofuel demand imports. The shift from domestic production to import from abroad would be rather significant in most European countries for ethanol, from around 95% of domestic production to an average 50% share in 2020 across member states. Trade amongst European member states would also decrease, from around 5% to 2%. For biodiesel, the impact should be much lower as import tariffs are much lower than for ethanol (Boeters et al., 2008).

If the IEA Alternative Policy Scenario was successfully implemented, energy-related CO_2 emissions could be cut by 1.7 Gt or 5% in 2015, and 6.3 Gt or 16% in 2030 relative to the Reference Scenario; 12% of these savings would come from renewables (including biofuels), 10% from nuclear power and the remaining from more efficient production and use of energy. Despite these savings, global CO_2 emissions would still be 8 Gt higher in 2030 than they are today. Going beyond the Alternative Policy Scenario to keep emissions at current levels, i.e. saving these 8 Gt, would mean increasing electricity efficiency by 50% over the alternative scenario and to implement new technologies such as CO_2 Capture and Storage. In this last scenario, 1Gt more would be saved in the transport sector thanks to more efficient and cleaner vehicles, notably using twice as much biofuel as in the Alternative Policy Scenario (IEA, 2006).

6.5 Impact of Biofuels on Agricultural Commodity Prices

As a background assumption in most potential assessments, energy crops should not compete with food and feed crops for land. In practice, land areas that are allocated in the supply-driven studies to food crops because of their high yield potential could also be allocated to energy crops by farmers depending on the markets. In a sensitivity analysis of the impact of land allocation on the bioenergy potential, 30% to 51% of the most productive land areas previously allocated to food and feed crops would then be allocated to energy crops, leading to an overall decrease in bioenergy potential in most cases due to the need for more agricultural land to produce enough food and feed on less productive areas. On the contrary, a global geographical optimisation of land-use patterns by allocating the most productive land areas to food crops would result in an increase in bioenergy potential in all scenarios (Smeets et al., 2007).

In 2006, global cereal stocks, especially wheat, were at their lowest levels since the early 1980s. As a result of reduced plantings and adverse weather in some major producing countries, world cereal production decreased by 2.4% between 2005 and 2006, coinciding with further expansion of the demand. Commodity prices hence rose steeply, further pushed up by speculative transactions adding to increased commodity-price volatility (Von Braun, 2007). Despite a more favourable global production outlook for the coming years, prices are unlikely to return to the low levels of previous years due to a host of reasons, notably the escalation of cost of inputs and the need to replenish stocks (FAO, 2008a). Biofuel production has contributed to the changed world food equation. While cereal use for food and feed has increased by 4% and 7%, respectively, since 2000, the use of cereals for industrial purposes, such as biofuel production, has increased by more than 25%; in the United States alone, the use of maize for ethanol production increased 150% between 2000 and 2006 (Earth Policy Institute and FAO in Von Braun, 2007). As a consequence, the prices of commodities used in biofuel production are becoming increasingly linked to energy prices. The coefficient of variation of oilseed price in the past five years was 0.2[64], compared with typical coefficients in the range of 0.08–0.12 in the past two decades; that of maize has increased from 0.09 to 0.22 in the past decade (Von Braun, 2007).

Since feedstock represents the principal share of total costs, the biofuel sector will both contribute to feedstock price changes and be affected by them. Hence, the impact of biofuel expansion on projected food prices is not yet well established (Von Braun, 2007). Furthermore, due to competitive land uses, biofuels may also impact prices of other food commodities than their own feedstock. Higher prices for maize, for instance, caused food consumers to shift to rice and wheat, while it was more profitable for producers to preferentially grow maize over these same crops. Prices of rice and wheat therefore increased. Comparing the expected grain price increase between 2000 and 2007 with and without the biofuel growth that occurred at that time, IFPRI estimated that biofuels were responsible for 30% of the increase in weighted average grain prices (Rosegrant, 2008).

Using the OECD/FAO Aglink/Cosimo/Sugar model (OECD, 2006), impacts on commodity prices until 2014 were modelled with three biofuel development scenarios compared with a baseline scenario, i.e. status quo agricultural policies, normal weather patterns, and biofuel growth in the US and Brazil only: the constant biofuel scenario with no growth in biofuel production, the policy-target scenario with projected biofuel growth in line with officially stated goals (in 2004), and a third scenario considering the policy-target scenario and higher oil prices (Fig. 28).

The biofuel growth in the baseline scenario has a relatively small impact on coarse grain, wheat and vegetable oil prices, increases of 2.5%, 1% and 1.5%, respectively, compared with the constant biofuel scenario. Impact on livestock markets prices is also limited despite slight price decreases due to the production of co-products such as oilseed meal for feed. The impact on sugar price is much larger, a 37% price increase up to 60% in the policy-target scenario.

The policy-target scenario especially requires enhanced biofuel production in the EU and Canada added to increased production in the US and Brazil. As a consequence, substantial growth in feedstock needs implies significant trade pattern changes. EU imports of vegetable oils would increase three-fold, while wheat exports would fall by 41%, and Canadian wheat and coarse grain exports would also decrease by 34% in 2014. Overall, world prices for most

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[64] i.e. 20% around the annual mean price.

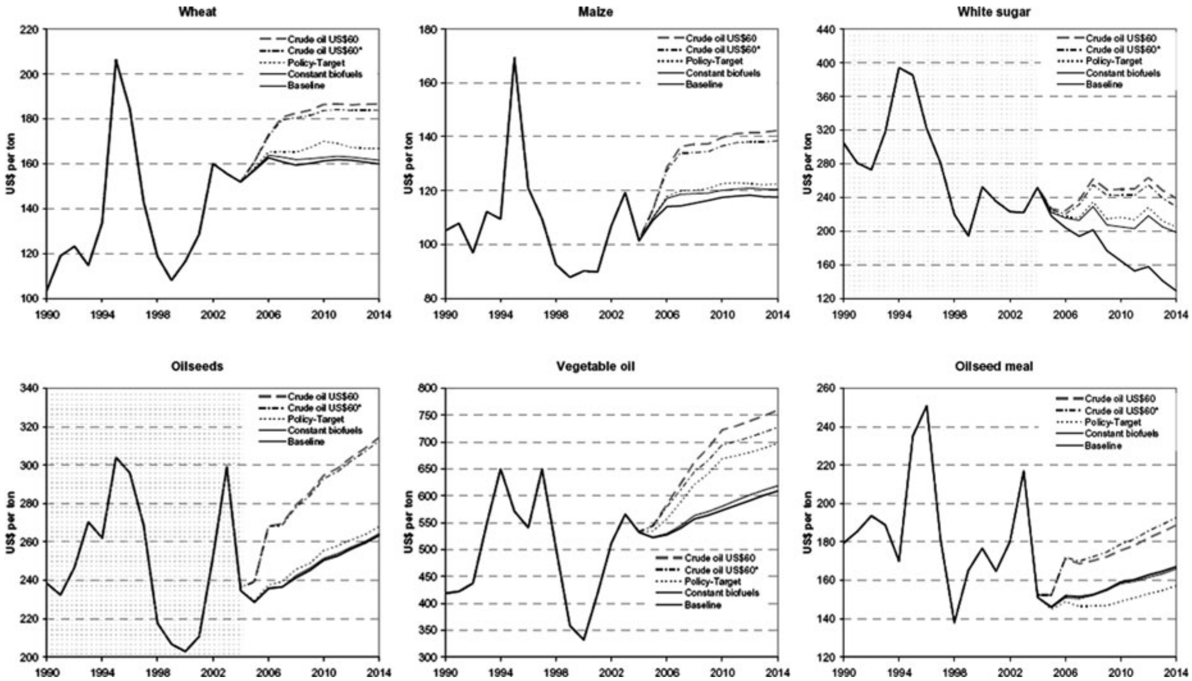

Fig. 28 World crop market prices under alternative scenario assumptions (OECD, 2006).
Note: "Crude oil US$60*" denotes a scenario assuming higher crude oil prices, but unchanged petrol-based fuel prices (and hence unchanged biofuel prices) relative to the policy-target scenario

commodities increase substantially compared with the constant biofuel scenario, by 4% for wheat, and 15% for vegetable oils, also inducing an increase in butter price of 3% as a substitute for oil (OECD, 2006). Despite a more significant decrease in oilseed meal price (–6%), meat market prices increase with and without growth in biofuel production. With crude oil price at a sustained level of US$ 60 per barrel from 2005 to 2014 instead of US$ 45 to US$ 35 in the other scenarios, commodity prices first increase due to the high production costs. Biofuel costs also increase, but compared with the policy-target scenario, at the same time oil price provides a further incentive for biofuels, whose share would increase to 6% in 2014, compared with 5.5% in the policy-target scenario. The increase in biofuel production would lead to a further increase in commodity prices (OECD, 2006). In comparison, IFPRI modelled that in a 2007-baseline biofuel production, maize and oil prices would be higher by 6% and wheat and sugar by 4% in 2014 compared with a constant biofuel scenario (Rosegrant, 2008).

By 2020, taking current biofuel investment plans (2007) into account, international prices could increase by 26% for maize, by 18% for oilseeds, by 11% for sugar, and by 8% for wheat (Von Braun, 2007). While the large response of sugar price to biofuel production is suspected to be inaccurate (note 35 in (OECD, 2006)), the lower response in the IMPACT-WATER model (IFPRI) is also surprising since sugar price is highly related to ethanol and energy prices, unless sugar price increase is limited in IMPACT-WATER following the WTO sugar reform or other factors. In Boeters et al. (2008), the impacts on food prices of a 10%-biofuel target turn out to be negligible on the European Union scale compared with the policy baseline, i.e. the current economic welfare already impacted by distortionary taxes.

Market price projections and comparisons remain superficial, as many factors are still highly uncertain, such as food and feed demand, speculations on commodity prices, and biofuel international trade, including the future role of developing countries, and the development of 2nd generation biofuels. The latter are expected to be produced mainly from residues or dedicated energy crops that will not lead to an additional demand for food commodities as feedstock. The use of marginal lands could lower the pressure on agricultural lands and therefore the land prices. However, this potential remains limited due to low productivity, high costs and potential impacts on landscape.

Where dedicated crops would take over food crops for the use of land, the impact on food prices would also depend on local policies. For instance, within the framework of the US 1996 Farm Bill, a scenario of switchgrass production substituting 4–9.5 Mha allocated to food crops between 1996 and 2000 could have led to a price increase of from 4–14% of maize, sorghum, wheat, soybean, cotton and rice. However, through higher farm income and reduced loan deficiency payments, switchgrass promotion could have led to significant savings for the treasury (Ugarte and Walsh, 2002), the bottom-line cost being passed on to the consumer prices. As an example, according to OECD estimations, the CAP cost to ordinary citizens is around 100 billion € each year (US$ 125 billion), half from taxpayers and half from consumers owing to higher food prices. This is an average cost to an EU family (4 people) of around 950 € a year (US$ 1190), with only around 20 € (US$ 25) of this spent as EU money on targeted environmental programmes. The CAP has been estimated to be equivalent to a value added tax on food of around 15% and removing market price support would bring a one-off reduction in inflation of 0.9% (OECD, 2005).

7 Conclusion

Although bioenergy and biofuels in particular have recently been high on the policy agenda and subject to a lot of discussion, they still only contribute a marginal share in the global energy supply. Some key points may help to figure out how bioenergy can play a bigger part in the years to come.

- When reviewing biomass potential assessments, a rather modest assumption would be that the share of bioenergy in the total energy consumption can be multiplied by at least a factor of 2.5, but such deployment scenarios are extremely hard to predict since technology evolution is non-linear[65]. This means breakthroughs may be expected along the way, notwithstanding the driving role of policies that are also constantly evolving. However, it remains certain that the contribution of biofuels will be limited because of the scarcity of available land, unless advances in technology make tremendously higher yields possible, in biomass production and conversion, with significantly lower costs. For second generation biofuels, a ten-fold increase in the plant productivity is still needed to reach commercial potential. A global 10% share of transportation fuels, excluding international aero-traffic, may be reached by 2020–2030, provided that biofuel chains are optimised in terms of both environmental and economic performance, and combined with changes in the automotive sector towards: lighter cars, hybrids, flex-fuel and city-vehicles. Taking the 13.5% contribution of transport to global CO_{2eq} emissions, total emissions in 2000 of 41.75 $GtCO_{2eq}$ (Baumert et al., 2005), and a range of greenhouse gas savings of 20–60% (FAO, 2008a), 10% biofuel share would result in a reduction of 113–340 $MtCO_{2eq}$ per year. If second generation biofuels become available by 2030 and are combined with hybrid technology, biofuels could save an additional 1 $GtCO_{2eq}$ per year (IEA, 2006). This may represent a small contribution, but still a necessary one given the current trends and the scope of the GHG reduction targets (Fig. 29).
- The sooner second generation biofuels are commercially available, the more likely the 10% target will be met. Although they out-perform first generation biofuels, the latter will develop further and serve as a springboard for the second generation biofuels to be quickly introduced within well-established biofuel chains. Therefore, attention must be paid to the diffusion of best available practices and to the attainment of sustainability standards of first generation biofuel chains.
- From a CO_2 perspective, the best mitigation pathway is to prevent emissions. A combusted litre of biofuel will never perform better for the environment that a non-combusted litre of fossil fuel. However, a significant part of global greenhouse gas emissions cannot be avoided and for those only CO_2 or C sequestration can have a mitigative effect. From a sequestration point of view, the interest of biofuels is to concentrate non-point sources from transport into facilities where they can be captured, as is the case with CO_2 from sugar fermentation, for instance. It can be expected that with the need for

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[65] The Schumpeterian vision of technology advances that evolve by plateaus punctuated by radical breakthroughs.

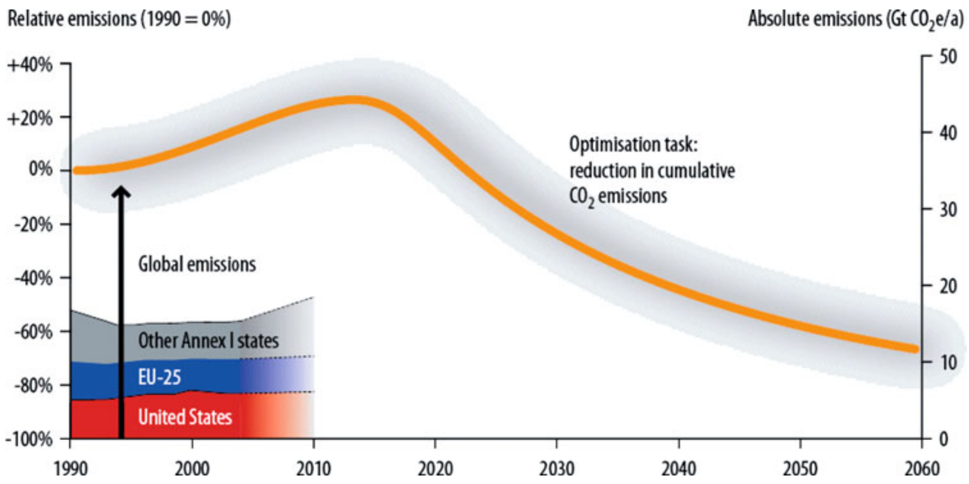

Fig. 29 Necessary reduction in greenhouse gas emissions (including from deforestation and other land use) to stay within the 2°C global warming target from today's perspective (Fischedick et al., 2007)

reducing industrial emissions, biofuel conversion facilities will keep improving in efficiency and gas savings or storage. Agricultural practices may also contribute to enhancing soil carbon sequestration; but while the residence time of stored soil carbon is not permanent and not easily controllable, the turnover of fixed atmospheric CO_2 by biomass and released through combustion can be quantified and in principle repeated indefinitely. Here, the intrinsic interest of biomass is that photosynthesis will be enhanced as atmospheric CO_2 concentration increases, provided that no other production factors are limiting, creating a negative feed-back loop. Finally, the overall interest of biofuels in terms of CO_{2eq} savings relies on the overall performances of agro-ecosystems, which in most cases and for all agricultural production can be largely improved.

- Soil organic carbon lost through deforestation negates the benefit of biofuel in terms of CO_2 savings. However, merely shifting the burden of deforestation and biodiversity losses onto biofuels will not stop land clearing for agricultural purposes. On the contrary, funds for biofuel development programmes could provide leverage to implement sustainability criteria for agricultural production worldwide, and to enhance the productivity of traditional slash and burn cultivation, thereby preserving the forest. Moreover, policies are also needed, notably in tropical regions, to empower local populations to prevent illegal logging and effectively urge forest preservation and investment in productive and environmental-friendly agro-ecosystems. In Brazil, for instance, it is cheaper to clear new land areas for the international beef and soya bean markets than to invest in already deforested regions[66].
- All bioenergy chains are not suitable for all locations. Bioenergy chains can bring benefits to the society in terms of fossil energy savings, as well as other positive environmental impacts, but only if the best appropriated bioenergy chain mix is chosen in accordance with local conditions, notably the biomass production systems and the types of primary energy inputs for conversion. Better knowledge of life-cycle greenhouse gas emissions from all energy uses of biomass, and strong sustainability criteria for biomass production, also addressing trade-off effects due to indirect land-use change, are still needed to fully assess the benefits and limitations of biomass use (EEA, 2008). Given the wide range of candidate biofuel chains, options will have to be identified and taken that minimise adverse environmental impacts while harnessing most of the advantages of biofuels. The resulting bioenergy mix will be systematically better for the environment than the business-as-usual scenario.

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[66] Dr. Emily Boyd, 25/11/2005 http://www.scidev.net/en/opinions/emissions-trading-cannot-solve-amazon-deforestatio.html.

In countries where land area is the main limiting factor, for instance, the priority should be given to biofuels from waste oils, animal grease, residues and municipal waste. The competition for the use of biomass for heat and power or biofuel should also be limited as far as possible by giving priority to the bioenergy chain that makes it possible to save the most greenhouse gases compared with the local substituted energy source.

- In the case of sustainable bioenergy chains, positive externalities ought to be given economic values so that bioenergy could be more competitive. Overall benefits from bioenergy chains have to be considered, taking into account the value of all co-products. Considering the competition for natural resources, the principle of "zero waste" within integrated biorefinery appears to be the best economic and environmental choice. The use of contaminated or degraded lands for bioenergy purposes is also essential. Economic incentives should thus aim at fostering these priorities.
- The "success story" of ethanol in Brazil suggests that further growth in biofuel production can be expected through intensive breeding programmes to foster the development of second generation biofuel feedstock. Indeed, the high productivity of sugar cane has benefited from decades of research and commercial cultivation. Nowadays, cane growers in Brazil use more than 500 commercial cane varieties that are resistant to many of the crop diseases found in the country. Between 1975 and 2000, in the São Paulo state, the sugar cane yield per hectare increased by 33%, ethanol yield from sugar by 14%, and fermentation productivity by 130% (Kojima and Johnson, 2005). Another key element is the flexibility of the production unit, that has to be found as a balance between complete integration to reach maximum efficiency, minimum losses and economies of scale, while this optimum-oriented specialisation will not prevent the unit from being flexible enough to adapt the processes to various feedstocks and end-products. Most distilleries in Brazil are part of sugar mill/distillery complexes capable of switching between 60%–40% and 40%–60% sugar-ethanol, which makes it possible to take advantage of fluctuations in the relative prices of sugar and ethanol. In France for instance, mill/distillery complexes are optimised to produce 66% sugar and 33% ethanol on a year-run basis, with very little flexibility[67]. The success of the mill/distillery complexes also relies on good integration within both the nationwide ethanol supply system and the electricity grid to sell the co-produced electricity, once their auto-consumption is satisfied (Kojima and Johnson, 2005).
- Bioenergy can contribute to tackling part of the energy dependency and the depletion of non-renewable resources, but they alone will not suffice. Their development would also be vain if at the same time energy efficiency was not drastically improved and energy consumption behaviours did not change radically. As the transport sector is the main growing source of energy consumption and greenhouse gas emissions, biofuels play a critical role as long as the bulk of vehicles is not electrified. Despite improvements in the energy efficiency of various transport modes and the introduction of non-fossil fuels, increased transport demand, especially increased car usage and a reduced number of passengers per car, is outweighing these benefits. Present knowledge indicates that it will not be possible to achieve ambitious targets comparable with the Bali roadmap without limiting transport demand (EEA, 2008). In a recent survey, responses from 25 767 EU citizens indicated that 54% would be willing to pay more for using less-polluting transport. The best ways to reduce transport CO_2 emissions and to promote biofuels would be through an interdiction to sell "polluting vehicles" that do not achieve state-of-the-art emission standards, and tax incentives to foster both fuel-efficient vehicles and biofuels (Fig. 30, Eurobarometer, 2007). However, evidence also suggests that only a minority of individuals actually take action to reduce private transport energy consumption and fewer may intend to take action in the future. Analysing "Special Eurobarometer" surveys from 1984, 1993 and 2002, it appears that action concerning reducing car fuel use has not increased between 1993 and 2002, despite an increase in real fuel prices, and intentions to take action to reduce energy use were generally lower in 2002 than in 1993 or 1984. According to the 2002 survey, more than half of all respondents (55%) reported having taken no action on energy

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[67] Personal communication by Dane Colbert, Director of Ethanol Union, 30/10/2008.

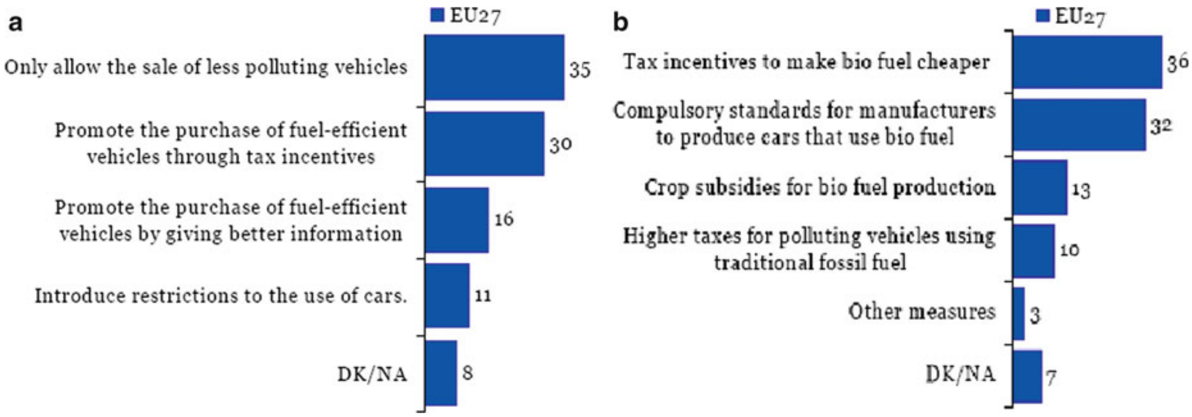

Fig. 30 (**a**) The best way to reverse the rise of CO_2 emissions from road transport in response to question Q5: road transport generates about one fifth of the European Union's harmful emissions. Between 1990 and 2004, CO_2 emissions from road transport rose by 26%. Which is the best way to reverse this trend? (**b**) The best way to encourage the use of biofuels in response to question Q8: biofuels are renewable fuels that can reduce fossil oil dependence of vehicles. Which is in your opinion the best measure to encourage the use of bio fuels? %, Base: all respondents DK: Don't Know/NA: No Answer (Eurobarometer, 2007)

efficiency in any of the four broad transport energy efficiency measures examined (reducing travel, cutting fuel use, buying a more efficient vehicle and using public transport) while almost two-thirds of all respondents (64%) report that they do not intend to take further action in any of these areas of energy efficiency (Stead, 2007). Regarding the fact that awareness of transport pollution issues is, moreover, likely to be lower on a global scale than in Europe, radical measures may be necessary in order to radically change transport consumer behaviours. To address transport demand, measures and policy instruments must hence also go beyond the transport sector itself and be introduced into sectors of the economy such as households, industry and service, within which the demand for transport actually originates (EEA, 2008).

- Harmonisation at an international level is crucial in order to ensure the overall complementarity of bioenergy chains, to provide a coherent framework for the markets and to control the sustainability of the systems. In particular, the framework for international biofuel trade is complex: trade barriers should be removed so that no artificial competitiveness would hamper the development of biofuels, but on the other hand, control of sustainability is necessary on a global scale and instruments should be put in place to ensure it. Voluntary schemes for the certification of sustainable biomass already exist, e.g. the Forest Stewardship Council (FSC) for forest product or the Roundtable Sustainable Palmoil (RSPO), and are currently being discussed as implementation options for bioenergy sustainability standards. Hence most of the key elements for such standards are available as well as experiences from existing voluntary schemes. Although legally binding standards are superior, pragmatically, voluntary schemes might provide a well-needed start ("entry option") (Fritsche et al., 2006). Finally, cross-sector integration of agriculture, energy and transport policies is also mandatory to make biofuel incentives coherent and to send a clear message to the population.
- Concerns about GMOs and rising food prices are justified. However, these are not exclusive to biofuels, and should be addressed in a wider perspective. Not mentioning the debate on GMO, competition for land uses between food and non-food crops should be minimised as much as possible. Where competition for land uses is critical, market distortion should not, as in the case of the cotton market, spoil opportunities given to developing countries. It is important that governmental support for biofuels as an infant industry remains temporary, or else the policy will result in inefficient allocation of resources in the long run, once costs decline as output expands and production experience is acquired. The extent to which biofuel programmes can contribute to rural development is dependent on the industry characteristics and, ultimately, whether

it is able to become financially viable without direct government support. However, if public funds are needed to support the industry, the question to be addressed in the first place is whether government resources will be diverted from other programmes and what would be the comparative impacts on rural development and the environment (Kojima and Johnson, 2005). From an agronomical point of view, promising options in developing countries are in particular those that introduce energy crops within agro-forestry systems. However, the need for an intensification of the production, and also the development of perennial plantations, may remain largely hampered as long as the lack of land property rights does not make it possible to empower the farmers. Biofuel programmes need to be integrated within a broader context of investment in rural infrastructure and human capital formation. Indeed, strengthening property rights, removing both international and domestic trade-barriers, access to education, water, electricity and networks, and developing transport infrastructure have proven better drivers of rural development than direct aid in providing farming equipment or inputs, for instance (Kojima and Johnson, 2005).

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Agronomic and Physiological Performances of Different Species of *Miscanthus*, a Major Energy Crop

H.W. Zub and M. Brancourt-Hulmel

Contents

Abstract The European Union recommends the use of lignocellulosic biomass to produce biofuels in order to reduce greenhouse gas emissions. *Miscanthus* × *giganteus*, a C_4 perennial and rhizomatous plant, has been identified as a good candidate for biomass production because of its high potential yield, of up to 49 t DM.ha^{-1} for autumn harvest and 26 t DM.ha^{-1} for winter harvest, under low input levels. Here, we review current knowledge on the biomass production in Europe of *M.* × *giganteus* and its two parental species, *M. sinensis* and *M. sacchariflorus*, under different stress conditions. This review identifies two key areas where *M.* × *giganteus* crops could be improved: (i) tolerance to frost during winter or early spring is essential, mainly in Northern Europe, in order to ensure overwintering and protect young shoots following early emergence. Susceptibility to winter frost at temperatures below −3.5°C for rhizomes and −8°C for young shoots of *M.* × *giganteus* can lead to significant plant losses and lower yields, and (ii) a good water supply is necessary to ensure good establishment rates and satisfactory biomass production. Reductions of up to 84% in above-ground dry matter production because of a lack of water for the autumn harvest, and up to 26% for the winter harvest have been observed. *M. sinensis*, which displays greater genetic variability than *M.* × *giganteus*, will provide the necessary genetic resources for frost and water stress tolerance. It is also necessary to either identify genotypes among *M. sinensis* species that are able to produce an above-ground biomass yield close to the biomass production of *M.* × *giganteus* under limited water supplies and/or low temperatures, or to generate new interspecific hybrids of *M.* × *giganteus* with greater tolerance. Particular attention should be paid

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_21,

from Zub and Brancourt-Hulmel, Agron. Sustain. Dev. 30 (2010) 201–214. DOI: 10.1051/agro:2009034

to nitrogen response; although no response to nitrogen supply has been observed in *M.* × *giganteus*, *M. sinensis* produces higher levels of biomass with nitrogen inputs.

Keywords *Miscanthus* • Biomass production • Chilling temperature • Frost • Nitrogen supply • Water supply • Improvement

Abbreviations

DM	dry matter
WUE	water-use efficiency
NUE	nitrogen-use efficiency
RUE	radiation-use efficiency

1 Introduction

Energy security and imperatives related to climate change require the large-scale substitution of petroleum-based fuel. Plant-based biofuels will achieve a reduction in greenhouse gas emissions through the fixation of atmospheric carbon in useful biomass (Ceotto, 2008) and the sequestration of carbon in the soil (Benbi and Brar, 2009). European Directive 2003/30/EC promoted the use of renewable sources by defining targets for biofuels in 2010: 5.75% of the fuel consumed in Europe should be produced from biomass. As ethanol production in France, this corresponds to about 2.5% of the total land planted to cereals to enable its production from conventional crops such as sugarbeet and winter wheat (Gurtler, 2007). To reduce this proportion, new "dedicated" energy crops are of particular interest for the production of biofuel using lignocelluloses, a structural material that accounts for much of the mass of plants. One of the benefits of lignocellulosic biofuel is that it reduces greenhouse gas emissions by 85% compared with reformulated gasoline (Wang et al., 2007). Crutzen et al. (2008) found that ethanol produced from corn and sugarcane had a "net climate warming" effect when compared with oil. Another advantage of lignocellolosic biomass is that it can be produced from abundant and diverse raw materials such as wood, grasses or the non-edible parts of plants.

Fig. 1 *Miscanthus* × *giganteus* during the third year of growth at Estrees-Mons, France (© INRA Lille)

Among these different raw materials, a good candidate is miscanthus, a tall, perennial, rhizomatous C_4 grass of the Poaceae Family (Hastings et al., 2008). It produces a large quantity of biomass under low input levels (Clifton-Brown et al., 2004). Its tropical and subtropical genotypes grow to 3 to 4 m when cultivated in Europe, and more in the warm and wet climates of southeast Asia (Fig. 1). Miscanthus rhizomes, or micro-plants, are planted in spring and the canes produced during the summer are harvested annually during the late autumn or winter, following the second or third growing season of the crop. The lifetime of the crop varies from 20 to 25 years (Lewandowski et al., 2003), and the long-term *Miscanthus* plantations enhance soil carbon sequestration (Hansen et al., 2004). Miscanthus spreads naturally *via* its underground storage organs or rhizomes, but some species can also be seed-propagated. Because miscanthus is propagated vegetatively, the clone is the variety type.

The cultivation of miscanthus in Europe is mainly based on one species, *M.* × *giganteus*, which is a natural triploid hybrid between a diploid *M. sinensis* and a tetraploid *M. sacchariflorus* (Greef and Deuter, 1993; Linde-Laursen, 1993). *M.* × *giganteus* production is characterised by low input levels, so that it is a relatively environmentally-friendly crop (Lewandowski et al., 2000). To date, no susceptibility to diseases or pests has been reported by the authorities in the UK (DEFRA, 2007), the European country where the crop is most widely cultivated.

The principal limitations to miscanthus production from *M.* × *giganteus* are its high establishment costs, its poor overwintering at some sites and the insufficient water supplies available in Southern regions of Europe. *M.* × *giganteus* has been found to display very little genetic diversity because of its sterility and vegetative propagation. Most of the clones found in this species were descended directly from the 'Aksel Olsen' clone, as shown by isozyme and DNA studies (Vonwuhlisch et al., 1994; Hodkinson et al., 2002). The only exception may be the 'Hervey' clone that came from Japan to the UK in the 1980s (Greef et al., 1997). The small genetic base of *M.* × *giganteus* explains why the same clone has nearly always been used during agronomy studies or for cultivation. The sterility of *M.* × *giganteus* is particularly interesting because it prevents putative invasion by the species; on the other hand, it is an issue in terms of improving biomass production and adapting it to a broad range of climatic conditions. Nevertheless, the *Miscanthus* genus contains more than 20 species, most of them originating from a broad range of geographic regions in Asia, from the sub-tropics to sub-arctic conditions (Numata, 1974, cited by Clifton-Brown and Lewandowski, 2002). Furthermore, the genetic diversity of the *M. sinensis* pool is relatively high compared with *M.* × *giganteus* accessions (Greef et al., 1997). *M. sinensis* is described as being self-incompatible (Greef and Deuter, 1993), which facilitates crossing and genetic studies; it can therefore be exploited to improve its resistance to abiotic factors or its industrial properties.

In order to highlight the need for research on the adaptation of miscanthus to European conditions, the present paper reviews the agronomic and physiological performance, and response to various stresses, of different *Miscanthus* that could be used to produce biomass in Europe. It focuses on the three species studied in Europe: *M.* × *giganteus, M. sinensis* and *M. sacchariflorus*. Stress conditions result from an excess or deficit in a physical or chemical environment. The environmental conditions that affect plant growth include high or low temperatures, excess rainfall, drought, or inadequate soil mineral nutrients such as nitrogen. Depending on when these environmental factors occur, their intensity and duration can reduce plant vitality and cause damage. The paper is presented in two sections; the first is devoted to a description of the plant, with particular emphasis on its agronomic potential and the variations affecting the production of biomass production by different *Miscanthus* species, while the second concerns the influence of stress conditions on plant traits.

2 Crop Physiology

Most agronomic and physiological research has focused on one clone of *M.* × *giganteus*. This section describes the life cycle of miscanthus, its requirements for establishment and growth and the efficiencies of the crop in terms of radiation, water and nitrogen (N) use. References to *M. sinensis* or *M. sacchariflorus* are mentioned when available.

2.1 Principal Features of a Miscanthus Crop

To establish a crop, the optimum planting date is dependent on the plant material. Whereas rhizomes can be planted from March to May (depending on the climate), micro-plants or plants established in pots should be planted later (late April–May) in order to avoid late frosts and improve establishment rates (Christian and Haase, 2001). In general, the irrigation of newly-planted rhizomes seems to improve the establishment rate under drier conditions, particularly in Southern Europe. Because of the high production cost of rhizomes or micro-plants, the optimum crop density is very low as the plant is able to produce several stems. The usual density is 2 plants.m^{-2}, but this can be increased to 5 plants.m^{-2}, the level studied in Europe (Lewandowski et al., 2000). However, Christian et al. (2009) recommended planting at least 14% extra rhizome fragments when using 5-year-old rhizomes in order to attain the required plant density.

After emergence, tillering, corresponding to the number of productive shoots per plant, increases rapidly during May, June and July (e.g. up to 40 stems.plant^{-1} for *M.* × *giganteus* during a UK experiment, Bullard et al., 1997). Rhizome-propagated plants may have fewer but stronger shoots than micropropagated plants (Lewandowski, 1998). During the growing period, the number of productive shoots falls to 25 stems.plant^{-1}. This phenomenon can be compared to tillering regression in wheat, barley or forage grasses (Aspinal, 1961; Thorne, 1962; Gillet, 1980). The youngest tillers decline while the oldest continue to grow in August, September or even October, depending on the climate and time elapsing between emergence and flowering.

During the growing period, leaf area development follows a rapid, one-peak curve. In the second year following establishment, it reaches a maximum leaf area index (LAI) of 6–10, depending on treatments and climate. Maximum LAI values have been observed during the flowering phase, after which the canopy starts to senesce (Cosentino et al., 2007). A mature stand of *M.* × *giganteus* is able to intercept around 90% of useful photosynthetic radiation when the LAI reaches 3.2 (Clifton-Brown et al., 2000). A similar value was found for *M. sinensis* Goliath (Vargas et al., 2002). The light extinction coefficient (k) through the leaf cover of the crop provides a measurement of the absorption of light by leaves: *M.* × *giganteus* achieves between 0.56 and 0.68 according to some authors (Cosentino et al., 2007; Clifton-Brown et al., 2000, respectively), whereas *M. sinensis* Goliath reaches 0.66 (Vargas et al., 2002). Most of these values are close to that of 0.67 reported for maize (Clifton-Brown and Jones, 1997).

The end of the growing period coincides with a drop in temperatures, and full senescence can occur with the first frost, as observed in the autumn in Denmark (Christian and Haase, 2001). However, the oldest leaves towards the base of stems start to senesce earlier. At the end of the growing season, nutrients and photosynthates are remobilised from the stems and leaves to the rhizomes (Christian and Haase, 2001). In *M. sinensis*, this accumulation is synchronised with flowering from July to October, depending on the genotype (Stewart et al., 2009). All stems left standing gradually dry out during the winter, until February/March when the crop is ready for winter harvest.

2.2 Threshold Temperature for Emergence and Thermal Time for the Growth and Development of a Miscanthus Crop

The threshold temperature corresponds to the temperature at which the plant can start to grow. Thermal time, which is the cumulated value of degree-days from emergence to flowering time, is often used in crop physiology to estimate or predict the length of different developmental phases under cultivated conditions. The degree-day is a function of baseline temperature. For *Miscanthus*, the threshold and baseline temperatures vary between studies (Clifton-Brown and Jones, 1997; Farrell et al., 2006).

Clifton-Brown and Jones (1997) demonstrated the growth of shoots in a controlled environment at temperatures between 6°C and 20°C, although the extension rate was lower at 6°C than at 15°C or 20°C. Price et al. (2004) concluded that the threshold temperature for growth was 6°C because *M.* × *giganteus* could grow at this temperature. When comparing different species, Koike et al. (1975) cited by Stewart et al. (2009) observed that a daily mean air temperature of 7°C was necessary to initiate growth of the above-ground organs of *M. sinensis.* Farrell et al. (2006) studied the effect of temperature on shoot emergence in four miscanthus genotypes: a clone of *M. sacchariflorus*, a clone of *M.* × *giganteus* and two hybrids of *M. sinensis*. Shoot production was markedly influenced by temperature in all genotypes. The rate of emergence increased in line with temperature (Table 1). Genotypes with a lower threshold temperature for emergence were thus able to emerge earlier. Similarly, the thermal responses for emergence in *M.* × *giganteus* and *M. sacchariflorus* (slope of regression fits of 0.01 and 0.008, respectively) were lower than those of *M. sinensis* hybrids (between 0.011 and 0.014). Higher emergence rates were associated with both a shorter thermal requirement for emergence and earlier emergence. Farrell et al. concluded as to the existence of genetic variability regarding the threshold temperature required for emergence. Hybrids of *M. sinensis* had a lower threshold temperature for emergence than *M.* × *giganteus* and *M. sacchariflorus* (Table 1).

Both Clifton-Brown et al. (2000 and 2004) and Hastings et al. (2008) preferred to use 10°C as the

Table 1 Different relationships between temperature ranges and shoot emergence, and plant and leaf extension rates as a function of different *miscanthus* species. Data from Clifton-Brown and Jones (1997) (1) and Farrell et al. (2006) (2)

		Shoot emergence rate		Leaf extension rate			Plant extension rate				
		best-fit regression model	a_1	best-fit regression model	a_2	b_2	best-fit regression model	a_3	b_3	c_3	d_3
M. sacchariflorus		linear $y = a_1x+b_1$ (2)	0.008	exponential $y = a_2 * e^{b^{x}_{2}}$ (2)	0.0007	0.4458					
M. × *giganteus*			0.01		0.0032	0.354					
M. sinensis Hybrid 6			0.011		0.0039	0.3485					
M. sinensis Hybrid 9			0.014		0.0262	0.2434					
M. condensatus (n = 1)	mean			$y = a_2$ * PER (1)	0.72			−0.0002	0.0107	−0.0645	0.2070
M. floridulus (n = 2)	min				0.81			−0.0002	0.0095	−0.0574	0.1443
M. saccharifer (n = 2)	max				0.88		third-order polynomial $y = a_3x^3 + b_3x^2 + c_3x + d_3$ (1)	−0.0006	0.0242	−0.1800	0.4225
M. sacchariflorus (n = 3)					0.83			−0.0002	0.0083	−0.0212	−0.0323
M. × *giganteus* (n = 2)					0.85			−0.0002	0.0084	−0.0099	−0.0990
					0.83			−0.0001	0.0074	−0.0237	0.0160
M. sinensis (n = 21)					0.69			−0.0004	−0.0034	−0.1060	−0.2950
					0.98			0.0002	0.0164	0.0733	0.3050
M. undine (n = 1)					0.86			0.0001	−0.0001	0.0534	−0.2130

x, temperature (°C); PER, plant extension rate; n, genotypes number.

baseline temperature in their model to predict potential yield without water stress (Clifton-Brown et al., 2000) or under water stress (Hastings et al., 2008). Indeed, Clifton-Brown et al. (2000) showed a better correlation between cumulative degree-days and leaf area index using a threshold temperature of 10°C rather than 6°C. It is necessary to carry out similar experiments with *M. sinensis* in order to determine the threshold temperature and thus compare the thermal times of different *Miscanthus* crops.

Applying a threshold temperature of 10°C for three species, the thermal time required to reach peak heading time was estimated at around 900°C for *M. sinensis* grasses in Japan (Shoji et al., 1990). According to Lewandowski and Clifton-Brown et al. (2000), a thermal time of 1800°C accurately predicted the time for flowering of the only *M.* × *giganteus* clone studied in Europe. Numata and Mitsudera (1969) cited by Stewart et al. (2009) reported a thermal time of 1500–4000°C for the flowering of *M. sinensis* grasses in Japan, and this was equivalent to a vegetative growth period of 6–9 months. Although one study with several *M. sinensis* genotypes was carried out at five sites in Europe (Clifton-Brown et al., 2001a), no data are available on the variations in the thermal time for growth of this species in Europe.

Clifton-Brown et al. (2001a) observed variations in flowering date among genotypes and across countries. The induction of the flowering is dependent on day length and variations are observed among the species. These can be related to the geographical origin of genotypes (Lewandowski et al., 2003). Experiments under artificial light conditions have been carried out to induce flowering. A short-day light period may be necessary to induce the flowering *of M. sacchariflorus* (Deuter, 2000). By contrast, day length may be less important than cumulative degree-days in *M. sinensis*. For instance, a particular genotype of *M. sinensis* flowers earlier in Portugal than in Sweden because it receives its sum of degree-days for flowering more rapidly (Clifton-Brown et al., 2001a).

2.3 Radiation-, Water- and Nitrogen-Use Efficiencies

Because of its C_4 photosynthetic pathway and perennial rhizome, miscanthus displays quite a good combination of radiation-, water- and nitrogen-use efficiencies for biomass production (Lewandowski et al., 2000; Heaton et al., 2004; Lewandowski and Schmidt, 2006). Several studies have been carried out to determine these efficiencies in one clone of *M.* × *giganteus,* and genetic variability has been reported with respect to the radiation- and water-use efficiencies of other *Miscanthus* species.

A linear relationship was found for miscanthus between cumulated above-ground dry biomass (or dry matter, DM) and global intercepted solar radiation (Clifton-Brown et al., 2000). Under water and nitrogen supplies, the radiation-use efficiency (RUE) of *M.* × *giganteus* reached 4.1 g $\mathrm{DM.MJ^{-1}}$ in France and the USA (Tayot et al., 1995; Heaton et al., 2008). Lower values of between 1.9 and 2.4 g $\mathrm{DM.MJ^{-1}}$ were observed, but under conditions of water stress (Bullard et al., 1997; Price et al., 2004; Clifton-Brown et al., 2000) or using a different method to calculate RUE values (Cosentino et al., 2007).

As for water-use efficiency (WUE), studies showed that an adult *M.* × *giganteus* stand reached between 9.1 and 9.5 g $\mathrm{DM.l^{-1}}$ in the UK (Beale et al., 1999) and between 6 and 10 g $\mathrm{DM.l^{-1}}$ in France (Cadoux et al., 2008). Lower values were found in a Mediterranean environment, with a negative correlation of −0.87 between water availability and WUE (Cosentino et al., 2007). Beale et al. (1999) showed that irrigation during dry periods reduced the WUE of the crop by 15%, with a higher water consumption of 45%.

The average nitrogen-use efficiency (NUE) of above-ground *M.* × *giganteus* biomass was calculated at about 277 kg $\mathrm{DM.kg^{-1}}$ of the N supply (total available nitrogen including soil N and N supply not being evaluated), but considerable variability was observed, ranging from 143 kg $\mathrm{DM.kg^{-1}}$ in Ireland to 613 kg $\mathrm{DM.kg^{-1}}$ in Portugal (Christian and Haase, 2001). The NUE calculated from the increase in biomass at different N input levels decreased continuously with an increasing N supply (Lewandowski and Schmidt, 2006; Cosentino et al., 2007). Furthermore, a 9% reduction in NUE was observed with an increase in the water supply (Cosentino et al., 2007).

Genotypic variability has been studied with respect to RUE and WUE, but not NUE. In field conditions under nitrogen supply and without irrigation, Jorgensen et al. (2003a) and Vargas et al. (2002) showed higher RUE values for *M. sinensis* (average: 1.62 g $\mathrm{DM.MJ^{-1}}$) than for hybrids between *M. sinensis* (with an intermediate RUE) and *M. sacchariflorus*. Under water stress conditions, RUE values for hybrids between *M. sinensis* and *M. sacchariflorus* were close to those of *M.* × *giganteus*. By contrast, under controlled conditions, Clifton-Brown et al. (2000) demonstrated similar WUE values for young shoots of *M.* × *giganteus* and *M. sinensis* (2 g $\mathrm{DM.l^{-1}}$), but the highest value was seen in *M. sacchariflorus* (3.8 g $\mathrm{DM.l^{-1}}$).

Several studies comparing the RUE (Beale and Long, 1995; Heaton et al., 2008), NUE (Boehmel et al., 2008; Lewandowski and Schmidt, 2006) and WUE (Beale et al., 1999) of miscanthus with other energy crops concluded as to the superiority of miscanthus efficiencies over other species. Firstly, Beale and Long (1995) and Heaton et al. (2008) found 50% higher RUE values for *Miscanthus* × *giganteus* than for two other rhizomatous perennial crops: *Spartina cynosuroides* and *switchgrass*. Secondly, Boehmel et al. (2008) compared the nitrogen-use efficiency of different annual and perennial energy cropping systems and concluded that miscanthus had a higher NUE value of 526 kg $\mathrm{DM.kg^{-1}}$ when compared with the NUE of energy maize (65 kg $\mathrm{DM.kg^{-1}}$). Lewandowski and Schmidt et al. (2006) found the same superiority of miscanthus NUE values over Reed canary grass and triticale, which displayed a stronger decrease in NUE values with nitrogen supply than miscanthus. Finally, Beale et al. (1999) showed than *Miscanthus* had a WUE value that was 35% to 55% higher than *Spartina cynosuroides* depending on water supply (rainfed and irrigated, respectively).

3 Above-Ground Biomass Production

Because miscanthus is a perennial grass, the first year concerns establishment of the crop, above-ground biomass production only being possible during subsequent years. The above-ground biomass potential of different species in Europe is discussed first of all, and then the components of this biomass are described.

3.1 Potential for Above-Ground Biomass Production by Different Species

The lifetime of the crop is estimated at between 20 and 25 years (Lewandowski et al., 2003), during which miscanthus biomass is produced during two phases: a yield-building phase, which in *M.* × *giganteus* lasts for two to five years, depending on climate and plant densities, and a plateau phase where the yield is maintained (Clifton-Brown et al., 2000, 2001b; Christian et al., 2008). Ceiling yields are attained more rapidly in warmer climates than in cooler climates, especially when crop water supplies are not limiting. Trials in Southern regions have shown that the plants tend to mature more rapidly than at Northern latitudes. The time required to reach the plateau phase is probably species-dependent, but no data are available.

Yield is very low during the first year (less than 10 $\mathrm{t.ha^{-1}}$ for *M.* × *giganteus*) but these figures are usually not known as the grass is not harvested. However, yields in the autumn of the first and second years are a good indicator of those for the third year. Clifton-Brown et al. (2001a) studied the performance of 15 *miscanthus* crops belonging to the *M.* × *giganteus* species (4 clones), *M. sacchariflorus* (1 clone), *M. sinensis* species (5 clones) and hybrids between *M. sacchariflorus* and *M. sinensis* (5 clones), at five sites in Europe during the first three years after establishment and observed a higher and significant correlation of 0.81 between the third-year and second-year yields, this correlation being 0.56 between the third-year and first-year yields.

During subsequent years, peak yields are obtained in the autumn, at the full plant flowering phase (Cosentino et al., 2007) and then decline through the winter due to leaf loss. Harvestable yields in the spring are 27%–50% lower than in the autumn (Clifton-Brown et al., 2001b; Cadoux et al., 2008; Himken et al., 1997; Jorgensen et al., 2003b; Ritchter et al., 2008). Jorgensen et al. (2003b) found similar losses of above-ground dry matter yields that reached 40% and 35% during the second and third years of growth when harvest was delayed from the autumn to the spring in one *M. sacchariflorus*, five clones of *M. sinensis* and five hybrids of *M. sinensis*. Greater above-ground dry matter yield losses were observed for hybrids of *M. sinensis* than with *M. sinensis* itself (48% and 35%, respectively, during the second year of growth and 45% and 29% during the third year of growth; Jorgensen et al., 2003b).

A review of autumn yields in Europe showed that the maxima from *M.* × *giganteus* were obtained in France in Lusignan (44 °N, 1 °E) and Grignon (48 °N, 2 °E) under irrigated and fertilised conditions: being, respectively, 49 and 42 t $\mathrm{DM.ha^{-1}}$ (Clifton-Brown et al., 2004). These yields ranged from 15 to 25 $\mathrm{t.ha^{-1}}$ without irrigation. For winter yields of *M.* × *giganteus*, the highest non-irrigated yields were found to be 15–19 t DM.ha^{-1} during the trials performed by the Miscanthus Productivity Network that involved 15 European sites. Yields ranged from 7 to 26 t DM.ha^{-1} following the third growing season, with some of the trial crops being irrigated (Clifton-Brown et al., 2001b). Outside this network, higher productivity was reported in Central and Southern Europe, but irrigation was always required (Clifton-Brown et al., 2001b; Cadoux et al., 2008).

In order to compare several species, a three-year study was carried out to evaluate the biomass production potential of four acquisitions of *M.* × *giganteus*, one clone of *M. sacchariflorus*, five clones of *M. sinensis* and five hybrids of *M. sinensis* (Clifton-Brown et al., 2001a). During the three years of production, the autumn yields demonstrated the higher productivity of *M.* × *giganteus* when it was able to survive the winter. Apart from *M.* × *giganteus*, higher yields were reported for *M. sinensis* hybrids, the highest being observed in Germany with mean yields of 11.1 t.ha^{-1} for *M. sinensis* and 15.1 t.ha^{-1} for hybrids. The mean yields of these hybrids could be between 6% and 90% higher than those of *M. sinensis*, depending on the country. Jezowski (2008) concluded as to the superiority of a hybrid between *M. sinensis* (2×) and *M. sacchariflorus* (4×) by comparison with a hybrid between *M. sinensis* (2×) and *M. sacchariflorus* (2×) or between two *M. sinensis* (2×): mean yields reached 20.5, 14.9 and 9.8 t DM.ha^{-1}, respectively.

3.2 Components of Above-Ground Biomass

These differences among genotypes and regions for above-ground biomass production could be explained by the difference in the duration of the growing period. Tiller densities, plant height and the proportion of leaves and stems were also key elements explaining the variability in biomass production among species. The next section focuses on above-ground biomass production.

3.2.1 Length of Growing Period

Above-ground biomass production is dependent on the duration of the growth period. After the first year, the start of the growing season is determined by the last spring frost, and the end of the growing season is determined by flowering or the first autumn or winter frost, depending on the harvest date or location.

In European environments, shoot emergence occurs between late March and late April, depending on the climate and the temperature required for the emergence of each genotype (Farrell et al., 2006) (see Sect. 2.2). Because *M. sinensis* needs fewer cumulative degree-days than *M.* × *giganteus* and *M. sacchariflorus* prior to emergence (60 vs. 90 and 120 degree-days, respectively), its growing season starts earlier.

The growing season cycle ranges from 6 to 9 months, depending on the genotype. *M. sinensis* genotypes, which flower rapidly, display a shorter growing season than hybrids of *M. sinensis* (which flower later), or *M.* × *giganteus*, which is generally unable to flower under Northern European conditions (Clifton-Brown et al., 2001a). During the growing season, *M. sinensis* and *M.* × *giganteus* normally produce new stems throughout the vegetation period (Lewandowski et al., 2003; Stewart et al., 2009) while *M. sacchariflorus* forms about 80% of its stems in the spring.

3.2.2 Plant Morphology

Jezowski (2008) studied two *M.* × *giganteus*, two hybrids of *M. sinensis* (2×) and *M. sacchariflorus* (2×), and two hybrids between two *M. sinensis*. Pooling of their observations revealed a good correlation between yield and shoot numbers and between yield and tuft diameter in 1-year-old plants (0.78 and 0.79, respectively), the mean of productive shoots per plant being 6.6. Stronger correlations were observed during subsequent years (more than 0.90 for 2- and 3-year-old plants; with mean numbers of productive shoots of up to 9.6 and 18.6, respectively), and yield was also correlated with plant height (0.67 in the second year and 0.91 in the third year). Angelini et al. (2009) obtained a correlation of 0.75 between stem numbers and the dry yield of above-ground biomass when they pooled their observations on *M.* × *giganteus* over a 10-year period.

Stems make a considerable contribution to above-ground biomass, but this is dependent on harvest date. In *M.* × *giganteus*, stems constitute between 67% and 75% of the standing crop biomass in the autumn, depending on crop age (Tayot et al., 1995; Bullard et al., 1997; Ercoli et al., 1999) and up to 77% or 98% in the winter, depending on location (Tayot et al., 1995; Cosentino et al., 2007; Christian et al., 2008). In *M. sinensis*, similar percentages of stems, between 57% and 78%, have been observed in the autumn (Kalembasa et al., 2005, Jorgensen et al., 2003b), and up to 94% in the winter (Jorgensen et al., 2003b).

4 Response of Miscanthus to Stress Conditions

Few studies have been devoted to stress conditions, the important one being the European Miscanthus Improvement Project which was designed to broaden the genetic base, test genotypes and develop breeding methods (Lewandowski and Clifton-Brown, 2000). It contributed to developing new screening techniques to determine genotypic variability for traits such as the response to effects of low temperatures, frost tolerance, mineral content and biomass yield (Lewandowski and Clifton-Brown, 2000). This project identified characteristics related to low-temperature growth and frost tolerance as being crucial to the improvement of miscanthus (Jorgensen and Muhs, 2001). Another project, the *Miscanthus* Productivity Network, studied the limitations of low temperatures and other abiotic factors on the growth of *M. × giganteus* under European climatic conditions (Walsh, 1998).

Studies on the use of environmental resources have focused on the effect of water availability (Christian and Haase, 2001; Clifton-Brown et al., 2002; Cosentino et al., 2007) and nitrogen availability (Lewandowski et al., 1995; Lewandowski and Kicherer, 1997; Lewandowski and Kauter, 2003; Christian et al., 2006; Lewandowski and Schmidt, 2006; Christian et al., 2008).

The effects of cold temperatures and frost are presented first of all, followed by the effects of water and nitrogen availabilities.

4.1 Effect of Chilling Temperatures

Several important air temperature effects on miscanthus growth have been distinguished. Air temperature affects canopy development through organ growth and expansion rates (Clifton-Brown and Jones, 1997; Clifton-Brown et al., 2001b), as well as canopy functioning through photosynthesis (Beale et al., 1996; Weng and Ueng, 1997; Wang et al., 2008a, b).

4.1.1 Effect on Leaf Expansion Rates

Thermal responses affecting the leaf expansion rate have been reported with respect to 32 genotypes grown under controlled conditions (Clifton-Brown and Jones, 1997). After the plants were kept in cabinets at 20 +/− 3°C with optimum supplies of water and nutrients, they were then subjected to repeated sequences of stepped temperature treatments during which they were exposed for 18 h to 20°C and for 11.5 h to 15, 10, 7.5 and 5°C, successively. Plant extension rates were measured mechanically by auxonometers attached to the youngest visible expanding leaf on a miscanthus plant. These auxonometers measured a combination of leaf and internodal extension. Leaf extension was estimated using ruler measurements of the leaf attached to the auxonometer. Leaf extension accounted for 83% of plant extension. The relationship between the temperature range and the plant extension rate fitted well with a third-order polynomial (Clifton-Brown and Jones, 1997). Farrell et al. (2006) determined the best fit relationship using an exponential regression model for the leaf extension rate (Table 1).

From the model developed by Clifton-Brown and Jones (1997), the estimated ratio between expansion rates at 10°C and 20°C varied among the genotypes and ranged from 3 to 4.7. All genotypes displayed predicted leaf extension at the lower temperature of 5°C that was always less than 0.1 mm.h^{-1}, except for two genotypes with a predicted rate of 0.19 mm.h^{-1} and two others which had a leaf extension rate of 0.13 mm.h^{-1} (calculated from Clifton-Brown and Jones, 1997). However, these advantages at the lower temperature were lost at the higher temperature.

During the same experiment, simulation with a simple growth model showed that genotypes with lower threshold temperatures for leaf expansion were unlikely to display greater productivity than genotypes with more rapid expansion rates at temperatures higher than 10°C (Clifton-Brown and Jones, 1997; Jorgensen and Muhs, 2001). Some genetic variability existed among genotypes with lower threshold temperatures for leaf expansion and others with a more rapid leaf extension rate when the air temperature was higher than 10°C. Improved biomass yields could thus be expected if these two traits were combined at the optimum level.

4.1.2 Effect on Light Interception and Photosynthesis

Final crop yields are closely linked to canopy photosynthesis during the growing season, which itself is dependent on the amount of radiation intercepted by leaves and the photosynthetic activity of individual leaves. Leaf area development and radiation-use efficiency have already been discussed in Sects. 2.1 and 2.3.

Weng and Ueng (1997) studied the optimum temperature for photosynthesis in *Miscanthus* in Taiwan and explained that it varied in line with habitats: it was higher than 35°C for lowland clones, which is close to the optimum temperature for maize and sugarcane (Yan and Hunt, 1999), but was 28°C for clones at altitudes above 2550 m.

In contrast to related C_4 species such as maize, *M.* × *giganteus* displays a notable ability to maintain high photosynthetic productivity at low temperatures of 14°C or 10°C (Beale et al., 1996; Naidu et al., 2003; Farage et al., 2006). The molecular mechanism underlying this tolerance remains unclear. *M.* × *giganteus* maintains a high rate of CO_2 uptake in order to increase its utilisation of absorbed light (Beale et al., 1996), and increases non-photochemical quenching (Farage et al., 2006) to prevent the marked reduction of CO_2 assimilation observed in chilling-intolerant C_4 species.

Nevertheless, the cold tolerance of C_4 photosynthesis in *M.* × *giganteus* may be related to the higher levels or activity of two major photosynthesis enzymes: Rubisco and Pyruvate Phosphate DiKinase (PPDK) (Sage and Kubien, 2007). Although no difference in Rubisco content and activity has been observed between *M.* × *giganteus* plants grown under cold and warm conditions (Wang et al., 2008a), the rise in PPDK content and activity per unit leaf area in the cold-grown genotype was related to the recovery and maintenance of photosynthetic activity (Wang et al., 2008b). Naidu et al. (2003) found a 28% increase in PPDK levels in cold-grown miscanthus.

4.2 Effect of Frost

Miscanthus may encounter two types of frost temperatures in Europe: negative temperatures during the winter and late frosts during the spring. The former may damage young plantings in Northern Europe and constitute an obstacle to crop establishment; in cooler areas, late frosts can damage newly-expanded leaves during the first year of the crop or just after the regrowth of older plantings.

A capacity for winter survival has been described as the principal obstacle to the establishment of *M.* × *giganteus* crops (Christian and Haase, 2001), particularly in Northern Europe (Denmark and Sweden), where no plants were able to regrow after the first winter (Lewandowski and Clifton-Brown et al. 2000). Similar mortality rates were observed in Ireland, the Netherlands, Belgium and Germany, as reported by Christian and Haase (2001). During these trials, plants started to grow during the spring after planting, but the first shoots produced were killed by late frosts and the rhizomes were unable to sprout again. The authors suggested that the winter survival of *M.* × *giganteus* was mainly dependent on the generation of sufficient metabolic reserves in the rhizome system to enable the emergence of sufficient shoots the next spring (Christian and Haase, 2001; Schwarz et al., 1994).

Christian and Haase (2001) suggested that winter survival might also be related to dormancy during frosty periods: frost tolerance may only develop in these organs once dormancy has been induced during the autumn or early winter. Rhizomes and appending buds can be expected to have a higher frost tolerance than overwintering organs. However, a study on the frost tolerance of buds and rhizomes compared with that of leaves showed that this was not the case (Clifton-Brown and Lewandowski, 2000b; Jorgensen and Muhs, 2001; Farrell et al., 2006). This finding may have been due to the protection and isolation offered by the typical micro-structure of plant molecules and the surrounding soil, rather than to the inherent frost tolerance of rhizomes.

Plazek et al. (2009) tested the effects on metabolic activity and rhizome regrowth of exposing leaves to a frost of up to −8°C or −15°C following a 14-day acclimation period at 12°C and a 21-day period at 5°C. Although no frozen plants could produce new shoots, the rhizomes displayed some metabolic vitality

Table 2 Coincidence between the lowest lethal temperature (LT_{50}) and lowest moisture content (MC) of leaves and rhizomes in four genotypes of miscanthus but no correlation between LT_{50} of leaves and LT_{50} of rhizomes

	Leaves (Farrell et al., 2006)		Rhizomes (Clifton-Brown and Lewandowski et al., 2000b)	
	LT_{50}(°C)	MC (%)	LT_{50}(°C)	MC (%)
M. sacchariflorus	–7.5 ab	87 a	–3.5 b	73 a
M. × *giganteus*	–8 b	85 a	–3.5 b	76 a
M. sinensis Hybrid 6	–6 a	85 a	–6 a	68 b
M. sinensis Hybrid 9	–9 c	80 b	–4.5 ab	71 b

LT_{50}, the lethal temperature at which 50% of shoots (or rhizomes) were considered as killed when the leaves were totally brown. MC, moisture content. Different letters indicate significant differences calculated from the least significant difference ($P < 0.05$).

after a 5-week regrowth period at 20°C. The authors concluded that frost susceptibility was due to the frost sensitivity of shoot apical meristems.

In terms of species variability, *M. sinensis* might be expected to be more frost-tolerant than *M. sacchariflorus* because in Asia, *M. sacchariflorus* is only found in warm regions, whereas *M. sinensis* is more ubiquitous (Clifton-Brown and Lewandowski, 2000b). Farrell et al. (2006) compared these species with *M.* × *giganteus* in terms of cold tolerance at the shoot level (young shoots of 3–4 leaves). Their screening method consisted of growing plants in cabinets at 20+/–3°C for 21 days with optimum supplies of water and nutrients. The plants were acclimated at 12+/–2°C for 8 days before their leaves were exposed to frost temperatures descending from −2°C to −10°C at a time-step of 2°C every 3 hours. Before this frost treatment, leaves were sampled from each genotype to determine their moisture content. Any frost damage was assessed 7 days later. The lethal temperature at which 50% of the shoots were killed (LT_{50}) was estimated for each genotype. The same experiment was carried out on the same genotypes as those studied by Clifton-Brown and Lewandowski (2000b) in order to determine the LT_{50} of rhizomes.

M. sinensis hybrids were found to have significantly lower rhizome moisture contents than *M.* × *giganteus* and *M. sacchariflorus*. These differences coincided with the lower LT_{50} of *M. sinensis* (Clifton-Brown and Lewandowski, 2000b; Farrell et al., 2006). Jorgensen et al. (2003b) found a higher rhizome moisture content in *M.* × *giganteus* (none of which survived the first winter) than in *M. sinensis* and most *M. sinensis* hybrids which displayed survival rates of up to 85–99%. However, the degree of resistance to negative temperatures differed in rhizomes and shoots. *M. sinensis* H6 had the lowest LT_{50} value for rhizomes while *M. sinensis* H9 had the lowest LT_{50} for shoots (−6°C and −9°C, respectively). Shoot and rhizome frost tolerance were not correlated in these studies (Table 2). Tissue moisture content is considered to be one of the best characteristics for use during plant screening programmes with the accumulation of osmotically active compounds during cold acclimation (Lewitt, 1980). However, no correlations with frost tolerance were found for either leaves or rhizomes (Clifton-Brown and Lewandowski, 2000b; Farrell et al., 2006).

4.3 Effect of Water Availability on Biomass Production and Its Components

Increased productivity will result in higher water demand, so that water may become a limiting factor to both crop productivity and the economic viability of the crop. Because of the climatic differences between Northern and Southern Europe, it is therefore necessary to select potential energy crops from species with a high water-use efficiency (Long and Beale, 2001), particularly when adapting the species to water-limited areas. The identification of drought-tolerant genotypes that can produce more biomass under water stress conditions remains an essential component in the improvement of miscanthus (Clifton-Brown and Lewandowski, 2000a).

The effects of water availability in the field are described with respect to *M.* × *giganteus,* particularly

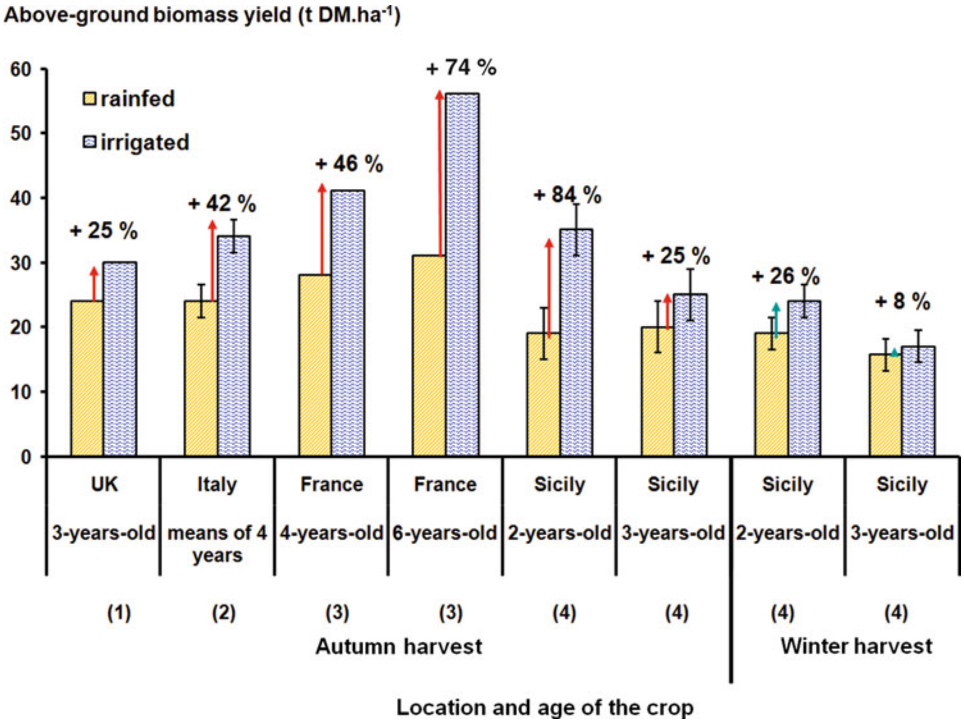

Fig. 2 Increase in dry matter content of above-ground biomass under a water supply in *M.* × *giganteus*. Values taken from Beale et al. (1999) (1); Ercoli et al. (1999) (2); Cadoux et al. (2008) (3) and Cosentino et al. (2007) (4). The more marked difference in France was due to an interaction with nitrogen supply, where available, errors bars indicate LSD ($P \leqslant 0.05$)

in terms of biomass production and its components. The response of other *Miscanthus* species to water availability is then described under controlled conditions.

Irrigation exerts an important influence on yield when in *M.* × *giganteus* is grown at sites with a poor water supply (Christian and Haase, 2001). Heaton et al. (2004) described a highly significant effect of water availability on biomass production ($P < 0.0001$) in a review of several experiments throughout Europe. Under varying levels of nitrogen inputs (between 60 and 240 kg N.ha^{-1}), biomass production increased by between 25% and 84% with irrigation (Fig. 2). The difference in yield between well-irrigated plots (100% of evapotranspiration restored)) and less irrigated plots (25% of evapotranspiration restored) was higher in autumn than in winter: +84% for an autumn harvest against 26% for a winter harvest (Fig. 2). Price et al. (2004) found similar losses of harvestable yield in the winter, of between 8% and 23%, by using a potential yield model simulating limited water supplies in England and Wales. These marked differences between rainfed and irrigated yields could be related to the period during which the drought occurred. Ritchter et al. (2008) determined the main growing season as the period of susceptibility to drought in miscanthus.

In terms of biomass components, water availability does not affect shoot production (Christian and Haase, 2001; Cosentino et al., 2007; Cadoux et al., 2008). However, this lack of effect is probably because shoot production takes place during the period of high soil water availability (at the beginning of the growing period). By contrast, the number of stems is more closely dependent on planting densities than on irrigation rate, *i.e.* water availability.

Plant height is not influenced by water availability at the beginning of the growing period. Nevertheless, a reduction in water availability towards the end of the growing period was found to markedly influence plant height (Christian and Haase, 2001; Cosentino et al., 2007; Cadoux et al., 2008). Irrigated plants were 49% taller than those without irrigation. A similar trend was observed for the leaf area index (Cosentino et al., 2007; Cadoux et al., 2008). Irrigation caused a 77% increase in the leaf area index compared with no irrigation. However, this effect was not reported by Christian and Haase (2001). In *Miscanthus* × *giganteus*, Cosentino et al. (2007) also observed a one month difference in the flowering date between irrigated and rainfed treatments.

During studies to compare *M.* × *giganteus*, *M. sinensis* and *M. sacchariflorus* under controlled conditions, a reduction in leaf area was observed in *M.* × *giganteus* and *M. sacchariflorus* but not in *M. sinensis* under water stress (Clifton-Brown and Lewandowski, 2000a; Clifton-Brown et al., 2002). However, the leaf area of *M. sinensis* was smaller before the water stress was applied. Furthermore, the water needs of this species may be less than in the other species. The authors concluded that *M. sinensis* might be less sensitive to water stress. In addition, *M. sacchariflorus* and *M.* × *giganteus* presented an increase in the senescence of green leaf area, while *M. sinensis* presented a lack of senescence under all treatments. This experiment suggested that the "stay green" mechanism in *M. sinensis* may be related to stomatal closure with a low soil moisture content. No data are available on the variability of the "stay green" mechanism observed in species of *M. sinensis*.

Photosynthetic activity contributes to adapting miscanthus to drought. Weng (1993) showed that both stomatal and non-stomatal photosynthesis factors were affected by water deficit, and suggested that genotypes displaying the highest degree of osmotic adjustment (OA) were the best at maintaining photosynthetic activity under water deficit. Nevertheless, no differences in OA were observed among *M. sinensis*, *M. sacchariflorus* and *M.* × *giganteus* under severe water deficit, although *M. sinensis* was more tolerant to water stress (Clifton-Brown et al., 2002). Leaf conductance was markedly reduced in *M. sinensis*, even under mild water stress, so that a completely green leaf area was maintained throughout the experiment.

4.4 Effect of Nitrogen Availability on Biomass and Its Components

Plant growth and development are dependent on nitrogen availability. Nitrogen forms part of living cells and is essential to all protein, enzymatic and metabolic processes involved in synthesis and transfer to energy. The response of biomass production and its components to nitrogen supply has been the subject of more study in *M.* × *giganteus* species (Ercoli et al., 1999; Christian and Haase, 2001; Cosentino et al., 2007; Danalatos et al., 2007; Christian et al., 2008) than in *M. sinensis* species (Obara, 1967; Matsumura et al., 1975; Sato et al., 1975 and Hoshimo, 1978 cited by Stewart et al., 2009).

Obara (1967), Matsumura et al. (1975), Sato et al. (1975) and Hoshimo (1978) cited by Stewart et al. (2009) showed that nitrogen is one of the most important nutrients required to increase the biomass production of *M. sinensis* grassland. A N input level of around 100 kg N.ha^{-1} can achieve a 50% to 500% higher biomass yield, and affect biomass components. Hoshimo (1978) observed a 16%–33% increase in plant height and leaf size under appropriate nitrogen supply.

By contrast, under non-limiting water conditions, nitrogen fertiliser rates of between 60 and 240 kg N.ha^{-1} generally had little or no effect on the biomass yield, stem number, plant height and leaf area index of *M.* × *giganteus* species (Ercoli et al., 1999; Christian and Haase, 2001; Danalatos et al., 2007; Cadoux et al., 2008; Christian et al., 2008).

During a multi-environment experiment, Lewandowski and Schmidt (2006) observed a trend towards increased biomass yields with a N input level of about 110 kg N.ha^{-1}, followed by a slight decline. However, these results varied considerably, as the same biomass of about 35 t.ha^{-1} could be produced with nitrogen inputs ranging from 90 to 150 kg N.ha^{-1}. This variability might have been explained by the soil nitrogen supply, but these data were not available.

In a Mediterranean environment but with a water supply, Cosentino et al. (2007) found an autumn biomass yield that was 21% higher (120 kg N.ha^{-1}) than without nitrogen during the second year of the crop. This increase even reached 35% for a winter harvest. It could be attributed to an 11% increase in plant height and a 27% increase in stem numbers par square metre.

Plant responses to nitrogen depend on the water availability for biomass production. During the same experiment, Cosentino et al. (2007) determined a marked nitrogen effect when the crop received less water during its third year of growth (Table 3). During an extended period of water stress in July, plant height was 53% higher under nitrogen input, but the difference was smaller (22%) when water was abundant during the second year of growth. The nitrogen

Table 3 Higher increase in plant height or above-ground dry biomass of *M.* × *giganteus* for plots with water supply (I_2) and nitrogen supply (N_1, N_2 or N_3) than for plots with nitrogen supply only. Data taken from Cosentino et al. (2007) (1) and Ercoli et al. (1999) (2)

		Water supply (mm)		Plant height	Above-ground dry biomass
2 years old (1)	I_0N_0	272 mm	+244%	+2%	+21%
	I_0N_2				
	I_2N_0	938 mm		+22%	+39%
	I_2N_2				
3 years old (1)	I_0N_0	228 mm	+93%	+47%	+8 %
	I_0N_2				
	I_2N_0	439 mm		+53%	+25%
	I_2N_2				
Means of 4 years (2)	I_0N_0	228 mm	+100%		+23%
	I_0N_1				
	I_2N_0	439 mm			+41%
	I_2N_1				
Means of 4 years (2)	I_0N_0	228 mm	+100%		+41%
	I_0N_3				
	I_2N_0	439 mm			+100%
	I_2N_3				

I_0, rainfall; I_2, Irrigation.
N_0, N_{soil}; N_1, N_{soil} + 100 kg N.ha^{-1}; N_2, N_{soil} + 120 kg N.ha^{-1}; N_3, N_{soil} + 200 kg N.ha^{-1}.

effect also appears to be dependent on crop age, as a weak effect of nitrogen was only observed in two- or three-year-old crops (Cosentino et al., 2007; Lewandowski and Schmidt, 2006). Contrary to what was expected, no nitrogen effect was detected when the crop was mature and after 14 successive spring harvests (Lewandowski et al., 2000; Danalatos et al., 2007; Christian et al., 2008). No data are available on the effect of successive autumn harvests, and it could be expected that these might increase crop nitrogen requirements; the allocation of nitrogen from leaves and stems to rhizomes occurs during the winter.

According to these results, *M.* × *giganteus* has low nitrogen needs for growth. For example, Long and Beale (2001) found that 93 kg of N availability per hectare enabled the production of 25 t.ha^{-1} of above-ground biomass. Nevertheless, Beale and Long (1997) recommended nitrogen input of between 50 and 90 kg.ha^{-1}.year^{-1} to enable rhizome development and prevent soil exhaustion.

Moreover, Beale et al. (1996) reported no difference in the photosynthetic potential of *M.* × *giganteus* under either 120 kg(N).ha^{-1} or no nitrogen applications under temperate field conditions. Weng and Hsu (2001) showed that the photosynthetic capacity of seventeen *Miscanthus* clones collected from lowland areas in Northern and Southern Taiwan and mountainous areas in central Taiwan increased slightly with N applications from 8–16 μmol.m^{-2}.s^{-1} with no nitrogen input to 12–21 μmol.m^{-2}.s^{-1} under 176 kg.ha^{-1} nitrogen. However, no correlation with biomass production was calculated during this study.

This low need for and response to nitrogen of *M.* × *giganteus* crops, even after 14 years of winter harvest, could be attributed to efficient nitrogen storage by the rhizome. By studying ^{15}N–amended soil, Christian et al. (1997) found that plant nitrogen uptake over 2 years was greater than the amount supplied as fertiliser and arising from soil nitrogen. The plants were thus able to obtain nitrogen from other sources. Eckert et al. (2001) identified one of these as a nitrogen-fixing bacterium, *Azospirillium*, associated with the roots of *M.* × *giganteus*. Another study found the same type of bacterium (*Clostridia*) associated with the roots of *M. sinensis* (Miyamoto et al., 2004) and its stems (Ye et al., 2005). No studies have yet been performed to determine the relationships and exchanges between miscanthus roots and these bacteria, but Ye et al. (2005) showed that the inoculation of this type of bacteria in biomass enhanced the tolerance of salinity. It is therefore possible that exchanges between bacteria and miscanthus exist. Moreover, Funatsu (2006) cited by Stewart et al. (2009) reported that AM fungi (Arbuscular mycorrhiza) associated with the roots of *M. sinensis* in volcanic and ashy soil might enhance nitrogen assimilation.

5 Conclusions and Prospects

Miscanthus has been identified as a good candidate for biomass production. Peak yields of 49 t DM.ha^{-1} have been observed in Europe during an autumn harvest of mature crops with irrigation. We observed smaller maximum yields of between 7 and 26 t DM.ha^{-1} for a winter harvest, this difference mainly being due to leaf loss. Because of its C_4 photosynthetic pathway and perennial rhizome, *M.* × *giganteus* displays a satisfactory combination of radiation-, water- and nitrogen-use efficiencies (see Sect. 2.3) when compared with several other bio-energy crops.

The slight effect of nitrogen input on yield increases under water stress. However, lower levels of nitrogen availability need to be tested in the future because soil nitrogen availability and mineralisation have not been addressed by most studies, suggesting that experiments have probably been carried out under nitrogen excess to demonstrate any effect in miscanthus.

The high potential for above-ground biomass production by *M.* × *giganteus*, and the efficiencies of this crop, nonetheless varied under different management systems and climates. Two key elements for improvement have been identified by the present review. Firstly, frost tolerance during the winter or early spring seems essential in Northern Europe in order to ensure overwintering and protect young shoots after early emergence. Although *M.* × *giganteus* has been identified as a C_4 cold-tolerant species by comparison with maize because of its higher PPDK protein content at cold temperatures, we did not observe any winter survival of *M.* × *giganteus* in Northern Europe (North Sweden and Denmark). Susceptibility to winter frost at temperatures below −3.5°C for the rhizome and −8°C for young shoots of *M.* × *giganteus* may lead to marked plant losses and lower yields. Secondly, water supply is necessary to ensure a good establishment rate and a high biomass yield. We observed a reduction of up to 84% in above-ground dry matter production under water stress for autumn harvests, and up to 26% for winter harvests. This reduction was mainly due to a 49% decrease in plant height and a 77% decrease in leaf area index. Insofar as a lack of water will become a crucial problem in some areas in the decades to come, it is essential to improve the tolerance of miscanthus to water stress and the water-use efficiency of this crop under diverse levels of water supply.

Because no genetic variability exists in *M.* × *giganteus*, and because the development of a single clone of this species throughout Europe might facilitate disease development, *M. sinensis* must provide the genetic resources for frost and water stress tolerance. Through the production of hybrids, this species displays a good potential for biomass yield under European conditions, as well as tolerance to frost and to limited water supplies. Firstly, its better frost tolerance than *M.* × *giganteus* has been observed in hybrids between two *M. sinensis* which have been able to resist temperatures as low as −6°C for rhizomes and −9°C for young shoots. And secondly, *M. sinensis* is able to maintain its leaf area index under water stress during the first stage of growth, even though it has a similar water-use efficiency.

However, to date it has not been possible to combine a high tolerance of abiotic stress with high levels of biomass production. It is now necessary either to identify *M. sinensis* genotypes that can produce an above-ground biomass yield close to that of *M.* × *giganteus* under water stress and/or low growth temperatures, or to generate new interspecific hybrids of *M.* × *giganteus* with higher tolerance thresholds. On a crop scale, the cultivation of sterile species, for example with unpaired ploidy levels, would be useful to prevent uncontrolled invasion. As in other genera, this variation in genome ploidy could lead to variations in species traits, particularly with respect to biomass production, but no studies have as yet been reported.

Acknowledgements The authors acknowledge the Picardie region (project PEL), which supported this work. We thank Prof. A. Gallais for his helpful comments on the manuscript. Thanks also to the anonymous reviewers for their valuable recommendations.

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Changes in Atmospheric Chemistry and Crop Health

Jürgen Bender and Hans-Joachim Weigel

Contents

Abstract The concentrations of atmospheric compounds such as greenhouse gases, heavy metals and trace gas air pollutants have rapidly changed. Many of these compounds interact with agricultural systems and influence crop performance, both directly by affecting growth and quality or indirectly by altering the plant's ability to cope with other abiotic and biotic stresses. Some atmospheric compounds have little or no discernible impact on the environment; others reach levels that exceed thresholds for damage to crops. In this review, we analyse the literature on airborne species that directly impact crop growth and health. In Europe and North America emissions of SO_2, NOx and heavy metals have declined during the past decades and are currently not considered as a major threat to crops. By contrast, air pollutant emissions have been increasing in rapidly growing regions of Asia, Africa and Latin America. Ozone is the most phytotoxic of the common air pollutants. The widespread distribution of O_3 already presents a risk to crop growth and health in many regions of the world. It is concluded that the continuous increase in background O_3 concentrations will pose a critical threat to future world food security. Interactions with both biotic and abiotic factors must be taken into account in assessing risks of air pollutants in the field. There is evidence that these indirect effects could be more important under certain circumstances than the direct effects of air pollutants on plants. The parallel rapid increase in atmospheric CO_2 concentrations accompanied by climate change has two major implications: (1) a possible benefit to crop growth by direct stimulation of photosynthesis and by mitigation of gaseous air pollutants and water stress; and (2) a threat to crop production due to an enhancement of crop quality losses.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_22,

from Bender and Weigel, Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010013

Keywords Atmospheric change • Carbon dioxide • Crops • Ozone • Pollutant interactions • Product quality • Yield

1 Introduction

Anthropogenic activities have significantly changed the composition of the global atmosphere. Especially, the concentrations of several trace gases have undergone significant changes during the past century and continue to change. Plants are important mediators in the exchange of the different gaseous and particulate compounds between the atmosphere and the biosphere (Table 1). The transport of these compounds from the atmosphere into vegetation is by dry and wet deposition of gases, aerosols and sedimenting particles. Many atmospheric constituents can influence crop performance, both directly by affecting growth and quality or indirectly by altering the plant's ability to cope with other abiotic and biotic stresses. In terms of their impact on agricultural ecosystems the atmospheric compounds (for abbreviations see Table 1) can be broadly divided into:

- compounds which act as macro- or micronutrients, e.g., the gases CO_2, SO_2, NO, NO_2 and NH_3 and particulate NH_4, NO_3-N, SO_4-S, P, Ca, Fe and Mg, and
- compounds which may cause adverse or toxic effects, e.g., the gaseous pollutants O_3, SO_2, NO_2, NH_3, HF, PAN, NMHC and VOC, metals such as Pb, Cd and Hg, or excess nutrient substances, e.g., N, S, Zn and Al, which alter normal patterns of growth and development in ecosystems (Dämmgen and Weigel, 1998).

There are both natural and human-made sources for most of the atmospheric constituents. An air pollutant is usually defined as "a chemical constituent added to the atmosphere through human activities resulting in the elevation of its concentration above a background" (Krupa, 1997).

In this review, we focus on those airborne species whose concentrations have a known trend and are directly interfering with agroecosystems. Ambient air is always composed of mixtures of different species, with the concentrations of individual species or pollutants, respectively, varying in time and with location. For example, a particular air pollutant such as SO_2, HF or NH_3 can be dominant only in the vicinity of its sources, i.e. those pollutants are primarily of local importance. In comparison, among secondary pollutants, O_3 is of widespread global occurrence and can currently be considered to be the most important air pollutant (Fuhrer, 2009). Among the different environmental factors which determine crop growth, recent and predicted further changes in climate such as increased temperature, altered pattern of rainfall intensity and

Table. 1 Atmospheric compounds involved in element flux between vegetation and the atmosphere (after Dämmgen and Weigel, 1998)

• H_2O vapour, CO_2, CH_4, N_2O, NO_2, O_3 →	trapping of infrared radiation, contribution to the greenhouse effect
• NH_3, CO, HC →	effects on reactivity of the atmosphere
• CH_4 CO_2, SO_2, NO_2, NO, NH_3 (gases) → NH_4-/NO_3-N, SO_4S, P, Ca, K, Fe, Mg (particles)	involved in nutrient cycling, act as macro- and micronutrients
• O_3, SO_2, NO_2, HF, H_2O_2, PAN, → NMHC/VOC (gases), heavy metals (e.g., Pb, Cd, Hg), surplus nutrients (bioavailable forms of N, S, Zn, Al)	potentially toxic, affecting "normal" growth and performance of organisms, populations and ecosystems

Abbreviations: Al: aluminum; Ca: calcium; Cd: cadmium; CH_4: methane; CO: carbon monoxide; CO_2: carbon dioxide; Fe: iron; HC: hydrocarbons; HF: fluoride; Hg: mercury; H_2O: water vapour; H_2O_2: hydrogen peroxide; H_2S: hydrogen sulphide; K: potassium; Mg: magnesium; N: nitrogen; N_2O: nitrous oxide; NH_3: ammonia; NH_4^+: ammonium; NO: nitrogen monoxide; NO_2: nitrogen dioxide; NO_3^-: nitrate; NO_X: NO + NO_2; NMHC: non-methane hydrocarbons; O_3: ozone; P: phosphorus; PAN: peroxyacetylnitrate; Pb: lead; S: sulphur; SO_2: sulphur dioxide; SO_4^{2-}: sulphate; VOC: volatile organic compounds; Zn: zinc.

frequency, and atmospheric CO_2 concentration as well as other atmospheric compounds have become and will be of growing importance in many parts of the world. Therefore, projections of future global food security must equally consider the likely impacts of climate change and air pollution. With respect to effects, responses of crops to O_3 and CO_2 are particularly considered here as these trace gases are key variables of climatic and atmospheric change for future global food production (Long et al., 2005; Vandermeiren et al., 2009).

2 Spatial and Temporal Trends of Atmospheric Changes

The concentrations of SO_2, nitrogen oxides (NOx) and VOCs as well as of heavy metals and photochemical oxidants such as O_3 in many parts of the industrialised world have changed significantly during the last century (Dämmgen and Weigel, 1998). While local emissions of urban or industrial sources still occur, emissions, particularly of SO_2 and to a smaller extent of NOx ($NO + NO_2$), VOCs and particulate matter, have declined during the past decades in Europe and North America. This was due to successful policies to reduce emissions, as well as a decline in polluting heavy industries (UNECE, 2007). For example, emissions of SO_2 throughout Western Europe have declined by approximately 80% since the peak in emissions during the 1970s (Fowler et al., 2001). SO_2 levels and sulphur bulk deposition are now usually low during the growth periods of crops. At present, annual mean SO_2 concentrations in Europe are less than 10 $\mu g\ m^{-3}$ and rarely exceed 50 $\mu g\ m^{-3}$ SO_2, and bulk S depositions are lower than 10–15 kg ha^{-1} a^{-1} (Fowler et al., 2001). Emission estimates of atmospheric nitrogen species (NOx, NH_3) are more uncertain because of the variety of gases and sources (Grübler, 2003). However, the concentrations in oxidised atmospheric N compounds also show a declining trend although NOx concentrations are highly variable due to different local traffic densities (UNECE, 2007). In rural regions of Europe annual mean concentrations for NO_2 range between 5 and 30 $\mu g\ m^{-3}$ and less than 5 $\mu g\ m^{-3}$ for NO (Dämmgen and Weigel 1998). Currently, the emissions of NH_3 from farm land determine the concentrations of this gas in the air above these systems. The concentrations range from 1 to 30 μg NH_3, if one excludes periods with applications of liquid manure or slurry. Concentrations of airborne VOCs are also highly diverse and the evaluation of their occurrence and distribution is difficult, because there are both anthropogenic and biogenic sources, the latter including emissions from plant, animal and microbial sources (Kesselmeier and Staudt, 1999; Cape, 2003; Krupa et al., 2008). Average annual concentrations of the major airborne VOCs benzene, toluene and ethylene are usually lower than 5 $\mu g\ m^{-3}$, although maximum hourly concentrations of some VOCs can be 100 times larger than the average (Collins and Bell, 2002; Cape, 2003). For the majority of heavy metals such as Pb, Cd, Ni, Hg and Zn a similar decline in emission and subsequent deposition has been observed since the late 1980s in most of Europe, although higher metal deposition is still found in some Eastern European countries (Harmens et al., 2008).

In contrast to the situation in Europe and North America, air pollutant emissions have been increasing over the last two decades in many developing countries, particularly in rapidly growing regions of Asia, Africa and Latin America, where rapid industrialisation and population growth is taking place accompanied by increasing energy demand and road traffic, but with poor emission controls (Emberson et al., 2003). China and India are now the leading emitters of SO_2 in the world (Marshall, 2002). Also, the predicted increase in global NOx emissions may be attributed largely to the high percentage increases in developing countries, such as China (Marshall, 2002; Ghude et al., 2009).

Nitrogen oxides and VOCs are important precursors for the formation of tropospheric O_3. Tropospheric O_3 is a widespread secondary air pollutant found in all industrialised countries worldwide, and meanwhile also in many of the developing countries in the world, where it has reached levels in ambient air which are of concern with respect to vegetation damage and human health effects (Emberson et al., 2003; Royal Society, 2008), and these trends are expected to continue as economies continue to expand. While at least in most parts of Western Europe there is a clear trend of decreasing O_3 peak values ("photosmog episodes"), predictive models indicate that background O_3 concentrations will continue to increase at a rate of 0.5% to 2% per year in the Northern Hemisphere during the

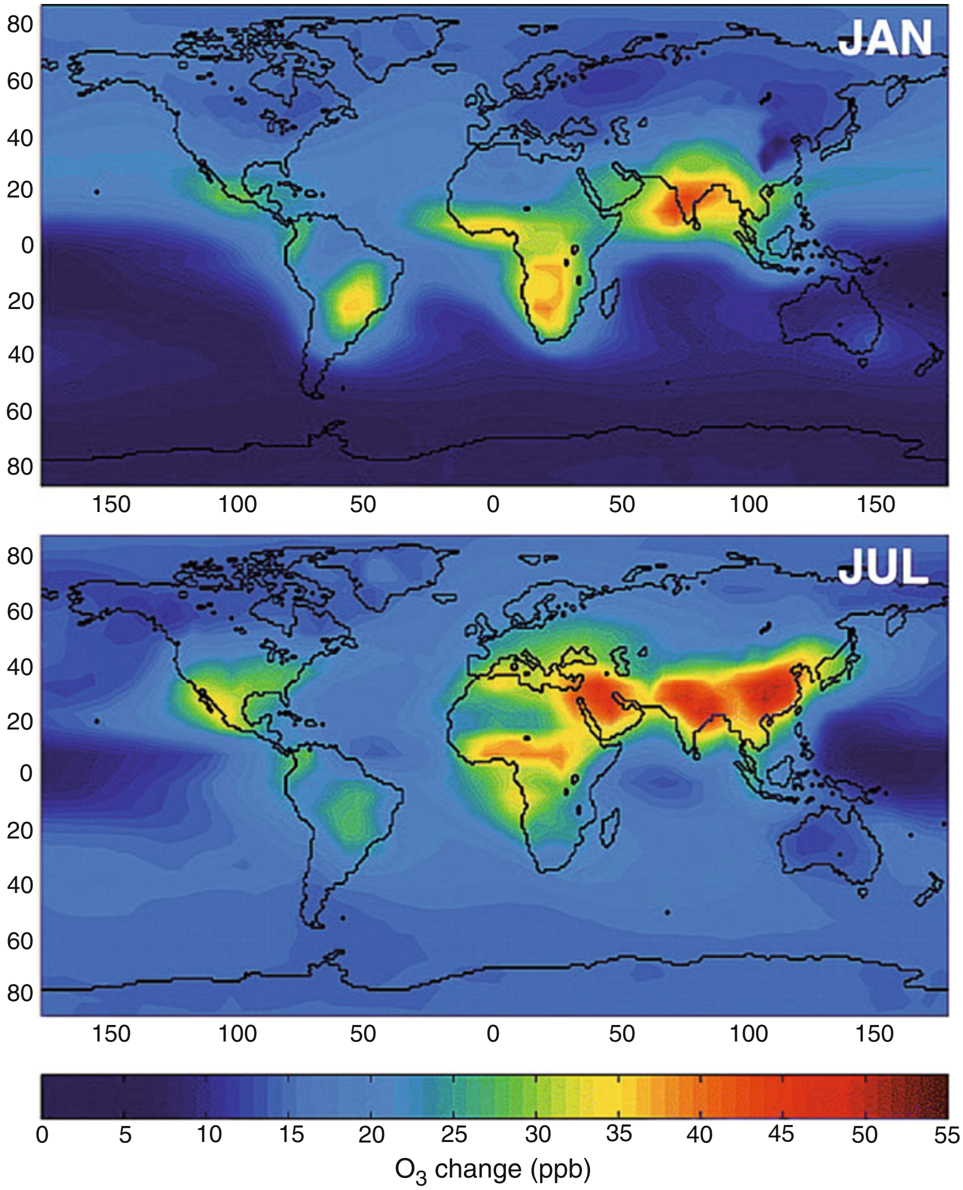

Fig. 1 Predicted changes in global surface O_3 concentration from 2000 to 2100. The figure shows the averaged changes in O_3 concentrations (ppb) for January and July. Blue colours denote no or little change while green to red colours point to a medium to severe increase in O_3 concentrations. Adapted from Prather et al. (2003)

next several decades. Currently, the background O_3 concentration in the Northern hemisphere is within the range of 23–34 ppb; however, global surface O_3 concentration is expected to be in the range of 42–84 ppb by 2100 (Vingarzan, 2004). Figure 1 shows the projected global increase in O_3 concentration over the next 100 years from Prather et al. (2003), based on IPCC global emission scenarios. According to these, the locations of the major O_3 increases ("hot-spots") in the future are expected to be Asia, Southern Africa, Southern Europe and the USA.

In contrast to the different temporal trends of the "classical" air pollutants such as SO_2 and NOx between industrialised and developing countries and the respective spatial variability in their concentration levels, atmospheric CO_2 concentration has risen steadily all over the globe from a pre-industrial concentration of about 280 ppm to a current value of about 385 ppm, and could reach more than 550 ppm by 2050 (IPCC, 2007). Due to the direct effects of rising CO_2 levels on crop photosynthesis, growth and quality, assessments of future air pollution effects on plants and crops have to consider this rapid change.

Overall, the concentrations of sulphur and nitrogen-based air pollutants and of heavy metals have declined during the past decades in many countries of Europe and North America. By contrast, air pollutant emissions have been increasing in rapidly growing regions of Asia, Africa and Latin America. Tropospheric O_3, however, is still at high levels worldwide.

3 Crop Responses to Atmospheric Trace Gases

3.1 *Direct Effects on Crop Growth and Yield*

Gaseous atmospheric compounds are transferred from the atmosphere onto plant canopies by diffusion, which is governed by micro-meteorological conditions (radiation, temperature, wind, etc.). The major path of entry into the leaf is through the stomata. The other two pathways are non-stomatal pathways, which include deposition on external plant surfaces including the cuticle and deposition on the soil (Fig. 2). Penetration of gases through plant cuticles is usually of minor importance (Lendzian and Kerstiens, 1991), although some pollutants such as SO_2 are able to damage the cuticle and gain entry to the internal leaf tissue to some extent (Wellburn, 1994). Aerosols and sedimenting particles containing nutrients and pollutants (e.g., heavy metals) are deposited on plant surfaces or on soil surfaces directly; matter deposited on plant surfaces indirectly can be transferred to the soil by run-off or by plant debris or litter. After entering the root zone they are available for plant uptake. The reaction of a plant to a given air pollutant depends on the exposure characteristics, plant properties and external growth conditions (Krupa, 1997; Bender and Weigel, 2003). Short-term exposures to relatively high concentrations generally result in acute visible foliar injury. Long-term chronic exposures to lower concentrations can cause alterations in physiological and biochemical processes that may result in chlorosis, premature senescence, and growth and yield reductions.

For example, while exposure to short-term high concentrations of SO_2 can lead to cellular death, exposure to low to moderate SO_2 concentrations over the long term can result in impaired cellular metabolism, an accumulation of sulphate in the vacuole, and a reduction in photosynthetic rate (Legge et al., 1998). Sensitivity of plants to SO_2 is generally determined by mesophyll resistance and by buffering capacities

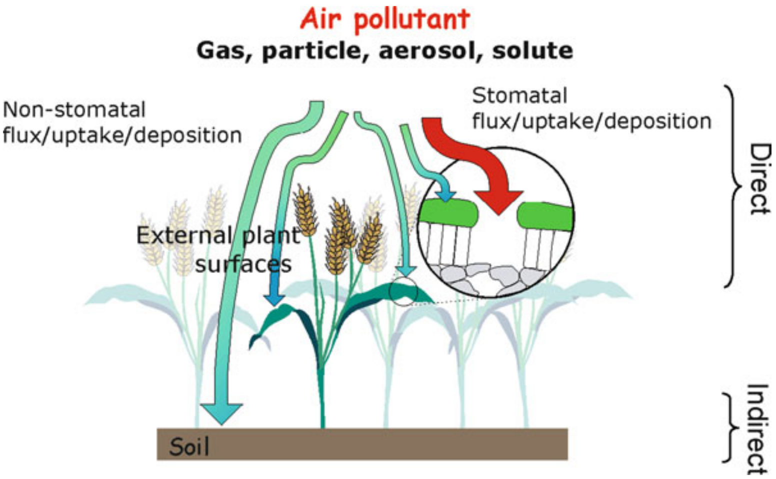

Fig. 2 Major pathways of the transfer of air pollutants to terrestrial surfaces (plant canopies and soil). The thickness of the arrows denotes the relative importance of the respective pathway. Redrawn by courtesy of Dr. Lisa Emberson, SEI York, UK

(i.e. the ability to counteract a change in pH from an optimal value; Wellburn, 1994). However, current ambient SO_2 concentrations in Europe can no longer be considered as a serious problem with respect to negative effects on plant performance. The few data describing exposure-response relationships for long-term (weeks to months) exposure to low and moderate SO_2 concentrations (50 to 100 $\mu g\ m^{-3}$) on crop growth and yield are somewhat variable and controversial (Weigel et al., 1990; Legge and Krupa, 2002). The overall evidence from fumigation studies shows that for most annual crop species ambient SO_2 concentrations are below the critical levels above which adverse effects occur (UNECE, 2004). The reduction in SO_2 emissions in Europe and North America has even resulted in the occurrence of sulphur deficiency in some agricultural species (e.g., rape) growing in sulphur-deficient areas (Legge and Krupa, 2002).

Similarly, phytotoxic effects of reactive nitrogen species (NO, NO_2, NH_3), particularly on crops, can only be observed at concentrations which are far above those occurring even in heavily polluted environments (Wellburn, 1994; UNECE, 2004). Exposure to moderate levels of NO_2 can stimulate certain metabolic processes (e.g., enzyme activities), which indicates that airborne nitrogen derived from NO_2 can be incorporated into the plant's nitrogen metabolism (Wellburn, 1990; Bender et al., 1991). At current rural concentrations, NO_2 is unlikely to be phytotoxic but may act, to some extent, as an additional source of N (Davison and Cape, 2003). Also, the concentrations of NH_3 in rural areas cannot be considered as a problem for crop growth, although there is some evidence that high and low temperatures or drought stress can considerably modify the effects of NH_3 (Cape et al., 2009). Overall, in highly fertilised agricultural systems across Europe atmospheric nitrogen (NO_2/NO, NH_3) and sulphur (SO_2, H_2S) compounds at current ambient levels cannot be considered as a direct threat for annual crops. By contrast, the few research findings from experiments performed in some developing countries of South and East Asia so far suggest that these pollutants can already lead to serious reductions of crop growth and yield, a situation which may be exacerbated in the future (Marshall, 2002; Ishii et al., 2004; Fu et al., 2007).

Considering current concentrations of VOCs, especially in rural areas, direct impacts of most of these compounds on crops are unlikely or unknown. Experiments to study the direct effects of VOCs on plants are usually performed in the laboratory and have used very high concentrations relative to ambient air (Cape, 2003). While a wide range of tolerance to airborne VOCs among plant species has been demonstrated, a few experiments have shown specific effects, particularly of ethylene (a plant hormone), on reproductive stages (seed germination, flowering, fruit ripening) (Collins and Bell, 2002; Cape, 2003).

For agriculture, chronic effects of air pollutants such as O_3 are of particular concern, because they are due to exposures for weeks, months, or over the entire lifecycle of the crop. It is well known that increasing O_3 levels cause a decline in the yield of many crop species, such as wheat, rice, soybean and cotton (Ashmore, 2005). Such yield losses have been attributed to reduced photosynthetic rate, altered carbon allocation and accelerated leaf senescence (Fiscus et al., 2005; Fuhrer, 2009). Recently, Mills et al. (2007) analysed O_3 exposure-response data for 19 agricultural and horticultural crops, respectively, and identified wheat, water melon, pulses, cotton, turnip, tomato, onion, soybean and lettuce as the most ozone-sensitive crops, while, for instance, barley was classified as O_3-resistant. Holland et al. (2006) estimated crop losses and the associated economic loss in Europe for 23 horticultural and agricultural crops for the base year 2000 and found an overall loss of 3% of all crop species considered, which would be equivalent to € 6.7 billion economic damage. The global impact of O_3 on crop yields was recently evaluated by Van Dingenen et al. (2009). Their estimates of present day global relative yield losses ranged between 7% and 12% for wheat, between 6% and 16% for soybean, between 3% and 4% for rice, and between 3% and 5% for maize. When translating the production losses into global economic damage for the four crops considered, they estimated an economic loss in the range of \$14–26 billion. About 40% of this damage is occurring in China and India. However, the uncertainty on these estimates is large. This is primarily due to the O_3 exposure metrics used in the estimates, which are based on the exposure concentrations in ambient air, either on a regional, national or global scale, rather than on the actual uptake of O_3 and thus do not account for the dose-specific nature of plant responses. In addition, only the direct O_3 effects on crop growth are considered, while indirect growth effects, e.g., mediated by phytosanitary problems, are not taken into account (see Sect. 3.3). Moreover, wide variability in O_3 sensitivity among various cultivars of a crop is common (USEPA, 2006). In summary, tropospheric O_3 remains the most important atmospheric pollutant that has direct negative effects on many crop species worldwide.

By contrast, the continuous rise in atmospheric CO_2 levels will principally have a positive effect on crop growth and yield, as CO_2 directly affects plant physiology and growth by serving as a primary substrate for photosynthesis. Generally, elevated CO_2 concentrations can increase biomass and yield in C_3 crops by increasing photosynthesis and decreasing photorespiration, but with large differences among species in the magnitude of the yield stimulation (Amthor, 2001; Kimball et al., 2002; Ainsworth and Long, 2004). No significant stimulation of yield has been found so far in C_4 crops, at least under well-watered conditions, because C_4 photosynthesis is saturated under ambient CO_2 (Long et al., 2005). However, in all crops (both C_3 and C_4), higher CO_2 concentrations reduce stomatal conductance and transpiration and improve water-use efficiency, i.e. crops will experience a reduced demand for water.

3.2 Direct Effects on Crop Quality

In comparison to air pollutant and climate change effects on crop growth and yield, much less is known about potential effects on the quality or the nutritive value, respectively, of agricultural and horticultural crops. Changes in crop quality due to O_3 exposure have been studied in a limited number of crops. For example, in wheat, O_3 reduced yield but increased grain protein concentration (Pleijel et al., 1999; Piikki et al., 2008). Moreover, O_3 was found to have positive effects on the quality of potato tubers by decreasing reducing sugars and increasing the vitamin C content (Vorne et al., 2002). In contrast, O_3 has been found to reduce the oil, protein and carbohydrate contents of the seeds of rape (Ollerenshaw et al., 1999). Recent evidence suggests that O_3 can also alter the plant food quality for ruminant animals. Decreased nutritive quality of forages was found in a number of pasture species (Krupa et al., 2004; Bender et al., 2006).

Pollutant-induced visible injury is of particular significance when the quality and the marketable value of the crop depend on the appearance of the foliage, as is the case for a number of horticultural crops. For example, Kostka-Rick et al. (2002) have shown that environmentally-relevant concentrations of O_3 can cause visible foliar injury on species such as lettuce, spinach or onion, which would make these crops unmarketable (Figs. 3 and 4).

Fig. 3 Ozone injury on spinach (*Spinacea oleracea* cv. Matador). Courtesy: J. Bender, vTI Braunschweig, Germany

Fig. 4 Ozone injury on spring onion (*Allium fistulosum* cv. Polo). Courtesy: J. Bender, vTI Braunschweig, Germany

A frequently observed phenomenon is that plants grown at high CO_2 levels exhibit significant changes in their chemical composition (Idso and Idso, 2001; Loladze, 2002). A prominent example of a CO_2 effect is the decrease in the nitrogen (N) concentration in vegetative plant parts as well as in seeds and grains and, related to this, the decrease in the protein concentrations (Cotrufo et al., 1998; Taub et al., 2008; Wieser et al., 2008). Other CO_2 enrichment studies have shown changes in the composition of other macro- and microelements (Ca, K, Mg, Fe, Zn) and in concentrations of secondary compounds, vitamins and sugars (Idso and Idso, 2001). Overall, these CO_2-induced changes may have negative consequences with respect to nutritional quality of foods and feeds, the plant-herbivore interaction and the element turnover of ecosystems, respectively. The examples above indicate that there may be economically important effects of air pollution and climate changes on the quality of crops and forage species, although the available information is still inconsistent.

3.3 Interactive Effects of Atmospheric Compounds

Under field conditions plants are exposed to different environmental factors including more than only one atmospheric compound. Based primarily on experimental work it has been shown that mixtures of atmospheric compounds and air pollutants, respectively, modify the magnitude and nature of the response to individual compounds. Generally, pollutant combinations may result in either more-than-additive (synergistic) or less-than-additive (antagonistic) effects. Based on the prevailing conditions at that time interactions of O_3 with other air pollutants (e.g., SO_2, NO_2) were studied quite frequently in the 1980s (reviewed by Fangmeier et al., 2002). Currently, at least for Europe and North America, a simultaneous occurrence of O_3, SO_2, NO_2 or NH_3 at phytotoxic levels is rather unusual and far less frequent than sequential or combined sequential/concurrent exposures. From experiments where exposure conditions have been more realistic in terms of their likelihood of occurrence in ambient air it can be concluded that: (1) antagonistic interactions tend to be found when gases were applied sequentially (e.g., O_3/NO_2) and/or when, e.g., nitrogenous or sulphurous air pollutants were combined with O_3 at relatively low levels, suggesting that plants were able to utilise the additional S or N source; and (2) synergistic interactions are more likely to be found when O_3 was applied simultaneously with another pollutant at high concentrations (Fangmeier and Bender, 2002). For the situation in Europe and North America this would imply that neither SO_2 nor NO_2 seems likely to pose an additional risk to that related to O_3. However, the effects of pollutant combinations on crop growth and yield should have a much higher significance in many developing countries where air pollutants such as SO_2, NOx and O_3 are rapidly increasing (Ishii et al., 2007).

With respect to the future there is some evidence that elevated CO_2 has the potential to mitigate negative effects of O_3(and other gaseous pollutants), mainly due to a CO_2-induced reduction in stomatal conductance, which reduces O_3 uptake. On the other hand, O_3 limits positive CO_2 responses in many plants as well (Fiscus et al., 2005). All climate change factors, such as CO_2, warming, changes in precipitation, etc., which may affect stomatal conductance and thus the flux of gaseous air compounds into leaves, will exert a modification on the effects of individual pollutants (Bender and Weigel, 2003; Harmens et al., 2007). In summary, the available information suggests that the continuing increases in the CO_2 component of climate change are likely to be ameliorative for the effects of air pollutants. Although there is some evidence that the concomitant increases in mean global temperature may decrease these protective effects (Fuhrer, 2003; USEPA, 2006), it is still not possible to predict the combined impacts of climate change and air pollutants on crops.

3.4 Indirect Effects

Atmospheric compounds and air pollutants, respectively, may interact with other biotic and abiotic growth or stress factors (e.g., water and nutrient supply; heat and water stress; salinity, pesticide application; pests and pathogens; symbiotic relationships) in a complex manner, thus causing indirect effects on crop performance. For example, while it is well accepted that reduced vitality due to O_3 stress can make plants more susceptible to plant pathogens, general predictions of O_3 effects on particular plant-pathogen systems are difficult, because the available data for specific pests and diseases are often controversial (USEPA, 2006; Fuhrer, 2009). Increased susceptibility after O_3 exposure has been reported for necrotrophic pathogens, while obligate biotrophic infections tend to be diminished by O_3 (Manning and von Tiedemann, 1995; USEPA, 2006). With regard to insect pathogens, there is a general trend that some pests may have a preference for and grow better when feeding on O_3-stressed plants, but there are also other observations where insect growth was not changed (USEPA, 2006). Viral infection often provides some protection from O_3 injury; however, the type and degree of protection depend on the specific host and virus (Manning and von Tiedemann, 1995).

The direct effects of elevated CO_2 levels on tissue chemical composition can have an indirect effect on plant-herbivore interactions, as host plants growing under enriched CO_2 environments usually exhibit, e.g., decreased tissue N concentration, increased C/N ratios and generally altered secondary metabolism of C-based secondary and structural compounds. This in turn may affect food consumption by herbivores and related population development (Stiling and Cornelissen, 2007). However, there is almost no information about how O_3 effects on plant-pathogen systems may be modified in a future climate with elevated CO_2 (Chakraborty et al., 2000; Fuhrer, 2009). For example, while host plants growing under enriched CO_2 environments usually exhibit larger biomass, increased C/N ratios and decreased tissue N concentration, O_3 has the opposite effect (Pleijel et al., 1999; Piikki et al., 2008). Hence, it remains open how food consumption by herbivores and population development are affected under future atmospheric conditions characterised by elevated O_3 and CO_2 concentrations (Stiling and Cornelissen, 2007).

Another important interaction may occur between the effects of air pollutants and soil moisture availability. Water supply directly affects stomatal conductance and hence the uptake and effects of gaseous air pollutants. For example, it is known that reduced soil moisture limits O_3 uptake by decreasing stomatal conductance, which increases O_3 tolerance (Bender and Weigel, 2003). However, other findings suggest that, in some species, soil moisture stress may reduce rather than increase O_3 tolerance (Bungener et al., 1999). The complex physiological and morphological changes due to water deficit impair plant vitality itself, e.g., by promoting senescence processes. Therefore, decreased pollutant uptake may not necessarily be connected with decreased pollutant injury. As outlined before (Sect. 3.1), elevated CO_2 concentrations often improve water-use efficiency, i.e. may mitigate drought stress effects (Manderscheid and Weigel, 2007), which is an important feedback effect in future climate change scenarios.

Although the available information is clearly insufficient to understand the importance of interactions between air pollutants and biotic or abiotic factors, it is suggested that these indirect effects could be more important under certain circumstances than the direct effects of the gases on plants.

4 Conclusion

Crops, similarly to all other types of vegetation, are closely linked to the exchange of matter between the atmosphere and biosphere. After deposition of atmospheric compounds on canopies, crop growth and quality may be affected in various ways. Regarding the situation in most parts of Europe and North America, exposure to compounds such as SO_2, NO_2/NO, VOCs and heavy metals is reduced and is currently not a major threat to crops. However, in many regions of both continents continuously increasing background levels of tropospheric O_3 remain a problem, which poses an additional risk to crop growth and health during the growing season. In the growing economies of many developing countries the concentrations of atmospheric compounds such as SO_2, NOx, NH_3 and particularly O_3 are rapidly increasing. Already, these pollutants can lead to serious reductions of crop growth and yields, a situation which may be exacerbated in the future. Interactions with both biotic and abiotic factors must be taken into account in assessing risks of air pollutants in the field. On a global scale the rapid change in atmospheric composition by the increase in the atmospheric CO_2 concentration accompanied by climate change has two major implications. A possible benefit to crop growth by direct stimulation of photosynthesis and by mitigation of gaseous air pollutants and water stress, but concomitantly a threat to crop production due to an enhancement of crop quality losses.

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Modelling Soil Carbon and Nitrogen Cycles During Land Use Change

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Contents

Abstract Forested soils are being increasingly transformed to agricultural fields in response to growing demands for food crop. This modification of the land use is known to result in deterioration of soil properties, in particular its fertility. To reduce the impact of the human activities and mitigate their effects on the soil, it is important to understand the factors responsible for the modification of soil properties. In this paper we reviewed the principal processes affecting soil quality during land use changes, focusing in particular on the effect of soil moisture dynamics on soil carbon (C) and nitrogen (N) cycles. Both physical and biological processes, including degradation of litter and humus, and soil moisture evolution at the diurnal and seasonal time scales were considered, highlighting the impact of hydroclimatic variability on nutrient turnover along with the consequences of land use changes from forest to agricultural soil and vice-versa. In order to identify to what extent different models are suitable for long-term predictions of soil turnover, and to understand whether some simulators are more suited to specific environmental conditions or ecosystems, we enumerated the principal features of the most popular existing models dealing with C and N turnover. Among these models, we considered in detail a mechanistic compartment-based model. To show the capabilities of the model and to demonstrate how it can be used as a predictive tool to forecast the effects of land use changes on C and N dynamics, four different scenarios were studied, intertwining two different climate conditions (with and without seasonality) with two contrasting soils having physical properties that are representative of forest and agricultural soils. The model incorporates synthetic time series of stochastic precipitation, and therefore soil moisture evolution through

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_23,

from Batlle-Aguilar et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010007

time. Our main findings in simulating these scenarios are that (1) forest soils have higher concentrations of C and N than agricultural soils as a result of higher litter decomposition; (2) high frequency changes in water saturations under seasonal climate scenarios are commensurate with C and N concentrations in agricultural soils; and (3) due to their different physical properties, forest soils attenuate the seasonal climate-induced frequency changes in water saturation, with accompanying changes in C and N concentrations. The model was shown to be capable of simulating the long term effects of modified physical properties of agricultural soils, being thus a promising tool to predict future consequences of practices affecting sustainable agriculture, such as tillage (leading to erosion), ploughing, harvesting, irrigation and fertilization, leading to C and N turnover changes and in consequence, in terms of agriculture production.

Keywords Soil organic matter • Biogeochemical cycles • Agricultural soil • Forest soil • Soil nutrients • Soil moisture dynamics • Soil restoration

1 Introduction

Soils are complex systems sustaining life on Earth. Among other functions, soils maintain plant and animal growth, recycle nutrients and organic wastes, filter and purify water. Precisely, soil quality refers to a combination of chemical, physical, and biological processes that confers to the soil the ability to carry out, among others, these particular ecological functions. Numerous human activities however utilise soil, modify its physical and chemical properties and change the composition of its ecosystems. As a result, in the last century a widespread decrease of soil quality has been observed, together with a deterioration of its functioning (Brady and Weil, 2004).

The main component of soils is organic matter (SOM), which shows a variable degree of decomposition, from fresh litter to highly decomposed humus. SOM stores three to four times the amount of carbon (C) than found in all living vegetation. Other than C, soils also contain nearly all the macro- (nitrogen, N, phosphorous, P, and potassium, K) and micronutrients required by living organisms. Among the macronutrients N plays a major role since it is essential for life but its bio-available forms are seldom abundant in the environment. Therefore in many ecosystems the N cycle controls the overall soil turnover and functioning. For these reasons and without neglecting the importance of other nutrients, in this paper the focus is on soil C and N cycles.

1.1 Land Use Change: Forest Versus Agricultural Soils

The increasing demand on food crops, pasture, firewood and timber is at the origin of worldwide changes of land-use in forested areas. This situation is worrying in some areas of the planet, such as South America, where 12% and 7% of forestland was converted to pasture and croplands, respectively, between 1850 and 1985 (Houghton et al., 1991). Land-use changes, and especially cultivation of previously forested land, reduce significantly the soil quality (e.g., changes in SOM content and decomposition rates, changes in soil chemical and physical properties), leading to a permanent degradation of land productivity (Nye and Greenland, 1964; Islam et al., 1999). Furthermore, it has been reported that deforestation increases carbon dioxide (CO_2) release to the atmosphere (Houghton, 2002), which contributes to global warming.

All studies that focused on the effects of land conversion from forest to cultivated land concluded that land-use change induces a reduction of the available soil C and a decrease in its quality. The maximum rate of loss occurs during the first 10 y of cultivation, with total C decrease up to 30% (Davidson and Ackerman, 1993; Lugo and Brown, 1993; Murty et al., 2002) followed by reduced but still significant reduction rate (Brams, 1971; Martins et al., 1991; Bonde et al., 1992; Motavalli et al., 2000). Furthermore, it was reported that the loss rate is highly variable and influenced by several factors such as the native vegetation, climate, soil type and management practices (Mann, 1986; Davidson and Ackerman, 1993; Bruce et al., 1999).

Contrasting with the conversion from forest to cultivated land, controversy exists when the change is from forest to pasture lands. The overall change in soil C has been shown to be either positive or negative. For instance, de Moraes et al. (1996) found an increase up to 20% in total soil C 20 y after the change in land use, while Veldkamp (1994) reported a net soil organic C loss up to 18% after 25 y. Johnson (1992) also observed that changes in soil C in both land-use cultivation and pasture were associated with changes in soil N. Reiners et al. (1994) found that the transformation of forest land to pasture led to important changes in the N cycling. For example, the ammonium (NH_4^+) pool was larger in pasture lands while the nitrate (NO_3^-) pool was less important in pasture than forest lands. This is consistent with a low rate of plant uptake of NH_4^+ and slow nitrification rates (Vitousek, 1984; Vitousek and Sanford, 1986).

One of the important aspects that affect SOM cycling in the transition from forest to cultivated soil is the removal of most of the fresh organic C (litter) due to harvesting (Smil, 1999). However, harvesting is not the only factor responsible for the soil organic C loss. Some other processes that were also recognized to contribute to change the amount of soil C are the changes in litter chemical properties (Feigl et al., 1995; Ellert and Gregorich, 1996; Scholes et al., 1997), soil type (Feller and Beare, 1997; Scholes et al., 1997; García-Oliva et al., 1999), microbial community (Prasad et al., 1995), changes in soil N cycling (Dalal and Mayeer, 1986; Brown and Lugo, 1990; Desjardins et al., 1994) and management practices (Feller and Beare, 1997; Fernandes et al., 1997; Bruce et al., 1999). Soil tillage and ploughing promote redistribution of residues and their decomposition. As a result, soil C and N pools are depleted and soil fertility is lost. Soil C is oxidized to CO_2 and lost to the atmosphere contributing to the increase of greenhouse gases in the atmosphere. Moreover, tillage improves soil aeration, destroys macro-aggregates and changes the hydrological cycle, with an increase of the respiration rates and ultimately an additional depletion of the C pool (Juo and Lal, 1979; Agboola, 1981; Ellert and Gregorich, 1996; Reicosky et al., 1997; Bruce et al., 1999).

In agricultural areas, the root zone (soil depth affected by plant roots) remains constant over time and is relatively shallow. Different rooting patterns have direct effects on the C flux, since they affect soil porosity and soil aeration (Berger et al., 2002). Therefore, changes in land use resulting in a modified rooting depth often have a direct influence on soil respiration and C mineralization rates, and thus on soil turnover (Howard and Howard, 1993).

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed as a consequence of human activities (Young et al., 2005), and typically involves a land use change. During the restoration, environmental conditions (e.g., type of vegetation, ecosystem corridors or soil practices) are manipulated to create ecological conditions suitable for the successful establishment of a target composition of species (Prober et al., 2005). The change from agricultural soil to the original forest is a typical example of soil restoration, where natural soil properties and vegetation are amended, resulting in an improvement of soil fertility and an ecosystem close to its former natural condition.

1.2 Modelling of Soil C and N Cycles

Numerical tools are becoming increasingly used to understand the modifications induced in ecosystems as a result of changes in land use, and it has been found that understanding the coupled N and C dynamics is of primary importance for predictive models of SOM evolution, for example to changes in land use and responses to global changes (Rodriguez-Iturbe et al., 2001). Modelling of soil biogeochemical processes dates back to the 1930s (Manzoni and Porporato, 2009), and nowadays an extended list of stochastic, empirical and mechanistic models incorporating soil nutrient dynamics is available. Models vary significantly in terms of complexity and mathematical description of the biological and geochemical processes involved. Manzoni and Porporato (2009) reviewed and classified about 250 different mathematical models developed over 80 y. Most of the models currently available evolved from early efforts to provide a concise mathematical description of the soil cycles, and have been adapted and improved to specific applications. The aims of the different models are numerous and include, for example: understanding and prediction of feedbacks between terrestrial ecosystems and global climate (e.g., estimate and predict climatological and biological effects of human activities) (Agren et al., 1991; Melillo, 1996; Moore et al., 2005); influence of climate changes on nutrient cycling in soils (Pastor and Post, 1986; Hunt et al., 1991; Moorhead et al., 1999; Eckersten et al., 2001; Ito, 2007); prediction of changes in soil C and N cycles related to possible land use changes (Eckersten and Beier, 1998; Paul and Polglase, 2004; Christiansen et al., 2006; Findeling et al., 2007; Pansu et al., 2007; Post et al., 2007; Kaonga and Coleman, 2008); and forecasts of crop productivity and system response under specific physical soil changes (Wolf et al., 1989; Wolf and Van Keulen, 1989; Matus and Rodríguez, 1994; Parton and Rassmussen, 1994; Henriksen and Breland, 1999; Nicolardot et al., 2001).

The aim of this manuscript is to provide an overview of the main processes, mechanisms and parameters affecting the evolution of selected soil nutrient cycles (soil C and N) and to provide a modelling framework that incorporates the key mechanisms. Both physical and biological processes, including degradation of litter and humus, and soil moisture evolution on diurnal and seasonal time scales are considered. In the first part of the manuscript, soil C and N cycles are summarized, followed by an overview of the most popular models dealing with soil nutrient turnover. In the second part, a compartment model based on Porporato et al. (2003) is described and applied to simulate soil C and N dynamics, as well as degradation and transformation processes occurring under different precipitation and soil scenarios. Contrasting soil types and precipitation regimes are considered, to illustrate modelling capabilities and to show how numerical tools can be used to understand effects of land use changes over soil C and N fluxes and, thus, the feasibility and viability of ecological restoration regarding the modelled ecosystem and surroundings.

2 C and N Cycles in Soil

The global C cycle can be depicted as consisting of a series of interconnected compartments (terrestrial, aquatic and atmospheric) where C is stored and transformed. Soils are part of the terrestrial C pool (Fig. 1). The amount of C stored in the (living and dead) organic matter in soils is three to four times higher than that in the atmosphere (Bruce et al., 1999). The circulation rates are also high. For these reasons, soil C turnover is of primary importance to developing understanding and forecasting global changes in biogeochemical cycles and climate change (Stevenson and Cole, 1999; Rodriguez-Iturbe and Porporato, 2004). The total global emission of CO_2 from soils is probably the largest flux in the global C cycle, and small changes in the magnitude of soil respiration, if they take place at large scale, could have a tremendous effect on the concentration of CO_2 in the atmosphere (Schlesinger and Andrews, 2000; Murty et al., 2002). By the same argument, soils have also a great potential for long-term C storage. Whether a soil will act as a sink or source of CO_2 depends on a number of environmental factors, including climatic variability and anthropogenic changes in land use, which for example may result in a modified composition of the vegetation and therefore of the quality and quantity of litter inputs (Gignoux et al., 2001).

The principal C exchange processes between soil and atmosphere are *photosynthesis* and *respiration*. Photosynthetic C fixation by plants – often named

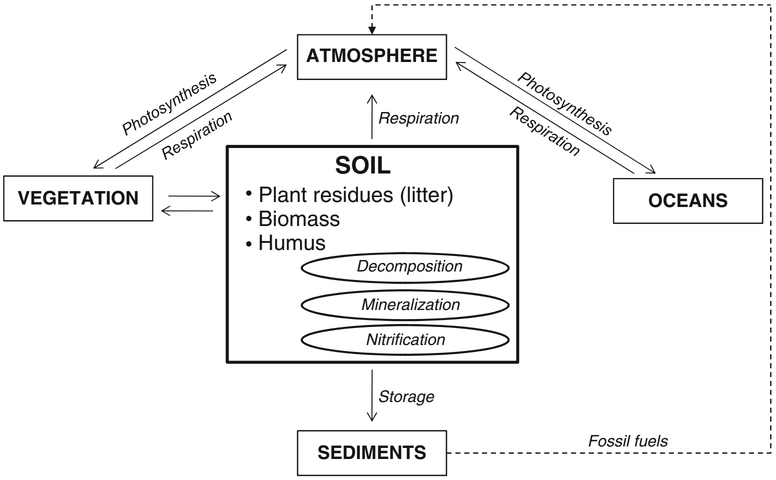

Fig. 1 Simplified representation of the global carbon cycle

primary producers – converts atmospheric CO_2 and is the main source of soil organic C. Briefly, during photosynthesis CO_2 is used as a C source to produce complex organic molecules, using sunlight as an energy source (e.g., Killham and Foster, 1994):

$$CO_2 + H_2O + \text{Energy} \rightarrow CH_2O + O_2. \tag{1}$$

The complex organic molecules produced by plants enter the soil C cycle as decaying organic matter (litter) and are progressively converted to simpler molecules. A significant fraction of the organic C introduced in the soil is directly used as an energy source to sustain pedofauna metabolism, and is released again to the atmosphere in form of CO_2 through respiration:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O + \text{Energy}. \tag{2}$$

Another part of the soil C is assimilated by vegetation and finally transferred to the soil as plant litter, becoming part of SOM (Porporato et al., 2003). Organic C is available in soils in a large variety of forms. Killham and Foster (1994) partitioned the soil organic C into three main pools: insoluble, soluble and biomass. Insoluble soil organic C includes plant residues and partially decomposed material, which forms the litter and the humus. Soluble C is a fraction of the humus further decomposed and is rapidly assimilated as a substrate by the pedofauna. The fast consumption of soluble C explains its often low concentration in the soil (1%) in comparison to insoluble organic C (90%). Soil biomass (9%) consists of microbes and animals (e.g., macroinvertebrates), the decomposition activity of which is mostly responsible for the C decomposition and recycling (Killham and Foster, 1994).

Within the soil, organic C is transferred between the different pools (or compartments) by means of *decomposition* processes, which are regulated by environmental conditions (e.g., soil moisture) and the C/N ratio (Brady and Weil, 2004). These factors will be discussed subsequently. Litter undergoing decomposition is mainly composed of plant residues (fallen leaves, roots, etc.). Decomposition rates are highly variable in time, and are mainly controlled by the environmental conditions (e.g., soil moisture level, aeration, soil temperature) and the quality of the added litter. Complex organic molecules can be decomposed under either aerobic or anaerobic conditions. Under normal conditions, soils are unsaturated and thus O_2 is likely to be always available. However, even in the vadose zone saturated conditions can result from, for example, significant precipitation events. Wetlands are a particular case where saturated conditions are found permanently or seasonally. In general, microbial decomposition rates are larger under aerobic conditions (Brady and Weil, 2004), where O_2 acts as the electron acceptor during oxidation of organic compounds (Barry et al., 2002). On the other hand, slow decomposition rates under anaerobic conditions can result in accumulation of considerable amounts of partially decomposed organic matter (Fig. 2).

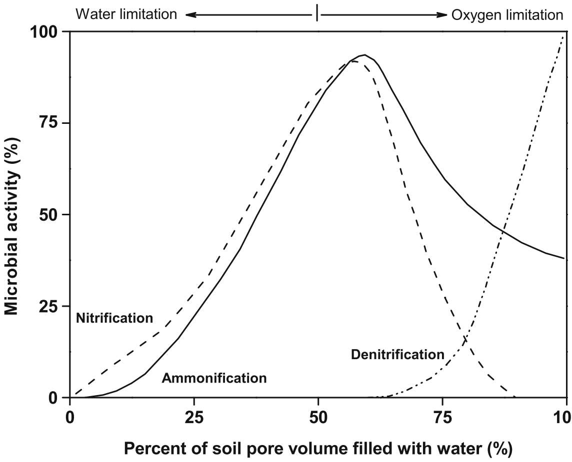

Fig. 2 Influence of the soil water content on bacterial activity in different processes of nitrogen transformations (after Fenchel et al., 1998)

Soil N comes mainly from the atmosphere, which is the largest N pool and contains almost 75% of the total N available on Earth (Barbour et al., 1999). In brief, the soil N cycle is based upon the uptake of the inorganic forms (NO_3^-, NH_4^+) by plants. N returns to the soil in organic form as plant residues, which are decomposed by the soil pedofauna (e.g., invertebrates, microbes, fungi) and are made available to plants in inorganic form.

The total amount of organic N in soils varies greatly and is influenced by the soil-forming factors likely climate, topography, vegetation, parent material and age. The N cycle is tightly coupled to the C cycle, since most of the microbial N transformations (e.g., nitrification) use energy supplied by C (Paul, 1976). Although locally N is also incorporated into soils through dry or wet direct deposition, the largest fraction of soil organic N fixation is done biologically (conversion from N_2 gas to organic forms, mediated by specific microbial strains). N is found in soils mainly within the organic matter fraction, for example humic compounds, plant roots, microbial biomass and decomposing organic materials. The amount of organic N contained in soils far exceeds that which is present in plant-available inorganic forms.

The soil N, mainly present in organic form as previously mentioned, is almost unavailable for plants. The vegetation mainly uses inorganic forms of N, which are made available by the SOM decomposition. Soil microorganisms convert the N contained in the organic matter to NH_4^+ in a process named *mineralization* (Schinner et al., 1995), further subdivided into two processes. The organic N is initially transformed via *ammonification*, and – if O_2 is available – NH_4^+ is subsequently oxidized to nitrite (NO_2^-) and NO_3^-, through *nitrification*:

$$\begin{aligned} NH_4^+ + O_2 + H^+ + 2e^- &\rightarrow NH_2OH + H_2O \\ &\rightarrow NO_2^- + 5H^+ + 4e^-, \end{aligned} \tag{3}$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-. \tag{4}$$

Although plants can use both forms of inorganic N, NO_3^- is used in preference to NH_4^+ because of its greater solubility in water. In other words, nitrates quickly dissolve in the pore solution, which is taken up by plants. On the other hand, however, this also means that NO_3^- is easily flushed to groundwater. NH_4^+ is instead less mobile because it is strongly adsorbed on clay minerals due to its positive charge.

Denitrification is the anaerobic microbial reduction of N, and NO_3^- is used as an electron acceptor (i.e., source of energy), resulting in a transfer of soil N to the atmosphere (Groffman et al., 2002):

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2(g) + 6H_2O. \tag{5}$$

Immobilisation is a process involving microbial uptake of nutrients, where inorganic N is converted into organic form, such as amino acids and biological macromolecules.

The carbon-to-nitrogen ratio (C/N) is an important factor affecting the overall turnover rates of SOM (Young and Young, 2001). Bacterial sensitivity to the C/N ratio is due to the fact that bacteria need a constant C/N ratio, while this ratio is highly variable in substrate. For example, intense competition among microorganisms for available N occurs when soil residues have a high C/N ratio, i.e., the substrate is poor in N making it the limiting factor (Brady and Weil, 2004). Environmental conditions (e.g., soil moisture and temperature) have a direct influence on bacterial activity and thus on this ratio (Koch et al., 2007). The C/N ratio of the substrate tends to decrease as the SOM becomes more decomposed – from fresh litter to highly transformed humus – when microbes are solely responsible for decomposition (Zheng et al., 1999) because the microbial C/N ratio is lower than that of litter (Persson, 1983). In other words, the humus is enriched in N compared to the litter. For this reason the C/N ratio of the litter pool controls the rates of mineralization/immobilisation. Young and Young (2001) identified a threshold of the C/N ratio which determines the bacterial activity. When C/N > 25, microbes respire completely using the available C and thus assimilate the entire N mineralized, and consequently N is immobilised. In contrast, if C/N < 25, the SOM N content far exceeds the immobilisation capacity of microbial populations and the result is a net mineralization. Although this threshold seems to be directly related to the C/N ratio needs of bacteria, White (1997) argued that this threshold value is variable among different ecosystems, for example because the C/N ratio of the vegetation changes depending on the composition and relative frequency of each species. For example, pines produce litter with C/N ratio as high as 90, while litter originating from cereal crops has C/N ratio of 80 and tropical forest trees produce litter with C/N ratios around 30 (Young and Young, 2001).

3 Moisture Dynamics as a Controlling Factor of Soil Carbon and Nitrogen Cycles

Soil moisture results from the interactions between climate, soil type (texture, granulometry, organic matter content) and vegetation, and it is consequently variable both in space and time. Among the possible physical processes the dynamics of soil moisture exerts the greatest influence over SOM turnover, mineralization, decomposition, leaching and uptake, and its effects are complex and non-linear. As an example to illustrate this complexity, the production of plant residues

– the main source of litter and therefore of energy for the pedofauna – depends on the growth rate of vegetation, which is controlled by water availability. Accumulation of SOM can increase the water retention capacity of the soil, with a positive feed-back on the vegetation. Moreover, the soil biota activity depends on the soil water content, and optimal decomposition rates are only achieved within a relatively narrow soil moisture range.

Soil biota is sensitive to moisture level for several reasons. In order to preserve cell integrity, when the soil water content decreases bacteria increase the intracellular solute concentration to compensate for the extracellular concentration and counterbalance the increased osmotic pressure (Stark and Firestone, 1995; Bell et al., 2008). Therefore, a high concentration of solutes results in an inhibition of the enzymatic activity and therefore decreased cellular activity. Additionally, as the soil becomes drier, water in soil pores becomes a thin layer covering soil grains and substrate availability becomes diffusion-limited. In consequence, microbial activity is further reduced (Csonka, 1989; Stark and Firestone, 1995; Fenchel et al., 1998).

It is however difficult to identify a unique threshold moisture level under which soil respiration (or microbial activity) diminishes. Davidson et al. (1998) and Rey et al. (2002) estimated that 75% of the soil field capacity corresponds to the soil moisture level below which soil respiration decreases, while according to Xu et al. (2004) a more likely value is 42%.

A number of studies have shown that soil moisture effects on soil C and N turnover also depend on the time-scale of interest. Curiel Yuste et al. (2007) found that, at the seasonal scale, the effect of temperature and soil moisture on CO_2 efflux (e.g., soil respiration) was very similar for ponderosa pine and oak savannah ecosystems. For shorter time scales (e.g., daily), decomposition of organic matter was mainly controlled by temperature during wet periods and a combination of temperature and soil moisture during dry periods. Soil bacterial growth (or soil respiration) – a parameter often used as a measure of microbial activity – shows a maximum at about 30°C (Pietikåinen et al., 2005). Nevertheless, the influence of temperature on microbial activity is generally considered much less important than soil moisture (Rodriguez-Iturbe and Porporato, 2004) because, although important differences in soil temperature are likely to occur at a daily and seasonal scale in the uppermost soil (e.g., first few centimetres), yearly average values at depth are much more constant than those of soil moisture.

Typically, summer drought decreases substantially decomposition rates (Curiel Yuste et al., 2007), but it has been observed that sporadic rains during these dry periods tends to increase the decomposition efficiency of the bacterial communities (Borken et al. 1999, 2002; Savage and Davidson, 2001; Goulden et al., 2004; Xu et al., 2004; Misson et al., 2005; Scott-Denton et al., 2006). Kieft et al. (1998) and Moore et al. (2008) observed an increase of root density and soil microbial activity rate in response to isolated moisture pulses in arid soils, although the response of root density occurred at longer time-scale. A fast rewetting of the soil profile is likely to have negative consequences on microbial populations in that it can generate an osmotic shock and result in cell lyses (Kieft et al., 1987; Van Gestel et al., 1993). In contrast, Ryel et al. (2004), Schwinning and Sala (2004) and Bell et al. (2008) found that, in arid soils, while plants usually do not take advantage of brief pulses of moisture generated by short precipitation events, microbial mineralization is stimulated. Consequently, short-term increases in soil microbial activity triggered by moisture pulses will not typically correlate with an increase in primary production at the same time scale, confirming that plant growth is not only dependent on soil microbial activity, but also on other factors such as the precipitation event duration, amount of soil water infiltrated and the overall change in soil moisture. The magnitude and timing of intra-seasonal precipitation becomes therefore a key regulator for microbial activity (Bell et al., 2008). Since decomposition and consequent mineralization can be stimulated by moisture pulses that are too brief to benefit primary producers (e.g., plants) (Cui and Caldwell, 1997; Schwinning et al., 2003; Austin et al., 2004), in arid soils there is potential for soil nutrient pools to accumulate over time and become available to plants as heavier precipitation occurs. The influence of soil moisture over soil nutrient dynamics has been also studied in temperate (Davidson et al., 1998; Buchmann, 2000; Reichstein et al., 2003) and tropical forests (Conant et al., 2000; Davidson et al., 2000; Kiese and Butterbach-Bahl, 2002; Epron et al., 2004). It was concluded that a strong influence of the soil moisture over microbial activity exists, but that the degree of correlation varies strongly among different ecosystems (Buchmann, 2000; Rustad et al., 2000).

4 Existing Models of Soil Carbon and Nitrogen Turnover

At least 250 models dealing with soil C and nutrient turnover exist (Manzoni and Porporato, 2009). Classification of all these simulators is difficult because they are based on a wide range of physical and biogeochemical descriptions of the processes and the underlying assumptions vary significantly. Nevertheless, based on their internal structure models describing SOM dynamics can be divided into (1) process-oriented, (multi)-compartment models; (2) organism-oriented (food-web) models; (3) cohort models describing decomposition as a continuum; and (4) a combination of model types (1) and (2) (Brussaard, 1998; Smith et al., 1998; Post et al., 2007). Process-oriented or compartment models (each compartment or pool is a fraction of SOM with similar chemical and physical characteristics) are built considering the processes involved in the migration of SOM across the soil profile and its transformations (Smith et al., 1998). Models belonging to this class can potentially have a variable degree of complexity, from the simplest case with no compartments (considering degradation as a continuum) to more refined, multi-compartment models, with each compartment composed of organic matter with similar chemical composition or degradability. Process-oriented models can be combined with GIS software, giving a modelling platform well suited for regional-scale studies. Examples of successful coupling between soil turnover and GIS software are CANDY (Franko, 1996), CENTURY (Schimel et al., 1994) and RothC (Post et al., 1982; Jenkinson et al., 1991). On the other hand, the theoretical compartments that define the structure of multi-compartment process-oriented models are difficult to compare with the measurements of SOM fractions, and therefore the testing and validation is difficult and limited (Christensen, 1996; Elliott et al., 1996). Among the most popular process-oriented models are also DAISY (Hansen et al., 1991), NCSOIL (Molina et al., 1983) and SOILN (Johnson et al. 1987).

In organism-based models the SOM flows from one organism pool to another, which in turn are classified depending upon their taxonomy or metabolism. The main advantage of organism-oriented models is that the main drivers of SOM fluxes and transformations – the pedofauna – are explicitly accounted for. However, as noted in Post et al. (2007), to date there is no general acceptance of the existence of a relation between soil biota abundance and degradation rates. In contrast, the relationship between degradation rate and amount (or concentration) of substrate, as in process-based models, is well recognized. Simple first-order kinetic rates are often suitable to model the transformations, and the reaction rates can be easily estimated from laboratory experiments and directly used in process-oriented models. Site-specific calibration of organism-oriented models involves the characterization of the soil microbial consortia and therefore requires more complex techniques, while process-oriented models are less influenced by the features of the microbial communities, and have a larger range of application to different environments. To summarize, process-oriented models are easier to apply and calibrate than organism-oriented, which explains their greater popularity. Nevertheless, organism-oriented models have been proposed by several authors, including Moore et al. (2004), Kuijper et al. (2005), Zelenev et al. (2006) and Cherif and Loreau (2009).

A cohort is a set of items sharing some particular characteristic. Cohort models divide SOM into cohorts, which are further divided into different pools (e.g., C, N). Contrary to process-based models where decay is usually treated as a purely physical or biochemical process, e.g., described by a first-order rate, cohort models consider explicitly microbial physiology as the driving factor of decomposition. An example of a cohort model was proposed by Furniss et al. (1982), where SOM was divided into three cohorts considering age, origin and size, with each cohort subdivided into a number of chemical constituents. Gignoux et al. (2001) developed SOMCO (soil organic matter cohort), where SOM is divided into different cohorts in a demographic sense, meaning that a cohort is a set of items of the same age. At each time step a new cohort is defined and its fate is followed until its relative amount to total SOM becomes negligible. Other examples of models belonging to this class are those of Pastor and Post (1986), Bosatta and Ågren (1991, 1994) and Frolking et al. (2001).

The last group of models consists of a combination of process- and organism-oriented models, which are seldom used because their applicability is limited by the data required to define the organism-oriented components (Smith et al., 1998). Some examples of combined models are proposed by O'Brien (1984) and Paustian et al. (1990).

In order to identify to what extent different models are suitable for long-term predictions of soil turnover, and to understand whether some simulators are more suited to specific environmental conditions or ecosystems, model comparisons were conducted using long-term experiments and multi-annual datasets. De Willigen (1991) tested 14 different models comparing their ability to simulate soil N turnover (e.g., mineralization and plant uptake). It was concluded that aboveground processes (e.g., plant growth) were easier to simulate than belowground transformations (e.g., soil water and N content), and that the more complex, multi-compartment models do not necessarily provide better results in terms of predictive capabilities. Rodrigo et al. (1997) compared the effects of soil moisture and temperature variations on nine different models (NCSOIL; SOILN; DAISY; Kersebaum's model, Kersebaum and Richter, 1991; MATHILD, Lafolie, 1991; TRITSIM, Mirschel et al., 1991; NLEAP, Shaffer et al., 1991; SUNDIAL, Bradbury et al., 1993; CANTIS, Neel, 1996) on predictions of soil C and N turnover. Not surprisingly, they observed the highest C decomposition and N mineralisation rates close to field capacity conditions and decreasing rates during soil drying. In this study good agreement between the different models for low moisture conditions was observed, whereas poor agreement was found in wet soils, with water saturation equal or above field capacity. A complete comparison of nine process-oriented multi-compartment models (CANDY; NCSOIL; RothC; DAISY; CENTURY, Parton et al., 1987; Verberne model, Verberne et al., 1990; ITE, Thornley, 1991; DNDC, Li et al., 1994; SOMM, Chertov and Komarov, 1997) was presented by Smith et al. (1997). A qualitative and quantitative evaluation of the performance of the models was carried out by comparing their ability to simulate observed data from seven different sites in temperate regions. A general conclusion of all these comparisons was that the errors derived from the tested models are not significantly different, meaning that the models provide consistent results except when a model is used for an application for which it was not developed. For example, the ITE and SOMM models were developed for grasslands while in the study of Smith et al. (1997) they were applied to crops. Model calibration is an additional source of uncertainties and makes the comparison of different models difficult. Pansu et al. (2004) presented a qualitative comparison of the predictive performance of a family of five multi-compartment models, MOMOS-2 to -6, using ^{14}C- and ^{15}N-labelled species in field experiments. These models use the same conceptual approach but have different complexity, in that the number of compartments varies from 3 to 5 and the description of the biochemical transformation uses a different level of detail and simplification. Pansu et al. (2004) concluded that the simplifications do not decrease significantly model accuracy, but that the use of additional compartments results in improved long-term predictions.

Most of the currently available models are updated versions of earlier and original versions that have been modified to extend the applicability to specific ecosystems. Table 1 presents a list of the five most popular models, their main features together with the key references. The popular CENTURY model, originally devised for modelling soil nutrient dynamics in grassland systems, has been considerably modified since its first version. Smith et al. (1997) and Parton and Rassmussen (1994) modified the CENTURY model for application to crop and pasture systems, while Kelly et al. (1997), Peng et al. (1998) and Kirschbaum and Paul (2002) modified the model to be applied to forest ecosystems. Despite the ad hoc modifications, contrasting results in terms of predictive capabilities were obtained. The RothC model of Jenkinson et al. (1990), is an evolution of the model previously presented by Jenkinson and Rayner (1977), named Rothamsted. TOUGHREACT-N (Maggi et al., 2008) was developed to study the biogeochemical soil N cycle under different conditions of fertilization and irrigation. It is based on the multi-phase, multi-component reactive transport model TOUGHREACT (Xu et al., 2006), in turn an evolved and improved version of TOUGH2 (Pruess et al., 1999). SWIM (Krysanova et al., 1998), based on previously developed tools (MATSALU, Krysanova et al., 1989; SWAT, Arnold et al., 1993) and originally devised for modelling soil N cycle in mesoscale watersheds (10^2 to 10^4 km^2), has recently been extended to better describe groundwater dynamics and processes in riparian zones (Hattermann et al., 2004; Wattenbach et al., 2005). FullCAM (Richards, 2001) accounts for full C turnover in forests, and is an integrated suite of sub-models: the empirical C tracking model CAMFor (Richards and Evans, 2000), the tree growth model 3PG (Landsberg and Waring, 1997), the

Table 1 Main characteristics of the five most popular models

Model	Type	Characteristics	References to updated versions
CENTURY Parton et al. (1987)	Process-oriented multi-compartment	• Long-term (decades to centuries) of SOM dynamics, plant growth and cycling of N, P and S, using monthly input data; Three organic compartments: active, slow and passive; Soil texture regulates the C transfer from one compartment to another.	Parton and Rassmussen 1994 Fernandes et al. (1997) Kelly et al. (1997) Smith et al. (1997) Bolker et al. (1998) Peng et al. (1998) Kirschbaum and Paul (2002) Easter et al. (2007)
DAISY Hansen et al. (1991)	Process-oriented multi-compartment	• Simulates crop production, soil water and N dynamics; • Four different modules: hydrological, soil temperature, soil nitrogen, and crop models; • Decomposition rate constants depend on clay content; • Vertical soil profile divided in layers considering physical and chemical characteristics.	Jensen et al. (1997) Smith et al. (1997)
NCSOIL Molina et al. (1983)	Process-oriented multi-compartment	• Simulates C and N fluxes through soil microbes and organic components; • Four organic compartments: plant residues, microbial biomass, humus and stable organic matter.	Smith et al. (1997) Hadas et al. (2004)
CANDY (CArbon and Nitrogen DYnamics) Franko et al. (1995)	Process-oriented multi-compartment	• Composed of four different submodels: soil temperature, hydrological, crop and organic matter turnover models; • Daily time step; • Simulates soil N dynamics, temperature and moisture.	Franko (1996) Franko et al. (1997) Smith et al. (1997)
RothC (Rothamsted model) Jenkinson and Rayner (1977)	Process-oriented multi-compartment	• N and C are not interconnected; • C turnover sensitive to soil type, temperature, moisture and plant cover; • Monthly input data.	Smith et al. (1997) Easter et al. (2007) Kaonga and Coleman (2008) Jenkinson and Coleman (2008)

litter decomposition model GENDEC (Moorhead and Reynolds, 1991) and the soil C turnover model RothC (Jenkinson, 1990). PASTIS (Lafolie, 1991; Garnier et al., 2001, 2003) is a one-dimensional mechanistic model that simulates the transport of water, solutes and heat using Richards' equation for water flow, the advection-dispersion equation for solute transport and the diffusion equation for heat flow. Some variations to this model have been implemented, such as PASTIS$_{\text{mulch}}$ (Findeling et al., 2007), which extends the original capabilities by including the physical effects of a surface residue mulch on rain interception and evaporation. Another example of model evolution is the family of models MOMOS-2 to -6, which are modified versions from the initial MOMOS-C (Sallih and Pansu, 1993) and MOMOS-N (Pansu et al., 1998) models. TRIPLEX (Peng et al., 2002) is a model of forest growth and C and N dynamics, and is a combination of three prior well-established models: 3PG (Landsberg and Waring, 1997), TREEDYN3.0 (Bossel, 1996) and CENTURY4.0 (Parton et al., 1993). Easter et al. (2007) developed a soil C modelling system, GEFSOC, aimed at modelling soil C stocks and exchange rates at regional or country scales in response to land use changes. The developed tool is based on three well-recognized models: the CENTURY general ecosystem model, the RothC soil C decomposition model and the empirical IPCC method (IPCC, 2003) for assessing soil C stock changes at regional scales. The model can be coupled to a soil and terrain digital database to include the topography and spatial soil variability of the studied area.

5 Description of the C-N Model

The model we describe and use in this work was presented by Porporato et al. (2003). It belongs to the group of process-based models, with the soil organic matter and nutrients divided into five pools. Three pools consist of SOM (litter, humus and microbial biomass), while the remainder are for inorganic N. The model is applied to the root zone treated as a single unit, i.e., spatial variations are ignored.

The framework with three organic pools is in good agreement with Jenkinson (1990), who proposed that process-based models should have between two and four pools to obtain reliable results. These pools represent the main components of the system, and C and N concentrations correspond to average values over the rooting depth (Z_r) (Rodriguez-Iturbe and Porporato, 2004). This simplification is justified because soils have often a uniform distribution of SOM and inorganic N over the whole rooting depth (Porporato et al., 2003). This is not true however for the uppermost soil layer, where organic residues tend to accumulate, and acts as a source of litter to the layers beneath.

Additionally, some other simplifications were made during the development. First, SOM decomposition rates are known to vary over orders of magnitude among the different components and, as already described, each functional group of organisms has specific and highly variable decomposition and mineralization rates. In the model however no distinction is made between different microbial populations. Rather, for each pool, a single, first-order kinetic rate is used, which represents an average transformation rate. This approach, although approximate, reduces the number of model parameters and therefore simplifies its calibration. Decomposition rates vary however among the different pools: litter has faster decomposition than the humus pool. The second approximation concerns the C/N ratio. As for the transformation rates, the model considers a single C/N ratio for each pool, again representing an average value. In this case, the litter C/N ratio can be computed, for example, as the weighted average of the C/N ratios of the different species, weighted by their relative amount in the ecosystem. Other than this, vegetation characteristics (maximum evapotranspiration, wilting point, incipient stress point, etc.) are assumed constant. This is an important simplification, since in previous sections it was pointed out that climatic conditions influence vegetation growth and deposition of fresh organic matter. The advantage is that we reduce and simplify the external factors influencing C and N turnover to soil type and moisture content dynamics.

Model inputs are precipitation and litter fall rates, while on output the extent of soil respiration, plant uptake, transpiration and leaching are recovered. The amount and frequency of precipitation are the only climatic variables considered. Isothermal conditions are assumed, meaning that variations of the average daily temperature within the year are limited. This assumption is clearly not satisfied in many climatic regions (e.g., at high latitude). On the other hand, the model can still be applicable given that, during the unfavourable season (too high or low temperature), the moisture content becomes an additional limiting factor, thus inhibiting soil respiration and transformations.

The model of Porporato et al. (2003) is comprised of a set of coupled non-linear ordinary differential equations. Each equation describes the mass balance of C and N in the five pools. An overview of the reaction network is given in Fig. 3. Moreover, since the soil moisture is the key factor in this model, and influences the decomposition and turnover rates as outlined above, soil water variations are computed from the water balance at one point. In order to facilitate model understanding and comparison with previous works, here we use the same notation as in Porporato et al. (2003), D'Odorico et al. (2004) and Rodriguez-Iturbe and Porporato (2004).

The evolution of C in the litter, humus and biomass pools is given by:

$$\frac{d\mathrm{C}_l}{dt} = ADD + BD - DEC_l, \tag{6}$$

$$\frac{d\mathrm{C}_h}{dt} = r_h DEC_l - DEC_h, \tag{7}$$

$$\frac{d\mathrm{C}_b}{dt} = (1 - r_h - r_r)DEC_l + (1 - r_r)DEC_h - BD, \tag{8}$$

where C_l, C_h and C_b are the C concentrations in the litter, humus and biomass pools respectively [M L^3], ADD is the external input of C to the system [M L^{-2} T^{-1}], BD is the recycling rate of decaying biomass in the litter pool [M L^{-3} T^{-1}], DEC_l and DEC_h are the C fluxes leaving the litter and humus pools due to microbial decomposition [M L^{-3} T^{-1}], while r_h and r_r are non-dimensional coefficients representing the fractions

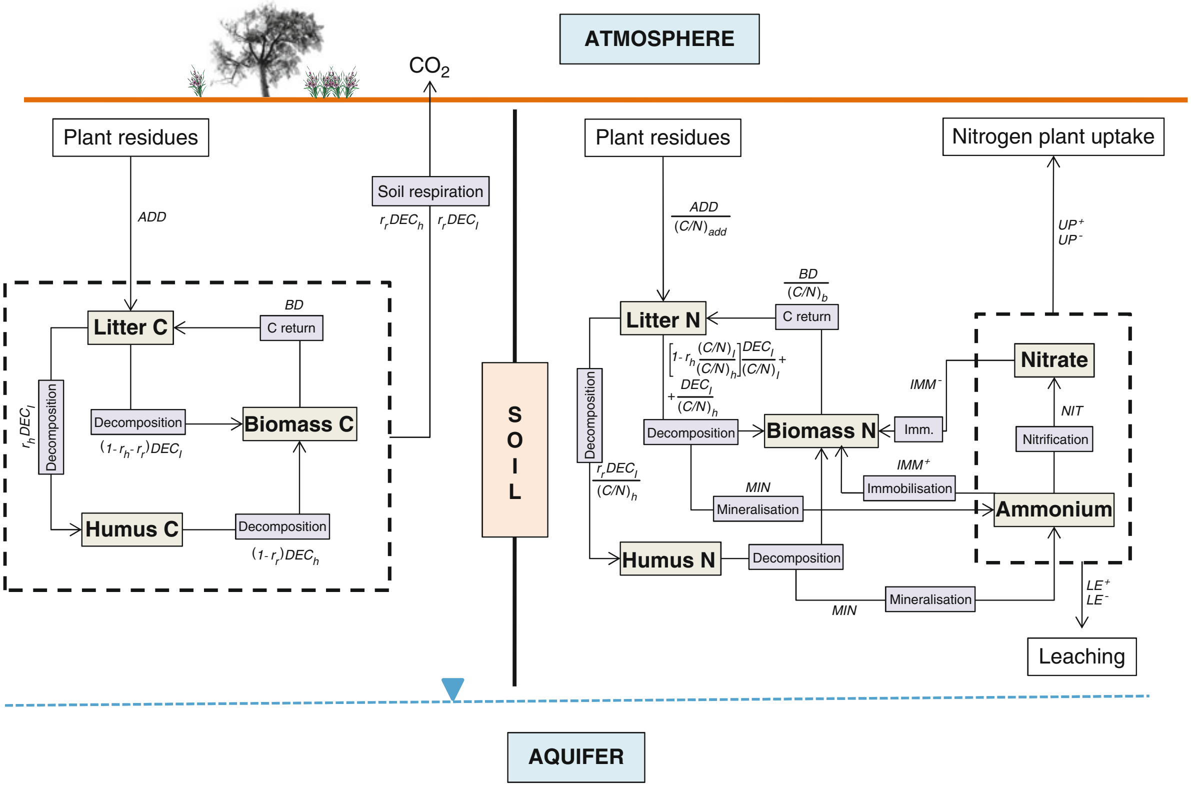

Fig. 3 Schematic representation of soil C and N cycles (after Rodriguez-Iturbe and Porporato, 2004)

of decomposed organic C that go into the humus pool and to respiration, respectively.

The combination of equations (6)–(8) gives the overall C balance equation (C_{tot}) in the system:

$$\frac{dC_{tot}}{dt} = ADD - r_r DEC_l - r_r DEC_h. \qquad (9)$$

The flux of C between two pools is described by first-order kinetic equations (DEC_l, DEC_h and BD), where the reaction rates (k_l, k_h and k_d, respectively) are weighted averages of the decomposition rates of the different organic molecules. The first-order kinetic equations of C decomposition and microbial death for the litter, humus and biomass pool are:

$$DEC_l = \phi\ f_d(s)\ C_b\ k_l\ C_l, \qquad (10)$$

$$DEC_h = \phi\ f_d(s)\ C_b\ k_h\ C_h, \qquad (11)$$

$$BD = C_b\ k_d, \qquad (12)$$

where ϕ is a non-dimensional factor that accounts for a possible reduction of the decomposition rate when the litter is very poor in N (high C/N ratio) and the immobilization is not sufficient to integrate the required N by the bacteria. This factor has an important influence on the dynamics of the biomass evolution, and details on how it is defined and computed can be found in Porporato et al. (2003). The term $f_d(s)$ is a non-dimensional parameter that describes soil moisture effects on decomposition:

$$f_d(s) = \begin{cases} \dfrac{s}{s_{fc}}, & s \leqslant s_{fc}, \\ \dfrac{s_{fc}}{s}, & s > s_{fc}, \end{cases} \qquad (13)$$

where S_{fc} is the soil field capacity (water content held in soil after excess water drained away by gravity). The main model parameters are listed in Table 2.

The N balance in the litter, humus and biomass pools is computed from the C balance equations, scaled by the appropriate C/N ratio:

$$\frac{dN_l}{dt} = \frac{ADD}{(C/N)_{add}} + \frac{BD}{(C/N)_b} - \frac{DEC_l}{(C/N)_l}, \qquad (14)$$

Table 2 Summary of the main parameters considered in the soil C-N model

Symbol	Representative model parameters	Dimensions
C_l	Carbon concentration in the litter pool	$[M\ L^{-3}]$
C_h	Carbon concentration in the humus pool	$[M\ L^{-3}]$
C_b	Carbon concentration in the biomass pool	$[M\ L^{-3}]$
N_l	Organic nitrogen concentration in the litter pool	$[M\ L^{-3}]$
N_h	Organic nitrogen concentration in the humus pool	$[M\ L^{-3}]$
N_b	Organic nitrogen concentration in the biomass pool	$[M\ L^{-3}]$
N^+	Ammonium concentration in the soil	$[M\ L^{-3}]$
N^-	Nitrate concentration in the soil	$[M\ L^{-3}]$
ADD	External carbon input	$[M\ L^{-2}T^{-1}]$
BD	Carbon return to the litter pool due to death of microbial biomass	$[M\ L^{-1}]$
DEC_l	Carbon output due to microbial decomposition	$[M\ L^{-3}T^{-1}]$
DEC_h	Carbon output due to humus decomposition	$[M\ L^{-3}T^{-1}]$
r_r	Portion of decomposing carbon that is lost by respiration	–
r_h	Fraction of decomposing litter which undergoes humification	–
k_l	Litter decomposition rate	$[L^3T^{-1}M^{-1}]$
k_h	Humus decomposition rate	$[L^3T^{-1}M^{-1}]$
k_d	Biomass death rate	$[T^{-1}]$
$(C/N)_{add}$	Carbon to nitrogen ratio in the external added litter	–
$(C/N)_l$	Carbon to nitrogen ratio in the litter pool	–
$(C/N)_h$	Carbon to nitrogen ratio in the humus pool	–
$(C/N)_b$	Carbon to nitrogen ratio in the biomass pool	–
MIN	Mineralization rate	$[T^{-1}M^{-1}L^3]$
NIT	Nitrification rate	$[T^{-1}M^{-1}L^3]$
IMM^+	Ammonium immobilization rate	$[M^{-1}L^3T^{-1}]$
IMM^-	Nitrate immobilization rate	$[M^{-1}L^3T^{-1}]$
LE^+	Ammonium leaching rate	$[M^{-1}L^3T^{-1}]$
LE^-	Nitrate leaching rate	$[M^{-1}L^3T^{-1}]$
UP^+	Ammonium plant uptake rate	$[M^{-1}L^3T^{-1}]$
UP^-	Nitrate plant uptake rate	$[M^{-1}L^3T^{-1}]$
Φ	Non-dimensional factor that takes into account the contribution due to either the net mineralization or to the immobilization	–
ϕ	Non-dimensional factor that accounts for a possible reduction of the decomposition rate when the litter is very poor in N and the immobilization is not sufficient to integrate the required N by the bacteria	–
$f_d(s)$	Non-dimensional factor (soil moisture effect on decomposition)	–
s	Soil moisture	–
Z_r	Rooting depth	$[L]$

$$\frac{dN_h}{dt} = r_h \frac{DEC_l}{(C/N)_h} - \frac{DEC_h}{(C/N)_h}, \tag{15}$$

$$\frac{dN_b}{dt} = \left[1 - r_h \frac{(C/N)_l}{(C/N)_h}\right] \frac{DEC_l}{(C/N)_l} + \frac{DEC_h}{(C/N)_h} - \frac{BD}{(C/N)_b} - \Phi, \tag{16}$$

where N_l, N_h and N_b are the N concentrations in the litter, humus and biomass pools, respectively $[M\ L^{-3}\ T^{-1}]$, $(C/N)_{add}$, $(C/N)_l$, $(C/N)_h$ and $(C/N)_b$ are the C to N ratios of added organic matter, litter, humus and biomass pools, respectively, and Φ is a term that takes into account the contribution due to either the net mineralization or to the immobilization $[M\ L^{-3}\ T^{-1}]$. This term relates the total mineralization and immobilization rates:

$$\Phi = MIN - IMM, \tag{17}$$

where MIN expresses the mineralization rate [M L^{-3} T^{-1}] and IMM is the total rate of immobilization (sum of the N immobilization rate in the NH_4^+ IMM^+ and NO_3^- IMM^- pools, respectively) [M L^{-3} T^{-1}]. When IMM is equal to zero, MIN is equal to Φ, while when MIN is zero IMM is equal to $-\Phi$. The $(C/N)_b$ ratio is one of the most important parameters in the model, since the switch between mineralization and immobilization is defined in order to maintain as constant the C/N ratio of the biomass pool. If the organic matter is rich in N (and $(C/N)_b$ is smaller than the value required to sustain growth of microbial biomass), decomposition results in surplus N. This is used by the microorganisms, and mineralization occurs. In contrast, if decomposition produces an environment poor in N, microorganisms will increase the immobilization rate of NH_4^+ and NO_3^- in order to meet their requirements. This process is rather complex and very dynamic, as explained in Porporato et al. (2003).

N transfer between the pools is described by the same first-order kinetic transfer parameters used for C, with each term scaled by the corresponding C/N ratio (Fig. 3). The balance equations of inorganic N are:

$$\frac{dN^+}{dt} = MIN + IMM^+ - NIT - LE^+ - UP^+, \tag{18}$$

$$\frac{dN^-}{dt} = NIT - IMM^- - LE^- - UP^-, \tag{19}$$

where N^+ and N^- are the inorganic N concentrations in the NH_4^+ and NO_3^- pools, respectively [M L^3], NIT is the nitrification rate [M L^{-3} T^{-1}], UP^+ and UP^- are the N uptake by plants from the NH_4^+ and NO_3^- pools, respectively [M L^{-3} T^{-1}], and LE^+ and LE^- are N fluxes from the root zone towards the groundwater [M L^{-3} T^{-1}].

The combination of equations (14–16, 18, 19) gives the overall evolution of total N (N_{tot}) in the system:

$$\frac{dN_{tot}}{dt} = \frac{ADD}{(C/N)_{add}} - LE^+ - UP^+ - LE^- - UP^-. \tag{20}$$

Equations (9) and (20) represent the total C and N sinks and sources of the system depicted in Fig. 3.

Although we have described the main elements of the model here, further descriptions – for example the rates of mineralization (MIN), immobilization (IMM^+ and IMM^-), nitrification (NIT), plant uptake (UP^+ and UP^-) and leaching (LE^+ and LE^-) and their associated variables – can be found in Porporato et al. (2003), D'Odorico et al. (2004) and Rodriguez-Iturbe and Porporato (2004), together with additional discussion about the underlying assumptions and simplifications introduced in this model.

6 Modelling Scenarios

It has been shown in previous sections that land use change and hydroclimatic conditions are the main factors contributing to changes in soil C and N turnover. To test the relevance of mechanisms and parameters contributing to the fate of soil C and N, different modelling scenarios were simulated, for which the main variables are presented in Table 3. The combination of two different soils and two different climatic conditions gives four different scenarios, the results of which are presented subsequently. Due to the high frequency of NO_3^- variations and their importance to plant growth, D'Odorico et al. (2003) found that a daily temporal resolution was necessary to capture the impact of soil moisture on nutrient dynamics. A daily time step was used here also. The same initial C and N amounts in different pools, as well the same decomposition, mineralization and root uptake rates were considered in all scenarios, thereby allowing for a direct comparison between them. These values, presented in Table 4, were taken from D'Odorico et al. (2003).

6.1 Climate Scenarios

The occurrence and amount of precipitation are both intermittent and unpredictable. Precipitation scenarios were generated with a stochastic procedure described in Laio et al. (2001). Rainfall was assumed to follow a Poisson distribution with frequency λ [T^{-1}], and each rainfall event had infiltration sampled from an exponential distribution with mean α [L]. Two different climates were considered with a different occurrence of precipitation. Rainfall interception by canopy depends on the vegetation type and structure and cannot be neglected, especially in arid areas

Table 3 Soil and climate parameters corresponding to the modelled scenarios

Climatic parameters			Climate *A* (seasonality)	Climate *B* (no seasonality)
Mean storm frequency (λ)		d^{-1}	0.3, 0.1	0.2
Mean storm [–] depth (α)		m	1.55×10^{-2}, 4.8×10^{-3}	1.08×10^{-2}
Soil parameters			Soil I (agricultural)	Soil II (forest)
Saturated hydraulic conductivity (K)		m d^{-1}	1	2
Active root depth (Z_r)		m	0.3	0.7
Pore size distribution index (b)		–	4.05	5.39
Porosity (n)		–	0.35	0.45
Tortuosity (d)		–	1.5	3
Field capacity (s_{fc})		–	0.35	0.55
Vegetation parameters	Maximum transpiration (T_{max})	m	3.6×10^{-3}	4.63×10^{-3}
	Canopy interception threshold	m	5×10^{-4}	2×10^{-3}
	Maximum evapotranspiration (E_{max})	m d^{-1}	3.7×10^{-3}	4.76×10^{-3}
	Evapotranspiration wilting point (E_w)	m d^{-1}	10^{-4}	1.3×10^{-4}
	Hygroscopic point (s_h)	–	0.04	0.14
	Point of incipient stress ($s*$)	–	0.16	0.37
	Wilting point (s_w)	–	0.05	0.17
Modelled scenarios				
Scenario *A*I	Agricultural soil with climatic seasonality			
Scenario *B*I	Agricultural soil without seasonality			
Scenario *A*II	Forest soil with climatic seasonality			
Scenario *B*II	Forest soil without seasonality			

where the evaporation rate can be significant. Canopy interception is accounted for in the model by defining a threshold value (e.g., high values for forests and low for grasslands) below which no rainfall reaches the soil surface. If instead the rainfall depth is higher than the threshold value, the total amount of rainfall reaching the soil surface is equal to the rainfall depth reduced by the canopy interception.

Parameters representing the two climates considered are presented in Table 3. Climate A is characterized by seasonality represented by two wet and two dry seasons over a year. This climate can be considered comparable to a Mediterranean climate, with two wet seasons, spring and fall (e.g., high λ and α) and two dry seasons, summer and winter (e.g., low λ and α). In contrast, climate B is characterized by a lack of seasonality, with relatively low but homogeneous amount of precipitation randomly distributed over the year, using λ and α between those of the wet and dry seasons considered in climate A.

Although in Section 3 was pointed out that temperature exerts a control over soil C and N cycles, in this study only isothermal conditions were considered. This assumption was made for two reasons. First, the effect of temperature in many climates is less important than that of soil moisture and, second, because considering the effects of soil moisture alone the number of factors affecting soil nutrient cycles is reduced, and is therefore easier to understand the influence and feedbacks on soil changes and nutrient dynamics. Temperature variations are however closely related to climate conditions and therefore this factor should be considered in future analyses.

6.2 Soil Scenarios

We seek to identify whether different patterns of soil moisture evolve through time as a consequence of the combination of different processes in the soil-plant-atmosphere system. To this end, equation (21) is used to calculate the soil moisture balance at a point (Laio et al., 2001):

$$nZ_r \frac{ds(t)}{dt} = R(t) - I(t) - Q[s(t);t] - E[s(t)] - L[s(t)], \tag{21}$$

Table 4 Parameters related to carbon and nitrogen soil dynamics used in all model scenarios. Shaded parameters correspond to variables that are not focused upon in this manuscript. Their details can be found in Porporato et al. (2003)

Initial soil moisture	s	–	0.15
C litter pool	C_l	$g\ m^{-3}$	1200
C humus pool	C_h	$g\ m^{-3}$	8500
C biomass pool	C_b	$g\ m^{-3}$	50
N ammonium pool	N^+	$g\ m^{-3}$	0
N nitrate pool	N^-	$g\ m^{-3}$	1
Added litter	Add	$g\ C\ m^{-3}\ d^{-1}$	1.5
C/N ratio of added litter	$(C/N)_{add}$	–	58
C/N ratio of litter	$(C/N)_l$	–	22
C/N ratio of humus	$(C/N)_h$	–	22
C/N ratio of microbial biomass	$(C/N)_b$	–	11.5
C/N ratio of ammonium	$(C/N)^+$	–	1
C/N ration of nitrate	$(C/N)^-$	–	1
Isohumic coefficient	r_h	–	0.25
Respiration coefficient	r_r	–	0.6
Litter decomposition rate	k_l	$m^3\ d^{-1}\ g\ C^{-1}$	$6. 5 \times 10^{-5}$
Factor of carbon return to litter pool	k_d	d^{-1}	8.5×10^{-3}
Humus decomposition rate	k_h	$m^3\ d^{-1}\ g\ C^{-1}$	2.5×10^{-6}
Rate of nitrification	k_n	$m^3\ d^{-1}\ g\ N^{-1}$	0.6
Ammonium immobilization coefficient	k_{amm}	$m^3\ d^{-1}\ g\ N^{-1}$	1
Nitrate immobilization coefficient	k_{nit}	$m^3\ d^{-1}\ g\ N^{-1}$	1
Ammonium plant demand	DEM^+	$g\ N\ m^{-3}\ d^{-1}$	0.2
Nitrate plant demand	DEM^-	$g\ N\ m^{-3}\ d^{-1}$	0.5
Fraction of dissolved ammonium	a^+	–	0.05
Fraction of dissolved nitrate	a^-	–	0.1

where n is the porosity; Z_r is the depth of active soil or root depth [L]; $s(t)$ is the relative soil moisture content $(0 \leqslant s(t) \leqslant 1)$; $R(t)$ is the rainfall rate [L T^{-1}]; $I(t)$ is the amount of rainfall lost through interception by canopy cover [L T^{-1}]; $Q[s(t);t]$ is the rate of runoff [L T^{-1}]; $E[s(t)]$ is the evapotranspiration rate [L T^{-1}]; and $L[s(t)]$ is the leakage rate [L T^{-1}].

The soil was assumed as a horizontal and homogeneous layer of depth Z_r. This is an important assumption because soil depth depends in time and space on two main parameters, soil structure and vegetation. In the simulations we considered the same soil depth for water balance and nutrient cycles. Water infiltration into the soil and runoff are entirely controlled by soil moisture dynamics, since water will infiltrate into the soil if there is available storage. Excess rainfall that cannot be stored in the soil is converted into runoff.

Although the vegetation type depends on both climate and soil, here the vegetation parameters were fixed for each soil in order to reduce the number of variables affecting changes in C and N fluxes (Table 3). This is also justified by the fact that vegetation parameters mostly depends on soil moisture, which directly depends on soil characteristics. Evapotranspiration varies from a maximum value E_{max} when soil moisture ranges between the maximum, unity, and the point of incipient stress, $s*$ (soil moisture level at which the plants begin to close stomata in response to water stress). The evapotranspiration rate decreases linearly from E_{max} to E_w, the latter rate corresponding to the soil moisture at the wilting point s_w (soil water content at which plants wilt and can no longer recover or, in terms of water potential, is defined as the suction head beyond which the plant can no longer take up water). Below this value, only transpiration is active, and the water loss rate is linear from E_w to zero at the point of hygroscopic water s_h (microscopic film of water covering soil particles not available for plants). More details and assumptions concerning evapotranspiration are given by Laio et al. (2001).

Verhoef and Brussaard (1990) defined a series of functional groups of pedofauna based on their contribution to nutrient decomposition and mineralization. Organisms belonging to the same functional group play a similar role in decomposition-mineralization transformations. For example, there is a functional group that includes organisms that pulverize, mix and granulate the soil. Such organisms are rather important because (i) they contribute to incorporate the organic residues available on the surface into the lower horizons; and (ii) they create large pores and channels that guarantee aeration of the soil profile and eliminate excess water. Other functional groups include pedofauna specialized in breaking down woody recalcitrant materials, in degrading litter and digesting organic residues, etc. (Brady and Weil, 2004). Although the functional group concept is useful for modelling of soil nutrient cycles, here a more simplified approach considering a single value for nutrient decomposition and mineralization rates is used, representing the contribution of the entire pedofauna to these processes.

In combination with climates A and B, two soil types – named I and II – are considered, representative of agricultural and forest soils respectively (Table 3). Agricultural and forest soils have contrasting physical properties mainly due to management practices and the type of vegetation supported (Lutz and Chandler, 1955; Carmean, 1957; García-Oliva et al., 1994; de Moraes et al., 1996; Islam and Weil, 2000). The main differences between these soils are:

- *Silt and clay content*. Agricultural soils have lower amounts of silt and clay than natural forest soils, mostly as a result of preferential removal of these particles by water erosion (Islam and Weil, 2000).
- *Soil aggregate stability*. A higher input of litter fall combined with the absence of tillage and ploughing practices gives rise to forest soils with greater soil aggregate stability (Islam and Weil, 2000). Furthermore, forest soils are naturally protected from the impact of raindrops by the canopy and organic matter at the soil surface that absorbs raindrop energy (Carmean, 1957). In practice, the effect of raindrops is (i) removal of silt and clay particles and (ii) disruption of soil aggregates that subsequently can block large pores and reduce water percolation.
- *Bulk density and porosity*. Agricultural soils have higher bulk density and lower porosity than forest soils, mainly because of a greater residual sand content combined with poorer soil aggregation (García-Oliva et al., 1994; de Moraes et al., 1996).
- *Soil structure*. Agricultural soils often have a deteriorated structure in comparison to forest soils. This deterioration is apparent in pore modification, increased bulk density, increased compaction, and less stable aggregates (Carmean, 1957). In addition, compacted, impermeable layers or pans within the soil profile often develop as a consequence of repeated ploughing, mainly under wheel track patterns (Roger-Estrade et al., 2004; Coquet et al., 2005).
- *Infiltration rate*. As a consequence of above mentioned properties, which contribute to reduce the average pore size and their connectivity, the rate of infiltration is reduced in agricultural soils. Additionally, forest vegetation has more extensive root networks, leading to large number of interconnected channels leading to rapid water infiltration (Lutz and Chandler, 1955).
- *Runoff and soil erosion*. Low infiltration rates of agricultural soils contribute to increased runoff, which emphasizes soil erosion and removal of silt-clay soil particles (Lutz and Chandler, 1955).

Due to the above differences, the agricultural soil (soil I) is characterized by a relatively low saturated hydraulic conductivity (K), as well as lower values of pore size distribution (b), porosity (n) and soil field capacity (s_{fc}) than soil II, representative of a forest soil (Ndiaye et al., 2007). Furthermore, the soil tortuosity is likely to be affected by the loss of structure and by the less extended root network of agricultural soils, the loss of connected porosity due to tillage processes and disturbed aggregates clogging large pores. The soil tortuosity index for the agricultural soil is also thus decreased in comparison with the forest soil (Table 3).

The rooting depth (Z_r) considered is larger for forest soils than agricultural soils, since the root network is much more important for forest vegetation than agricultural. As previously mentioned, vegetation depends on both climate and soil. However, we have defined the vegetation parameters only in function of the soil type. As a consequence of the low C/N ratio of agricultural vegetation and microbial decomposers associated, cultivated soils typically have lower C/N ratio than forest soils (Zheng et al., 1999). Nevertheless, to facilitate the comparison between the four scenarios we assumed equal C/N ratios for both soils, as well as initial C and N concentrations.

7 Results

Figures 4a and 4d present the precipitation over 20 y for climates A (seasonality) and B (no seasonality), respectively, while the evolution of water saturation for the same period – as computed with equation (21) – for each climate and soil type is depicted in Figs. 4b–4f, respectively. There is a marked difference in precipitation distribution between climates A and B, with wet and dry seasons in climate A and random uniformly distributed precipitation in climate B. Soil water saturation follows the dynamics imposed by

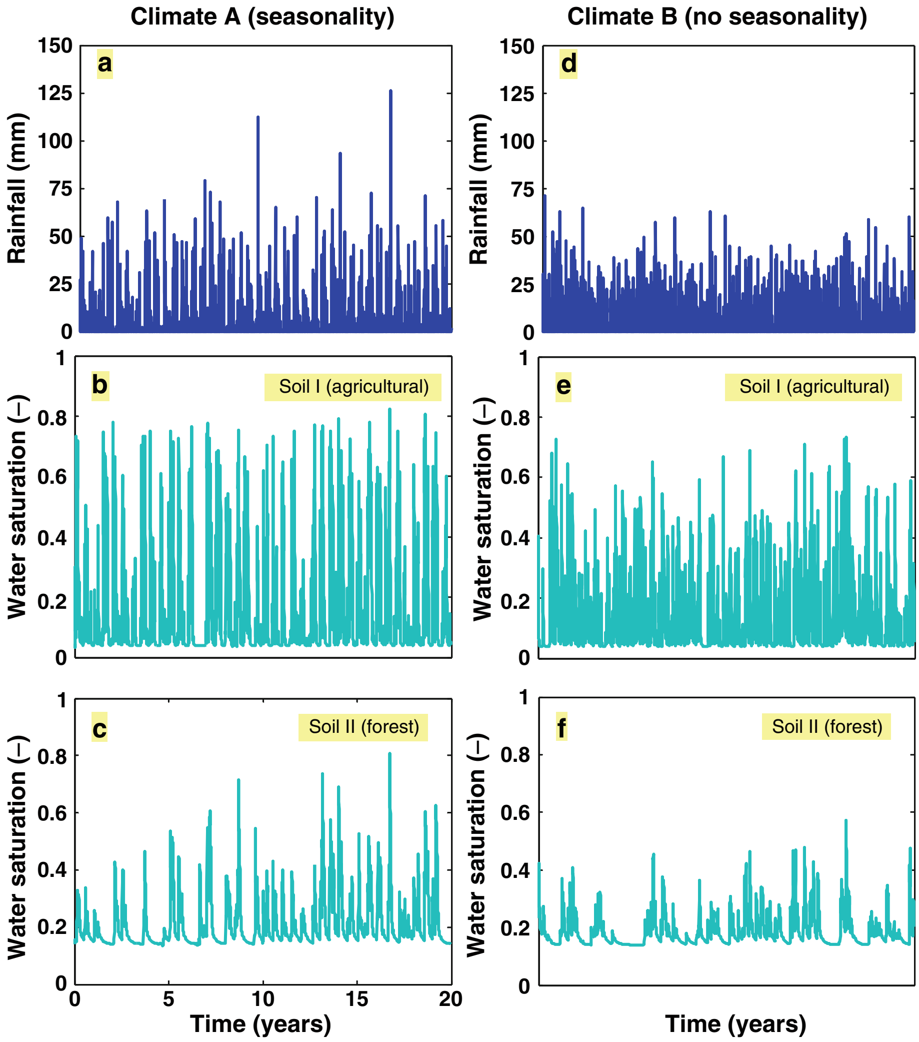

Fig. 4 Simulated rainfall and corresponding water saturation of climate A (with seasonal effects) and climate B (without seasonal effects) for idealized agricultural (**a**, **b** and **c**) and forest (**d**, **e** and **f**) soils

precipitation, more notably in agricultural soils while the trend in water saturation evolution is smoothed in forest soils. As expected, peaks of soil saturation are lower for those soils under the influence of climate B than climate A, while water saturation in agricultural soils is lower than those of forest soils. The latter is due to different vegetation parameters associated with each soil type, specifically to the wilting point (s_w), fixed at 0.05 and 0.17 for agricultural and forest soils, respectively. It is interesting to note that forest soils attenuate changes of soil saturation much more than agricultural soils (e.g., compare Figs. 4b to 4c or even Figs. 4e to 4f), and delays water saturation peaks, mostly due to difference rooting depth.

The evolution of the five different nutrient pools, for agricultural and forest soils under the conditions of climate A, are presented in Fig. 5. Figures corresponding to the same pool are depicted with the same vertical scale in order to facilitate comparison between them. From the comparison is evident that seasonal effects are much more visible in agricultural than in forest soils. The lower rooting depth (Z_r), soil hydraulic conductivity (K) and soil porosity (n) in the agricultural soil may be at the origin of these differences. Although forest soils present similar behaviour, small peaks observed in agricultural soils and corresponding to high frequency changes in precipitation, are not evident. As previously mentioned, litter decomposition

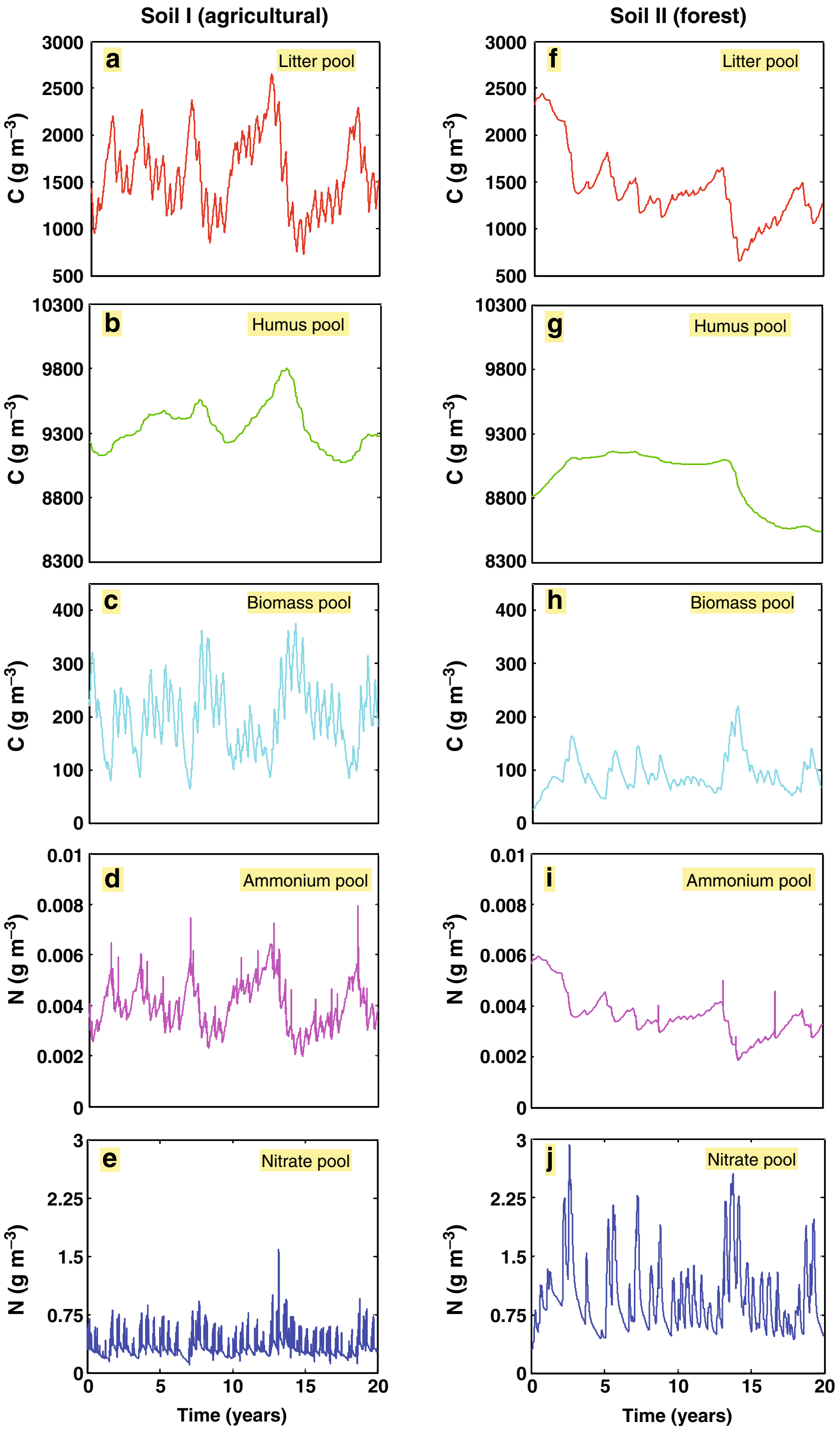

Fig. 5 Simulated organic carbon and inorganic nitrogen concentrations for agricultural (left side) and forest (right side) soils under climate A conditions

in agricultural soils is enhanced by tillage and plough practices, represented in the model by lower values of saturated hydraulic conductivity (K), pore size distribution (b), porosity (n) and soil field capacity (s_{fc}). This explains the lower values of C litter pool in agricultural soils (compare Figs. 5a and 5f), although the high influence of seasonal climates over agricultural soils is likely to hide this fact. Furthermore, this trend is likely to influence the evolution of subsequent pools (Figs. 5g–5j). Peaks of litter C match well the biomass pool decrease, while decreases of litter C concentrations well correlate with the peaks of C concentration in the biomass pool for both agricultural and forest soils. This is not surprising since the augmentation of the biomass pool is linked to degradation of the litter pool. C concentrations in the humus pool (C_h) for agricultural and forest soils are lower than in other pools, since this pool is an intermediate pool between litter and biomass and there is almost no interaction with other variables. Concentrations of NH_4^+ (Figs. 5d and 5i) show a trend similar to that of C in the litter pool, although NH_4^+ variations are almost negligible since its concentration is very low. The NO_3^- pool preserves much of the high-frequency variability imposed by the random forcing of precipitation, which is not surprising since NO_3^- dynamics are the final product of a number of intertwined processes in which both high- and low-frequency components interact. It is interesting to note the low levels of NO_3^- obtained for agricultural soils (Fig. 5e) compared with the relatively high levels in forest soils (Fig. 5j). This is reflected in Fig. 6d and 6h, where NO_3^- leaching is almost nonexistent in forest soils while it is relatively important for agricultural soils. The higher rates of mineralization and NO_3^- uptake in agricultural soils than in forest soils aid to explain this fact. Note that fertilization practices commonly undertaken in agricultural soils are not considered in the model. Thus, simulated results are in good agreement with the need of agricultural soils for regular fertilizer input, since high decomposition and mineralization rates (Figs. 6a and 6b), NO_3^- uptake by plants (Fig. 6c) and NO_3^- leaching (Fig. 6d) to lower layers results in low levels of NO_3^- available for plants.

The results obtained, especially in forest soils, provide evidence of a structure with temporal amplitudes considerably larger than the one induced by the stochastic hydrologic forcing. This behaviour was already observed by Thornley et al. (1995) and D'Odorico et al. (2003). The presence of this behaviour in soil nutrient dynamics (see Figs. 5f–5j), a priori not related to climatic conditions, is a manifestation of the nonlinearity and degree of complexity of the whole system. Thornley et al. (1995) pointed out that such behaviour suggests that soil nutrients cycles could show cases of richer (perhaps chaotic) dynamics enmeshed with the variability that is directly induced by the stochastic hydrologic fluctuations. This issue is not fully assessed yet to date, although a partial discussion is provided by Manzoni and Porporato (2007).

The evolution through time of the C and N pools in agricultural and forest soils under climate *B* (no seasonality) is presented in Fig. 7. A general trend for both agricultural and forest soil is that simulated concentrations in all C and N pools closely reproduce the concentrations observed in climate *A* (seasonal). This is supported by the similar decomposition rates between climate *A* (Fig. 6) and *B* (Fig. 8). Nevertheless, the absence of seasonality, which is reflected in agricultural and forest soils by a loss of the high frequency nutrient peaks, is finally translated in less nitrate leaching and less variability in the soil nutrients. While water saturation changes under seasonal climates contribute to hinder decomposition rates from time to time, this does not occur in climates without seasonal effects, where decomposition peaks are more continuous through time. The loss of climate seasonality affects neither the inverse trend between litter and biomass pools, nor the high frequency of NO_3^- changes in both agricultural and forest soils.

As observed under seasonal conditions, the frequency of changes in C and N concentrations is higher in agricultural than in forest soils, although the general trend remains quite similar for both soils. Again, C concentrations in the humus pool are relatively constant, and NH_4^+ concentrations are low. Concentrations of NO_3^- are lower in agricultural soils than in forest soils, as observed under seasonal climate conditions. This fact supports what was mentioned in previous sections that agricultural soils are relatively poor in nutrients, independently of climate conditions. Furthermore, this is consistent with the higher mineralization rate (Fig. 8b), higher NO_3^- uptake by plants (Fig. 8c) and higher loss of NO_3^- by leaching (Fig. 8d) in agricultural than in forest soils.

Finally, some general insights can be gained from the model results. Great variations of the average value of C and N concentrations in forest soils are obtained both under seasonal and uniformly variable climatic conditions. These results show that a point measurement of litter, for example, is almost meaningless, and that time-series are needed to provide useful information for evaluation of soil nutrient turnover. It is worth noting also that steady state concentrations are not always reached, which means that longer simulations should be applied to better evaluate long term nutrient concentrations, mainly under non-seasonal conditions (see for example Fig. 5g in comparison to 5b).

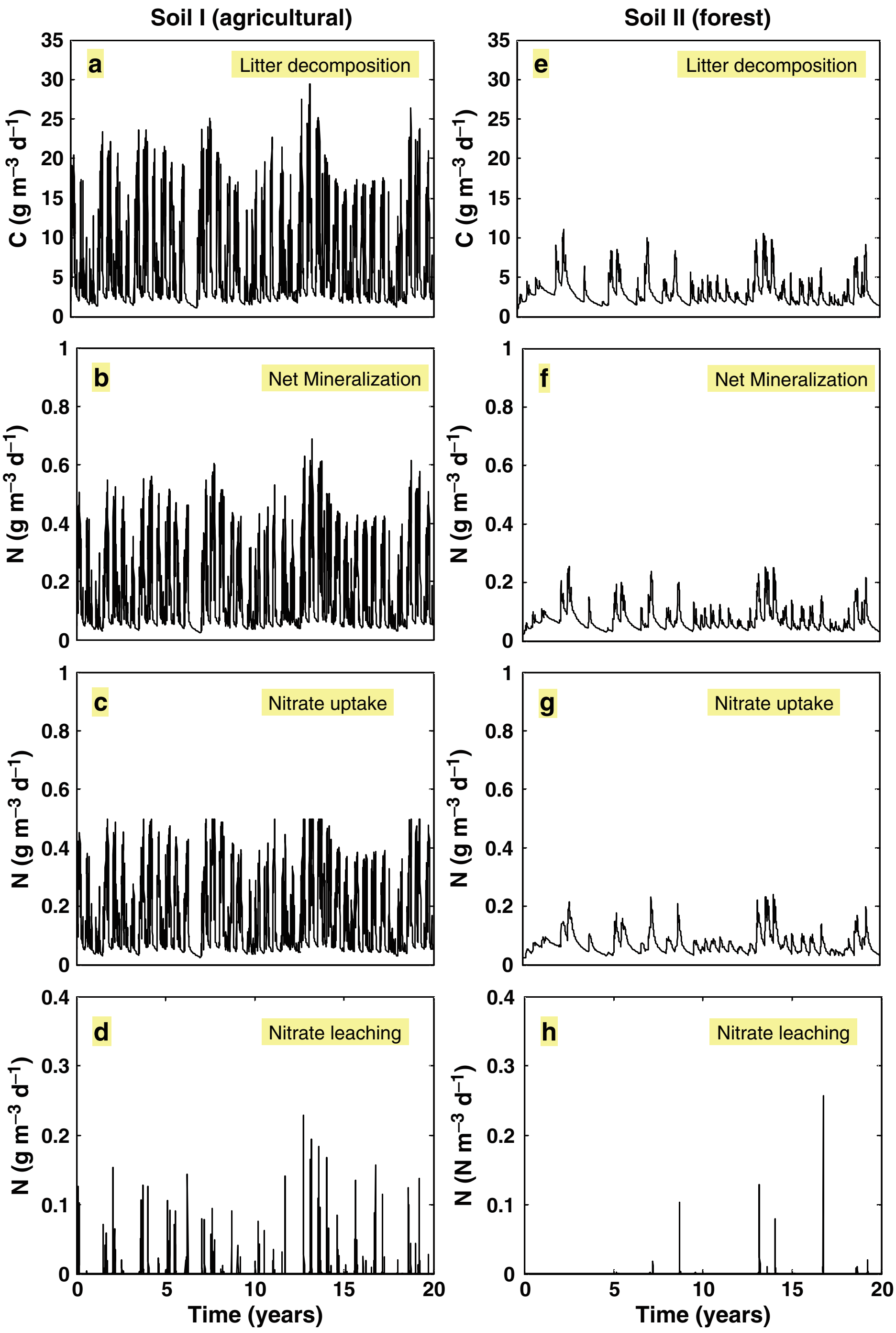

Fig. 6 Simulated rates of litter decomposition (**a**, **e**), net nitrogen mineralization (**b**, **f**), nitrate uptake (**c**, **g**) and nitrate leaching (**d**, **h**) for both agricultural and forest soils under climate *A* conditions

8 Conclusions

Land use changes affect soil properties and, thus, nutrient cycling dynamics. Changing soil properties means also changing the type of vegetation, altering even more the concomitant nutrient cycles. The example most widely observed is the change from forest to agricultural soils, due to increasing anthropogenic demands for food production. Agricultural practices generally cause changes in soil structure, compromising

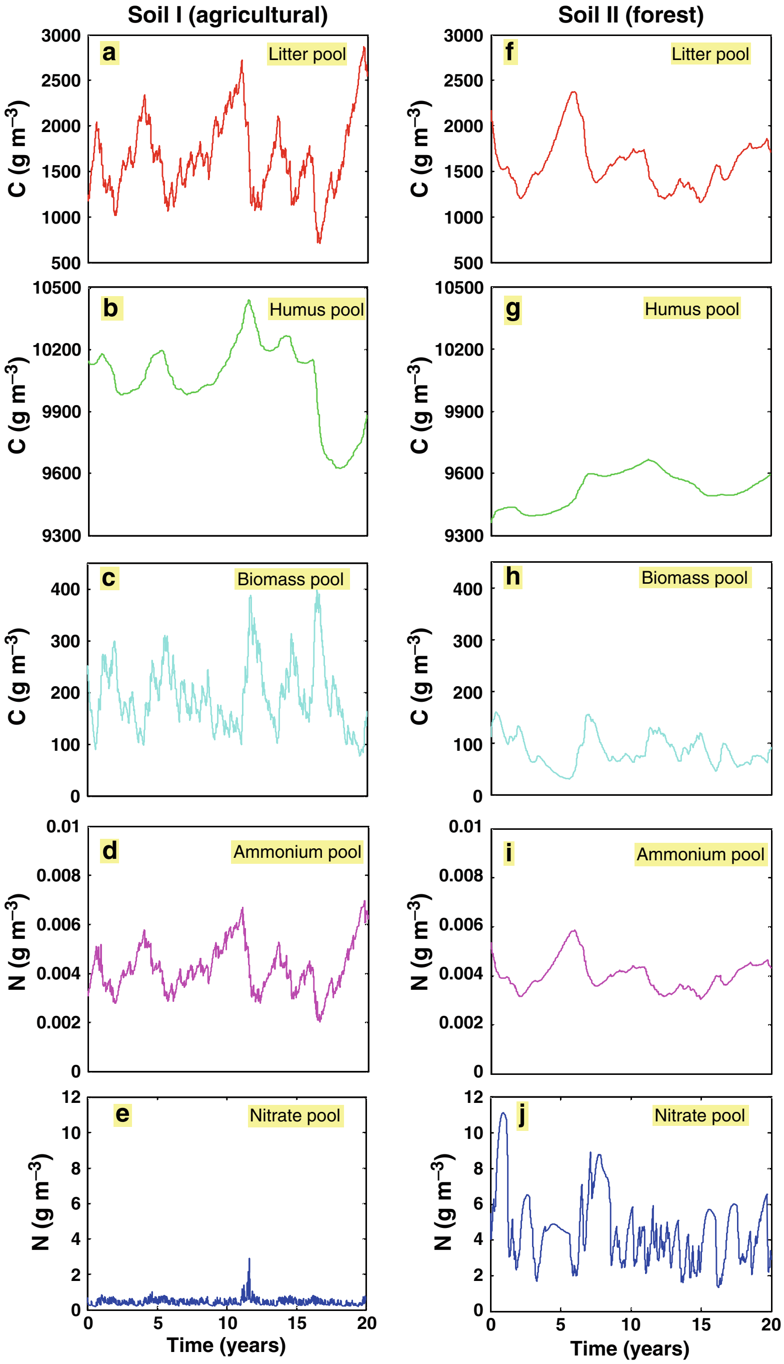

Fig. 7 Simulated organic carbon and inorganic nitrogen concentrations for agricultural (left side) and forest (right side) soils under climate *B* conditions

aggregation and porosity, leading to a soil structure decline. Tillage practices like mechanical mixing compact and reduce the size of aggregates and fills pore spaces with fines. The simulation of two different climates applied to two contrasting soil types (where specific vegetation properties were linked to each soil), showed that decomposition rates in agricultural soils are higher, which in addition to lower input of fresh

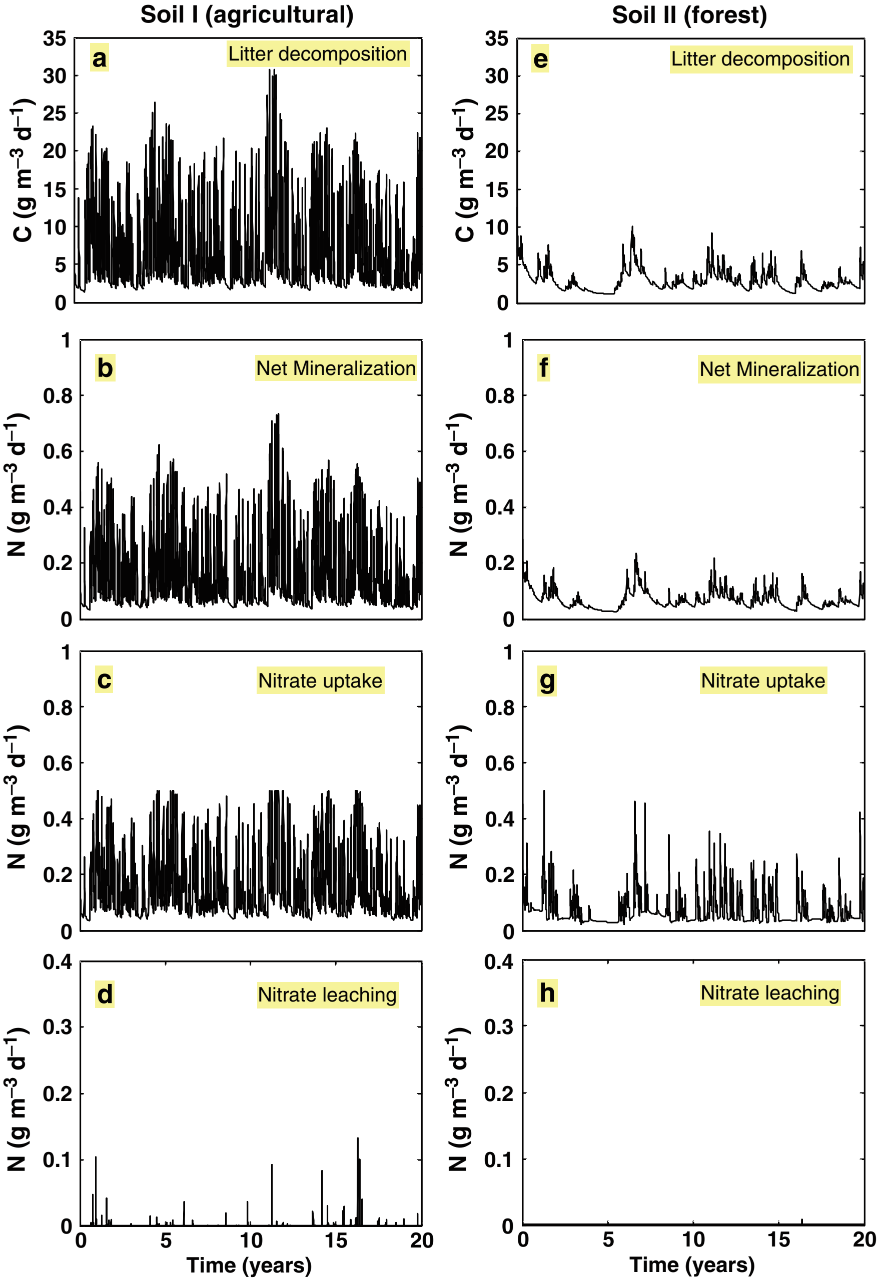

Fig. 8 Simulated rates of litter decomposition (**a**, **e**), net nitrogen mineralization (**b**, **f**), nitrate uptake (**c**, **g**) and nitrate leaching (**d**, **h**) for both agricultural and forest soils under climate *B* conditions

litter over a year in comparison to a forested area, results in lower C and N concentrations. In consequence, less NO_3^- is available to plants, since plant NO_3^- uptake is higher and leaching is enhanced under these conditions. The leached NO_3^- is compensated for by the input of extra nutrients in agricultural soils via fertilization, a practice that, if poorly managed, contributes to diminish the quality of aquifers.

Model application under seasonal and non-seasonal climatic conditions resulted in a higher attenuation of punctual growing concentrations of soil C and N under the effect of a seasonal climate, as well as average concentrations higher than those under the effect of non-seasonal climates. Furthermore, the high frequency imposed by seasonal climates is attenuated in forest soils, while this high frequency is reflected in agricultural soil nutrient cycles. Nevertheless, the general trend of the temporal dynamic is similar under both seasonal and non-seasonal conditions.

Land use changes should include long term practices to avoid the loss of soil properties, contributing to the maintenance of optimal conditions for long term agricultural production. Crop rotation is an important management practice to avoid soil C losses following conversion from forest to agricultural land (Murty et al., 2002). Furthermore, Agboola (1981) and Bruce et al. (1999) proposed that a diminution of tillage processes minimizes soil erosion and decomposition rates, and thus soil C losses. The results presented here underscore that models dealing with soil nutrient turnover are potentially promising tools to design new soil practices and predict long-term effects of these practices on soil fertility.

Acknowledgements This research is part of the RECORD project of the Competence Centre Environment and Sustainability (CCES, http://www.cces.ethz.ch/projects/nature/Record). Funding has been provided by the Swiss National Science Foundation under grant 200021-113296.

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Greenhouse Gases and Ammonia Emissions from Organic Mixed Crop-Dairy Systems: A Critical Review of Mitigation Options

S.M. Novak and J.L. Fiorelli

Contents

Abstract Dairy production systems represent a significant source of air pollutants such as greenhouse gases (GHG), that increase global warming, and ammonia (NH_3), that leads to eutrophication and acidification of natural ecosystems. Greenhouse gases and ammonia are emitted both by conventional and organic dairy systems. Several studies have already been conducted to design practices that reduce greenhouse gas and ammonia emissions from dairy systems. However, those studies did not consider options specifically applied to organic farming, as well as the multiple trade-offs occurring between these air pollutants. This article reviews agricultural practices that mitigate greenhouse gas and ammonia emissions. Those practices can be applied to the most common organic dairy systems in northern Europe such as organic mixed crop-dairy systems. The following major points of mitigation options for animal production, crop production and grasslands are discussed. Animal production: the most promising options for reducing greenhouse gas emissions at the livestock management level involve either the improvement of animal production through dietary changes and genetic improvement or the reduction of the replacement rate. The control of the protein intake of animals is an effective means to reduce gaseous emissions of nitrogen, but it is difficult to implement in organic dairy farming systems. Considering the manure handling chain, mitigation options involve housing, storage and application. For housing, an increase in the amounts of straw used for bedding reduces NH_3 emissions, while the limitation of CH_4 emissions from deep litter is achieved by avoiding anaerobic conditions. During the storage of solid manure, composting could be an efficient mitigation

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_24,

from Novak and Fiorelli, Agron. Sustain. Dev. 30 (2010) 215–236. DOI: 10.1051/agro:2009031

option, depending on its management. Addition of straw to solid manure was shown to reduce CH_4 and N_2O emissions from the manure heaps. During the storage of liquid manure, emptying the slurry store before late spring is an efficient mitigation option to limit both CH_4 and NH_3 emissions. Addition of a wooden cover also reduces these emissions more efficiently than a natural surface crust alone, but may increase N_2O emissions. Anaerobic digestion is the most promising way to reduce the overall greenhouse gas emissions from storage and land spreading, without increasing NH_3 emissions. At the application stage, NH_3 emissions may be reduced by spreading manure during the coolest part of the day, incorporating it quickly and in narrow bands. Crop production: the mitigation options for crop production focus on limiting CO_2 and N_2O emissions. The introduction of perennial crops or temporary leys of longer duration are promising options to limit CO_2 emissions by storing carbon in plants or soils. Reduced tillage or no tillage as well as the incorporation of crop residues also favour carbon sequestration in soils, but these practices may enhance N_2O emissions. Besides, the improvement of crop N-use efficiency through effective management of manure and slurry, by growing catch crops or by delaying the ploughing of leys, is of prime importance to reduce N_2O emissions. Grassland: concerning grassland and grazing management, permanent conversion from arable to grassland provides high soil carbon sequestration while increasing or decreasing the livestock density seems not to be an appropriate mitigation option. From the study of the multiple interrelations between gases and between farm compartments, the following mitigation options are advised for organic mixed crop-dairy systems: (1) actions for increasing energy efficiency or fuel savings because they are beneficial in any case, (2) techniques improving efficiency of N management at field and farm levels because they affect not only N_2O and NH_3 emissions, but also nitrate leaching, and (3) biogas production through anaerobic digestion of manure because it is a promising efficient method to mitigate greenhouse gas emissions, even if the profitability of this expensive investment needs to be carefully studied. Finally, the way the farmer implements the mitigation options, i.e. his practices, will be a determining factor in the reduction of greenhouse gas and NH_3 emissions.

Keywords Agriculture • Greenhouse gas • Ammonia • Abatement • Mixed crop-dairy systems • Organic • Livestock • Manure • Grassland • Carbon storage • Soil carbon sequestration

1 Introduction

There is currently a move towards more sustainable farming systems. One of the aims of sustainable agriculture is to establish environmentally-friendly production by limiting the adverse effects of agricultural activities on all the components of the environment. Regarding the atmosphere, agricultural activities may be a significant source of two major air pollutants: greenhouse gases (GHG), which contribute to global warming (IPCC, 2007), and ammonia (NH_3), which can lead to eutrophication and acidification of natural ecosystems (Ferm, 1998). Livestock production has recently been questioned because of its environmental damage, particularly in terms of climate change and air pollution (Steinfeld et al., 2006). Figure 1 summarises the GHG and NH_3 fluxes of a mixed crop-dairy system.

Dairy production systems represent the largest agricultural source of the greenhouse gases methane (CH_4) and nitrous oxide (N_2O) in Europe (Weiske et al., 2006). In dairy husbandry, methane is mainly produced from enteric fermentation in the rumen of cows - approximately 80% - and to a lesser extent by cattle manures (Monteny et al., 2006). Agricultural soils are a sink for methane rather than a source, consumption rates of atmospheric methane in soils being very low in European soil and climatic conditions (Le Mer and Roger, 2001; Oenema et al., 2001). Keppler et al. (2006) recently reported CH_4 emissions by plant tissues under aerobic conditions, but these emissions seem not to significantly affect the CH_4 budget of grazed grasslands (Allard et al., 2007). N_2O emissions are mainly derived from N inputs to agricultural land, i.e. from chemical fertilisers, manure, urine deposited by grazing animals, leguminous crops and crop residues, and from animal houses, i.e. from deep litter systems and solid manure heaps (Chadwick et al., 1999; Monteny et al., 2006; Petersen et al., 2006). In certain conditions, soils can also be a sink for N_2O, but the factors regulating N_2O consumption are not yet well understood (Chapuis-Lardy et al., 2007).

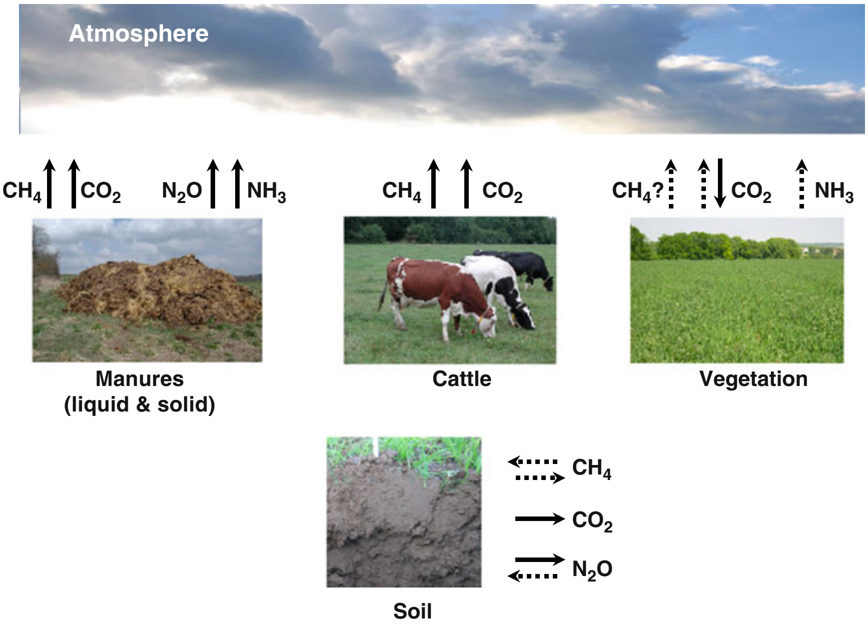

Fig. 1 Greenhouse gas and ammonia fluxes in the main compartments of a mixed crop-dairy system (adapted from Soussana et al., 2004). Broken arrows indicate small fluxes. Emissions from cattle excreta are indicated in the compartment "manures". Photos by D. Foissy, INRA Mirecourt

Carbon dioxide is another biogenic greenhouse gas playing a significant role in anthropogenic global warming. In dairy farming its major sources are soil and cattle. In soils, carbon dioxide is mainly released by microbial decay of plant litter and soil organic matter, and root respiration (Bahn et al., 2006). Cattle emit CO_2 through the respiration of organic carbon from ingested grass or fodder. However, this short-cycling carbon is generally seen as not relevant for the greenhouse effect, because it is assumed that carbon dioxide emissions have been fixed by plants through photosynthetic activity earlier in the farm cycle and thus make no net contribution to global warming (Schils et al., 2005). Soils can also act as a sink for CO_2; the CO_2 fixed in plant biomass through photosynthesis can be stored in the soil as organic C by converting plant residues into soil organic matter after being returned to the soil. Root systems also make a significant contribution to soil C inputs through rhizodeposition (Rees et al., 2005). If the input of C into the soil is greater than its losses as CO_2, C is stored in the soil.

In addition to their effect on global warming, agricultural activities and particularly livestock farming are the main source of atmospheric ammonia, around 50% of European ammonia emissions coming from cattle production (Ferm, 1998). In dairy farms, losses of NH_3 occur during slurry application, housing, slurry storage, grazing, fertiliser application and from crops, in descending order of importance (Bussink and Oenema, 1998). After deposition on land, ammonia can increase acidification and nutrient-N enrichment of sensitive habitats (Sutton et al., 1993). In addition, NH_3 reacts with atmospheric acids to form ammonium (NH_4^+)-containing aerosol, which may both directly and indirectly affect light scattering and global radiative forcing (Sutton et al., 2001), and which is likely to threaten human health in Europe (WHO, 2004). Furthermore, ammonia is an indirect source of N_2O: Mosier et al., (1998) assume that 1% of the atmospheric N deposited is converted into N_2O, and Ferm (1998) estimates that 5% of the global N_2O emissions come from NH_3 oxidised in the atmosphere. N_2O may also be indirectly emitted outside the farm gates from leached nitrate through denitrification.

To create more environmentally sustainable dairy systems regarding the air component, it is therefore necessary to limit the emissions of GHG, together with those of ammonia. Organic farming systems may be considered as the most developed form of sustainable farming systems, as their conception is based on objectives of environmental, social and economic sustainability. The fulfilment of the aim of environmental protection has already been studied by some authors assessing the effects of organic farming on various environmental parameters, including air quality (e.g. Stockdale et al., 2001; Shepherd et al., 2003), but to our knowledge, no literature study has reviewed agricultural practices that reduce emissions of GHG and NH_3 from organic dairy farming.

In this study we will analyse the mitigation options available for organic mixed crop-dairy systems, which are more common in Europe than specialised dairy or arable units (Stockdale et al., 2001). They are seen as systems having the potential to increase the efficiency of N cycling, thus reducing N losses to the environment (Ledgard, 2001). However, the combination of plant and animal production induces complex interrelations between the emissions of GHG and ammonia, which need to be studied before the implementation of mitigation options.

The mitigation options discussed in this article can also be applied beyond the scope of organic farming: they are relevant for all mixed crop-dairy systems which aim to be sustainable. Studies dealing with the mitigation of the three greenhouse gases N_2O, CH_4 and CO_2, as well as ammonia from dairy farming systems, are scarce (Brink et al., 2001; Wulf et al., 2002a), and consequences of mitigation options are mainly considered for individual sources or compartments of the farm and not at the whole-farm level. This article is a critical review of mitigation options accounting for direct N_2O, CH_4, CO_2 and NH_3 emissions as well as for indirect N_2O emissions through ammonia and nitrate losses and relevant for organic mixed crop-dairy systems in northern Europe.

2 Characteristics of Organic Mixed Crop-Dairy Systems

Organic systems aim to achieve a balanced relationship between soil, plants and animals (Vaarst et al., 2005). This is particularly the case in organic mixed crop-dairy systems, the crop production providing the animal feed and litter, while the animals provide the organic fertiliser through excreta (Fig. 2). Mixed crop-dairy systems will be defined here as dairy systems producing forage crops for on-farm feeding, and characterised by the integration of livestock and arable crop production, with interchange of nutrients between crop and livestock production, described as the "highly integrated system" by Watson et al. (2005).

We will consider organic farming systems as defined by European Union legislation (EU, 1991, 1999, 2006). The 1804/99 European Union regulation (EU, 1999) describes in detail the allowed organic livestock

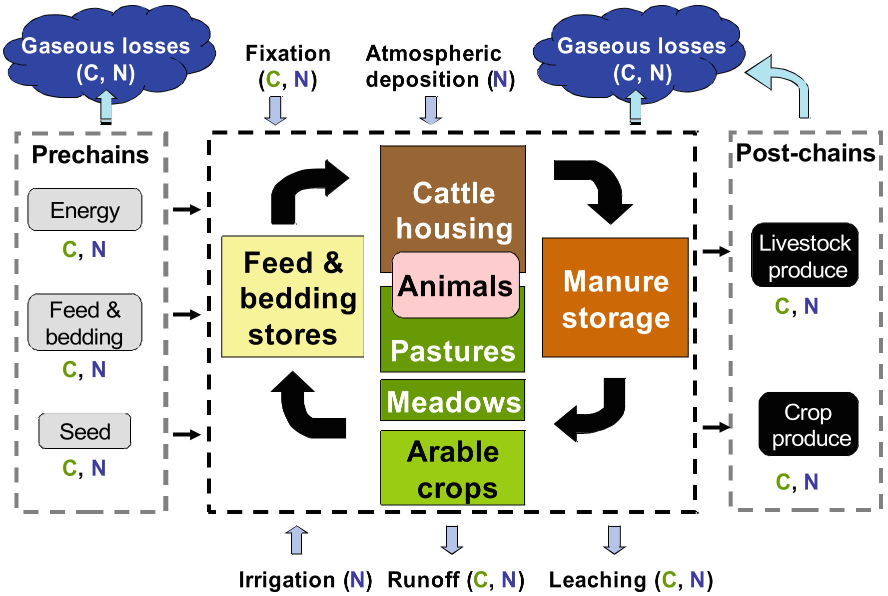

Fig. 2 Carbon and nitrogen flows in and out of a mixed crop-dairy system and between its main compartments. Gaseous losses from pre- and post-chains are outlined. Adapted from Olesen et al. (2006)

management in Europe. The main differences between organic and conventional systems concern housing and grazing conditions, animal nutrition, disease prevention and veterinary treatment. Zero grazing is not permitted and the maximum value of livestock density is limited to two livestock units per hectare for dairy cows. For livestock housing, at least half of the total floor must be solid and not of slatted or grid construction. Dry litter must be given in the rest area. Livestock must be fed on organically produced feedstuffs and 60% of the ruminant diet must come from forage. At least 50% of the feed shall come from the farm unit itself or if not, be produced in cooperation with other organic farms. The use of synthetic amino acids and growth promoters is forbidden, as well as the use of veterinary drugs in the absence of illness.

Regarding crop production and grasslands, the main differences between organic and conventional farming concern the ban on synthetic fertilisers and pesticides (EU, 1991). Organic systems are therefore characterised by low inputs of external nutrients and chemicals into the system. Nitrogen is brought from a legume "fertility-building" phase, through clover-based leys and arable grain legumes (Stopes et al., 2002), and also through lucerne (alfalfa)-based leys. The nitrogen fixed by legumes is made available to subsequent arable crops through mineralisation. Legume-based leys are utilised for grazing and fodder.

Grasslands are key components of organic mixed crop-dairy systems, by providing an important source of feed both through grazing and as hay and silage harvesting, and by contributing to building up the long-term fertility of the soil through organic matter deposition (Nicholas et al., 2004). Most organic systems have both temporary leys and permanent pastures (Nicholas et al., 2004).

Crop production is characterised by an increased diversity of cropping patterns in time and space compared with intensive conventional crop production systems (Stockdale et al., 2001). Intercrops or undersowing are often used to maintain soil cover year-round (Stolze et al., 2000).

The animal wastes provide organic fertiliser to croplands and grasslands, thus assuring the internal recycling of nutrients. As specified in Directive 91/676/EEC (EU, 1991), the total amount of manure applied on the farm may not exceed 170 kg N per year/ha of agricultural area used, which corresponds to 2 dairy cows/ha/yr as defined in Annex VII of the 2092/91 European Union regulation. The regulation also indicates that the number of livestock must be closely related to the area available in order to avoid problems of over-grazing and erosion, and to allow for the spreading of livestock manure, so that any adverse effect on the environment can be avoided.

The above-mentioned differences between the organic and conventional dairy systems imply that a lot of mitigation options proposed for conventional farming are not applicable to organic farms. For instance, products of synthesis which inhibit microorganisms or modify chemical reactions producing GHG such as methanogens in enteric fermentation or nitrification in soils are not allowed in organic farming. Besides, as organic systems and particularly mixed crop-dairy systems are extensive and use low external inputs, it does not leave much room for manoeuvre for lowering system nitrogen inputs or for reducing GHG emissions compared with intensive conventional systems which, for example, import energy-costly products such as pesticides or mineral nitrogen fertilisers.

Organic mixed crop-dairy systems can show some variations due to the environment in which they operate. The differences in livestock production will mainly consist of the land use (% of grasslands), the grassland management, the level of concentrates in the diet (between 0 and 40%), the dairy herd policy (size of the dairy herd, % of annual replacement, stocking density) and manure management. For instance, in the organic sector, Stolze et al. (2000) reported absolute livestock density of 1.6–1.8 livestock units (LU) per ha in the UK, but only 1.0 LU per ha on comparable organic farms in Germany. Some indications about the differences existing between organic dairy farming systems in Northern European countries can be found in Pflimlin and Kempf (2002), Weller (2002), Häring (2003), and Mosimann and Suter (2003). Crop production will mainly differ in crop rotation, soil tillage and fertilisation.

3 Review of Mitigation Options Available for Organic Mixed Crop-Dairy Systems

In general, mitigation measures may consist either of reducing or preventing the emissions of GHG and ammonia at source, or of favouring the storage of

Table 1 Effect of slurry aeration on greenhouse gas and ammonia emissions during storage and after field application (from the results of Amon et al., 2006). Note that CO_2 emissions mentioned here are not direct emissions, they correspond to a conversion of the energy requirement for the aeration process

	During storage				After applicaton	
	CH_4	CO_2	N_2O	NH_3	N_2O	NH_3
Slurry aeration	↘	↗	↗	↗	↗	↗

carbon in plants or soils. The mitigation measures for dairy production systems can be either technical (e.g. manure application techniques), management-based (e.g. changes in grazing/housing patterns) or system-orientated (e.g. shift from conventional to organic farming) Weiske et al., 2006).

Mitigation options show strong interrelations both between gases (more than one gas is affected either beneficially or adversely) and between farm compartments (the control of the emission of one compound may increase its emission at another stage of management). For instance, Amon et al. (2006) showed that intermittently aerating dairy cattle slurry had contrasting results on GHG and NH_3 emissions, and that slurry aeration affected the emissions not only during the storage period but also after slurry application (Table 1). It is therefore important to assess mitigation options for their impact upon the carbon and nitrogen cycles at the whole-farm level, by taking into account not only GHG and NH_3, but also NO_3^- leaching and C storage.

One must also consider that all the GHG do not affect global warming with the same intensity. Over the 100-year timescale, the Global Warming Potential from methane and nitrous oxide is estimated at 25 and 298, respectively, times that of CO_2 (Forster et al., 2007). A small increase in N_2O emissions may thus counterbalance a large decrease in CH_4 emissions. Therefore, to be effective, mitigation options have to induce an overall reduction of GHG emissions in terms of CO_2 equivalents in addition to a reduction in NH_3 losses.

A large number of studies on reducing emissions from dairy systems have already been carried out, either for greenhouse gases (e.g. Velthof et al., 1998; Amon et al., 2001a; Clemens and Ahlgrimm, 2001; Monteny et al., 2006; Schils et al., 2006; Weiske et al., 2006) or for ammonia (e.g. Bussink and Oenema, 1998; Malgeryd, 1998; Webb et al., 2005). We will focus here on the mitigation strategies suitable for organic mixed crop-dairy systems in northern Europe, by considering their different compartments (Fig. 2), grouped together into three subsystems: i/ animal production, which includes livestock and manure management, ii/ crop production dealing with crop rotation, fertilisation and soil tillage, and iii/ grasslands, grazed (pastures) or cut (meadows). Due to the lack of bibliographic data, we will not consider the emissions generated by the feed and bedding stores themselves.

3.1 Animal Production

3.1.1 Livestock Management

Feeding Strategies

Enteric fermentation in the cattle rumen is the major source of CH_4 emissions in dairy systems, but also the most difficult to reduce. The techniques currently being developed to reduce it by direct rumen manipulation (see e.g. Boadi et al., 2004; Martin et al., 2006) will not be discussed here as they are beyond the scope of organic farming. We will focus here on nutritional strategies consisting either of adding lipids to the diet, changing its fibre content or selective grazing. Linseed fatty acids supplemented at 6% were shown (Martin et al., 2008) to reduce dairy cow CH_4 emissions from 12 to 64% depending on the physical form of the lipid (crude linseed, extruded linseed or linseed oil). Furthermore, crude linseed increased milk yield, but not linseed oils. Linseed lipids are allowed as feed materials in organic dairy farming in the form of seeds or oil if the extraction process is physical (EU, 2006). Their promising results on CH_4 emissions need, however, to be confirmed by studies on more animals, taking into account the interaction with the nature of the basal diet and considering the effects of linseed on milk production.

Changing diet composition through the replacement of some of the grazing and roughage by concentrates (which have to represent less than 40% of the diet in organic dairy farming) increases animal productivity and may decrease the numbers of cows required to fill the annual milk quota (Lovett et al., 2006) and thus the CH_4 emissions per unit of milk. However, it goes against the general aim of organic dairy farming to produce milk with a minimum of external inputs. Increasing the proportion of concentrate in the diet also has other direct and indirect effects on GHG and ammonia emissions. For instance, although supplementation of diets with concentrates often diminishes enteric methane emissions from cows, it may simultaneously enhance slurry methanogenesis because this is associated with extra amounts of undigested fibre which may be a substrate for slurry microbes (Hindrichsen et al., 2006). The fibre and the protein content of cattle diets were also shown to significantly influence the ammonia emission rates of manure (Kulling et al., 2003), as well as the plant availability of cattle slurry N and the amount of residual slurry N remaining in the soil after the first growing season (Sorensen et al., 2003). Moreover, replacing roughage by concentrates also contradicts the European environmental policy to promote extensive use of maintained grasslands, which store significant amounts of carbon in soil (Freibauer et al., 2004). Besides, the production and transport of concentrates generate GHG.

Because of its high starch content, the increased use of maize silage relative to dry matter intake was also suggested to reduce enteric CH_4 (Mills et al., 2001) and to improve animal performance. However, there is a need for animal studies that directly compare maize and other cereal silage with grass silage to quantify the reduction in CH_4 that might be achieved (Beauchemin et al., 2008). Furthermore, these inputs must be evaluated in terms of the net contribution to total GHG to evaluate if the substitution of maize or cereal silage for grass silage will result in a net reduction in GHG emissions on the farm scale.

Forage species also affect CH_4 production in ruminants (McAllister et al., 1996). Measurements of CH_4 production from grazing beef cows indicated a 25% reduction in CH_4 losses with alfalfa-grass pastures (7.1% of gross energy intake) compared with grass-only pastures (9.5% of gross energy intake) (McCaughey et al., 1999). The introduction of legumes into grazed grasslands would therefore limit CH_4 emissions. Legume species also seem to affect the CH_4 production from ruminants. For instance, condensed tannin-containing legumes (such as sulla) were shown to reduce the CH_4 emissions of dairy cows and other ruminants (Woodward et al., 2002; Tavendale et al., 2005).

The control of the protein intake of animals to meet their requirements more precisely is proposed to reduce gaseous emissions of nitrogen in grazed pastures, since excess N in the diet of cattle is to a large extent excreted in the urine, which in turn can influence ammonia and N_2O emissions (Oenema et al., 2005; Watson et al., 2005; Kebreab et al., 2006). Controlling the N content of the diet is, however, not easy in organic dairy farming systems, since herbage plays an important role in the diet and because the proportion of legumes can vary greatly between swards through the seasons and between years. The N content of legumes is generally much higher than that required for optimum animal nutrition (Ledgard, 2001). However, as discussed above, CH_4 production (expressed relative to gross energy intake) from the rumen fermentation of legume forages is generally lower than the production from grass forage.

Genetic Selection

Genetic selection of cows based on an improvement of their ability to produce less methane or of their feed efficiency is still under validation (Boadi et al., 2004; Martin et al., 2006). The genetic selection of cows producing higher yield and thus less CH_4 per unit of milk often leads to a decrease in animal fertility and health (especially with Holstein cows) and to an increase in the overall replacement rate (Lovett et al., 2006). This may paradoxically involve an increase in herd size, with heifers emitting GHG and NH_3, which will increase the emissions from the whole farm.

Herd Characteristics

Options that increase lifetime efficiency or reduce the replacement rate are likely to reduce GHG emissions at the farm level, as shown in a modelling study from Weiske et al. (2006). This means that the decision to replace cows should not be based solely on economic considerations or fertility, but also on their capacity to produce milk for a long time. This could, for instance, be achieved by dairy cows resistant to illness and better adapted to the environment of their farm rather than high-yielding animals. Finally, reducing the milk production through the reduction of the number of milking cows certainly decreases the GHG and NH_3 emissions at the farm level, but this would mean a profound change in the farm economy.

Table 2 Effects on greenhouse gas and ammonia emissions of mitigation options reported for livestock management

Mitigation options for livestock management		CH_4	N_2O	CO_2	NH_3
Feeding strategy	Adding linseed lipids to the diet	↘ ?	–	–	–
	Increasing the proportion of concentrate in the diet	↘ from animals ↗ from slurry?	↗ or ↘ ? (from slurry[a])	↗ (fossil energy + soil)	↗ or ↘ ? (from slurry[a])
	Increasing the proportion of maize silage in the diet	↘ from animals	–	–	–
	Introducing legumes into grazed grasslands	↘	↗ ?	↘ ?	↗ ?
	Limiting excess N in the diet	↗ ?	↘	–	↘
Genetic selection	Selecting cows with low enteric CH_4 production	↘ ?	–	–	–
	Selecting high-yielding cows	↘ or ↗ ?	–	–	↘[b] or ↗ ?
Herd characteristics	Reducing the replacement rate	↘ ?	–	–	–
	Reducing the number of milking cows	↘	↘	↘	↘

[a] Depending on the N content of the concentrate compared to the roughage;
[b] Results from Lovett et al. (2006).
Caption:
↘ : the mitigation option decreases the emissions
↗ : the mitigation option increases the emissions
"↘ or ↗": both tendencies have been shown
– : no information was given on this compound
0: studies have shown that this option had no significant effect on this compound
?: the result needs to be confirmed by more studies.

In conclusion, Table 2 summarises the effects on GHG and NH_3 emissions of the different mitigation options discussed for livestock management. In the current state of knowledge, two main mitigation strategies can be drawn from the above-mentioned options, based on either: 1/ a herd with a limited number of high-yielding dairy cows fed with an energy-rich ration but probably needing a high replacement rate; 2/ a herd with animals bred for hardiness, less productive but more robust and long-lived.

As methane production from enteric fermentation is rather hard to reduce, mitigation strategies from organic dairy farming have focused on the reduction of CH_4 emissions from manure management.

3.1.2 Manure Management

All parts of the manure handling chain, i.e. housing, storage and application, need to be considered, since intervening in one part affects losses in another. As mentioned in the introduction, the manure handling chain may be a source of GHG as well as ammonia. The organic European Union standards require that straw-based housing systems have to be used in organic livestock production. Manure management in organic dairy farming is thus based on the farmyard manure system, which may vary according to the amount of straw used. This amount will particularly determine whether all urine and faeces are retained by straw and stored as farmyard manure or if part of the urine is stored as liquid manure.

Housing

Ammonia losses from buildings are usually the second largest sources of NH_3 after slurry application (Bussink and Oenema, 1998). NH_3 emissions from the buildings may be reduced by increasing the amounts of straw used for bedding, with the advantage of no subsequent increase in NH_3 losses during the storage or spreading of the manure, as all the ammoniacal nitrogen is immobilised in the straw (Webb et al., 2005).

Deep litter may result in significant emissions of N_2O and CH_4, depending on the rate of litter addition and mixing (Monteny et al., 2001; Oenema et al., 2005). Options to reduce CH_4 emissions consist here of avoiding anaerobic conditions in the bedding.

Manure Storage

Manure stores are the second largest source of methane emissions (after enteric fermentation) in European dairy farming (Sneath et al., 2006). CH_4 emissions arise mainly from slurry stores, whereas farmyard manure stores are a significant source of N_2O (Chadwick et al., 1999). Slurry and farmyard manure stored outside are also significant sources of NH_3 but they show great variations according to the temperature, the surface area, the duration of storage, and the occurrence of mechanical aeration (Bussink and Oenema, 1998).

The choice of a mitigation option at this stage of the manure handling chain will mainly depend on the nature of the effluent (liquid or solid manure).

Mitigation Options During the Storage of Liquid Manure

Overcoming the Effect of Storage Temperature In northern European conditions, grazing is generally not possible during late autumn and in winter and cows are kept in buildings during this period. Slurry is therefore collected from cowsheds under cold conditions. Several studies have shown that CH_4 and NH_3 emission rates for slurry increased significantly with storage temperatures (Husted, 1994; Bussink and Oenema, 1998; Sommer et al., 2000; Clemens et al., 2006). For instance, Husted (1994) reported an increase of 61% in the overall methane emissions if the slurry was applied in September instead of June. Emptying the slurry store before the increase in air temperature (i.e. before late spring) would therefore limit the emissions of NH_3 and CH_4 that occur during the storage of liquid manure, as well as NH_3 emissions resulting from field application which are also increased at higher air temperature (Sommer and Hutchings, 2001). However, other factors than air temperature must be considered, such as the load-bearing capacity of soils and the risk of nitrate leaching, which often limit the application of the stored slurry in autumn and winter. To overcome the effect of temperature when liquid manure is stored for a long time, some authors proposed cooling the manure tank (Clemens and Ahlgrimm, 2001; Monteny et al., 2001), but this requires fossil energy.

Favouring the Formation of a Surface Crust A natural crust generally forms at the slurry surface during storage as a result of evaporation, which promotes drying and binding of the particles if the slurry is not disturbed by mechanical mixing. The formation of this natural crust has been shown to greatly reduce NH_3 losses (by 50% in the study of Misselbrook et al. (2005) and by 80% in the study of Sommer et al. (1993)) and CH_4 emissions (by 38% in the study of Sommer et al. (2000) and by a factor of 12 in the study of Husted (1994)). Petersen et al. (2005) gave evidence for methanotrophic activity, i.e. methane oxidation, in surface crust materials. Measures to ensure crust formation are thus a cost-effective way of mitigating NH_3 and CH_4 emissions during the storage of slurry. They may, however, favour N_2O emissions, which were shown to increase under warm conditions for slurry covered with a natural crust (Sommer et al., 2000).

The concentration and nature of the solids present in the slurry, which are influenced by the cattle diet and bedding material used, are important in determining crust formation, as well as environmental factors such as temperature, wind speed and rainfall (Misselbrook et al., 2005). These authors found that slurry dry matter content was the most important factor influencing crust formation, with no crust formation on slurries with a dry matter content below 1%. They also observed large differences in crust formation on slurries from grass silage-fed cattle bedded on long straw compared with corn silage-fed cattle bedded on chopped straw, but with no significant differences in NH_3 emission rates. Disturbance of the crust during the regular transfer of slurry from buildings could be minimised by inserting the nozzle of the liquid manure spreader below the surface crust, and homogenisation before emptying can be achieved by mixing with or without destroying the surface crust (Petersen et al., 2005).

Covering Slurry Tanks A wooden cover on slurry tanks has also been reported as an effective technique to reduce CH_4 and NH_3 emissions (Amon et al., 2006; Clemens et al., 2006), and N_2O emissions under cold conditions. Covering the tank may increase N_2O emissions in warm conditions, but as CH_4 emissions during slurry storage contribute more to total GHG emissions than N_2O emissions, a lid has benefits as regards climate change. It may also shelter the natural surface crust from rain and help to keep it dry during winter. On the other hand, excluding rainwater from the slurry store may decrease the slurry water content, thus making its field application more difficult. Addition of a wooden cover reduces CH_4 and NH_3 emissions from slurry more than a natural surface crust alone. Covers made with other materials, such a chopped straw or wood, or expanded clay, have been shown to reduce NH_3 losses but not overall GHG (and particularly CH_4) emissions (Amon et al., 2006; Clemens et al., 2006; Guarino et al., 2006).

Performing Anaerobic Digestion Anaerobic digestion of manure has been proposed by many authors (e.g. Clemens et al., 2006; Monteny et al., 2006; Weiske et al., 2006) as a measure to abate CH_4 emissions from manure storage. Anaerobic digestion is a natural process whereby bacteria existing in oxygen-free environments decompose organic matter, resulting in a biogas (a mixture of CH_4, CO_2 and some trace gases) and a sludge that is stable and nearly odourless (Kebreab et al., 2006). Methane produced by digesters can be captured and burned as fuel. Anaerobic digestion in a covered gas-tight plant thus offers the benefits of reducing trace gas emissions and substituting fossil fuels by renewable energy (biogas).

Few studies have been done on the GHG and NH_3 emissions of slurry after its anaerobic digestion. During winter storage in pilot-scale slurry tanks, Clemens et al. (2006) reported significantly lower CH_4 emissions from digested dairy cattle slurry than from the same untreated slurry (emissions of N_2O and NH_3 were similar for the two slurries). These authors emphasise the importance of the digestion's duration (hydraulic retention time), which must be sufficiently long for a complete degradation of fermentable organic matter, so as to exploit the potential for gas production without increasing CH_4 emissions during subsequent storage.

However, even with a sufficiently long hydraulic retention time, CH_4 is still produced after anaerobic digestion. Clemens et al. (2006) therefore recommend including all potentially gas-producing compounds within biogas plants for complete collection of CH_4 and optimum environmental and economic benefit. It is also important to prevent uncontrolled losses of methane from biogas plants (which occur, e.g., by mean of small leakages) which can considerably alter the environmental balance of a biogas production system (Borjesson and Berglund, 2006).

After field application, Clemens et al. (2006) found no significant differences in GHG emissions between untreated and digested cattle slurry, whereas Petersen (1999) reported lower N_2O emissions from digested slurry compared with untreated slurry. NH_3 losses after field application of digested slurry have been reported to be similar to (Rubaek et al., 1996; Clemens et al., 2006; Wulf et al., 2002b), lower than (Rubaek et al., 1996) or higher than (Amon et al., 2006) those from raw slurry. These contrasting results are probably attributable to the effects of anaerobic digestion on both chemical and physical properties of the slurry. Anaerobic digestion reduces manure carbon and dry matter content by about 50% but generally results in an increase in NH_4^+ content and pH (Wulf et al., 2002b; Amon et al., 2006). The reduced viscosity of digested slurry improves its infiltration, thus limiting the NH_3 volatilisation, whereas its higher NH_4^+ content and pH promote the NH_3 losses. NH_3 emissions from digested slurry can therefore be reduced if the increased potential loss due to chemical factors is compensated for by its faster infiltration (Wulf et al., 2002b). Techniques to reduce NH_3 emissions from field application will be discussed later.

In France, Ademe et al. (2006) estimated 200–250 k euros as the cost of a biogas installation producing 30 kW of electricity. In organic dairy farming, two other limitations must be taken into account: 1/ slurry is only collected at one time of the year (i.e. during housing) and 2/ co-digestion with other waste products to increase gas production is not feasible, particularly because they should also come from organic farming unless the digested product is not used for an organic farm. The economical feasibility of on-farm power/heat generation with biogas will depend to a great extent on the energy price and on subsidies.

Performing a Mechanical Separation Mechanical separation is another technique used to treat slurry, resulting in a liquid fraction with low dry matter content reduced by 40–45%, and a solid fraction that can be stored in heaps. Separation is performed with a screw sieve separator and uses little energy. The separated slurry has a lower viscosity and flows more easily through band-spreading hoses. The study of Amon et al. (2006) indicates that slurry separation reduces CH_4 emissions, but is likely to result in an increase in N_2O and NH_3 emissions during composting of the solid fraction.

Lowering the pH of Slurry Lowering the pH of slurry with additives such as lactic acid has also been proposed to avoid the production of methane, this acid being authorised in organic farming not intentionally for that purpose but for the cleaning and the disinfection of livestock buildings and installations. Lowering the pH of the slurry with lactic acid can indeed reduce both CH_4 and N_2O emissions, but Berg et al. (2006) showed that this reduction is effective only if the pH is below 6.0, and that a lower pH would be necessary to reduce ammonia emissions. This technique seems therefore not to be practically possible.

Aerating the Slurry As oxygen is a strong inhibitor of methane production and an easily available product, aeration seems an attractive method to limit CH_4 emissions. Aeration was indeed shown to reduce CH_4 emissions of slurry during its storage (Martinez et al., 2003; Amon et al., 2006), but N_2O and NH_3 emissions may be greatly increased when the slurry is aerobically treated (Béline et al., 1999; Amon et al., 2006). N_2O may also be emitted by the aerated slurry during its subsequent storage (Béline et al., 1999). Besides, slurry aeration results in energy consumption. Nevertheless, laboratory studies showed that the aeration strategy (intermittent or continuous, length of the anoxic period) would play an important role in the emissions of N_2O (Béline et al., 2002). However, these results need to be confirmed by experiments on farm-scale units.

Comparison of Treatments Comparing the effect of different treatments of dairy cattle slurry (wooden or straw cover, mechanical separation, anaerobic digestion, aeration), Amon et al. (2006) found that anaerobic digestion reduced the overall GHG emissions from storage and land spreading the most, and had hardly any influence on NH_3 emissions from these processes (Fig. 3). This study has the asset of having been conducted in pilot-scale tanks whose conditions are nearer to field conditions than studies with laboratory reactors.

Mitigation Options During the Storage of Solid Manure

The use of straw for bedding in organic dairy housing systems produces solid manure, which can be either composted or stored outdoors stockpiled.

Composting Solid Manure Organic systems encourage the composting of solid manure, i.e. aerobic decomposition at temperatures of around 60°C, which offers the advantages of producing a more stable, uniform product, free of weeds and toxins and easier to spread (Mustin, 1987; Peigne and Girardin, 2004). However, the heat generated and the regular aeration by turning of the heap during the early stages of composting favour ammonia losses which are generally greater than for stockpiled farmyard manure (Amon et al., 2001b; Gibbs et al., 2002; Peigne and Girardin, 2004). Although NH_3 losses are large during the composting phase, they are low after subsequent soil application, because the remaining N is mainly in organically bound forms and thus concentrations of inorganic N are very low (Sommer and Hutchings, 2001; Amon et al., 2001b; McNeill et al., 2005).

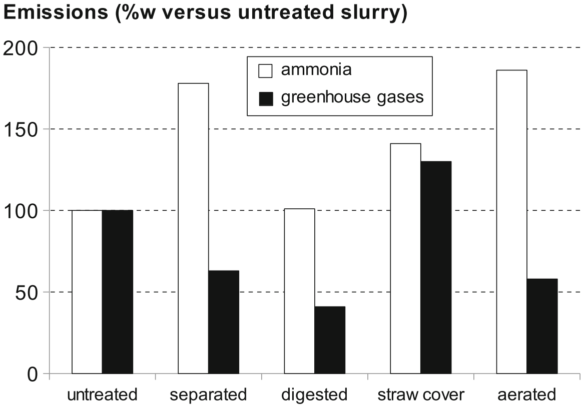

Fig. 3 Relative ammonia and greenhouse gas emissions from storage and land spreading for different treatments of dairy cattle slurry compared with untreated slurry with a wooden cover (from Amon et al., 2006)

Mechanical turning of composted manure generally results in lower N_2O and CH_4 emissions than for anaerobically stacked farmyard manure (Amon et al., 2001b; Peigne and Girardin, 2004). Considering the whole management system (housing, storage and spreading), Amon et al. (2001b) reported GHG ($CH_4 + N_2O$) emissions 25% lower from a composting system than from an anaerobic stacking system. Significant amounts of CO_2 may, however, be emitted during the composting process, but it is not a net source of CO_2 along the recycling chain of agricultural wastes, since well-composted material contains less easily decomposable carbon compounds than non-decomposed material (Peigne and Girardin, 2004). Composted manures being less degradable than fresh manures, their application permits greater carbon storage in the soil (Kirchmann and Bernal, 1997).

GHG and ammonia emissions during composting are greatly influenced by its management (turning method and frequency, duration of the composting operations) and by the physical and chemical characteristics of the raw material (C/N ratio) (Peigne and Girardin, 2004). A high porosity of the initial material provides sufficient aeration, which is essential for a good composting process and for limiting N_2O and CH_4 emissions. An increase in the C/N ratio significantly reduces N losses during storage and composting of livestock solid manure (Sommer, 2001). If the density of the farmyard manure is high (low porosity) or the C/N ratio is low (<20), straw may be added to the farmyard manure to improve its composting. Furthermore, the number and frequency of turnings can be reduced if the raw material has good characteristics (Peigne and Girardin, 2004), thus limiting NH_3 losses.

Considering indirect N_2O emissions, a concrete platform with water recovery is the best system to prevent nitrate losses by leaching or runoff from the compost heap to surface or groundwater (Peigne and Girardin, 2004). Covering the pile with a waterproof but air-permeable cover is another way to avoid contamination by runoff (Peigne and Girardin, 2004). However, nitrate leaching losses from compost with a high C/N ratio are generally low (Sommer, 2001).

Compacting and Covering Manure Heaps Compacting and covering manure heaps has the potential to reduce emissions of both NH_3 and N_2O when the manure contains relatively high ammonium-N contents (Chadwick, 2005). Compaction may, however, increase CH_4 emissions (Chadwick, 2005; Oenema et al., 2005). Covering manure heaps may also not always be effective, because the mineralisation rate increases with temperature, and so the effect of increased mineralisation may exceed the effect of reduced exchange rates of NH_3 from the manure with the atmosphere (Bussink and Oenema, 1998). Addition of straw to solid manure is another way of reducing NH_3 emissions by improving the C/N ratio (Clemens and Ahlgrimm, 2001), and it was also shown to reduce CH_4 and N_2O emissions (Yamulki, 2006).

Reductions in NH_3 emissions at the manure storage stage will result in increased manure total ammoniacal nitrogen content and therefore in potentially larger losses following land spreading (Misselbrook et al., 2005). It is therefore important that suitable application or incorporation methods are used to minimise losses.

Application Techniques

Slurry application is the major source of ammonia emissions in dairy farming systems (Bussink and Oenema, 1998) and it is to a lesser extent a direct source of N_2O (Oenema et al., 2005). Significant NH_3 and N_2O emissions also occur following the application of farmyard manure (Webb et al., 2004). In addition to being a direct source of N_2O, manure spreading may be an indirect source of this gas through ammonia losses and nitrate leaching. In general, the reduction of NH_3 emissions is more effective at the spreading phase than from buildings (Webb et al., 2005).

Spreading Manure During the Coolest Part of the Day

A “simple” mitigation method is to limit the application of manure to when conditions do not favour NH_3 volatilisation, e.g. during the coolest part of the day. Sommer and Olesen (2000) have calculated that avoiding applications during times of the day with a high potential for NH_3 losses could reduce the total emission of NH_3 from applied slurry by half. However, the efficiency of this technique depends on the farmer’s flexibility in the choice of application date and time (Sommer and Hutchings, 2001).

Incorporating Manure

Several techniques of manure spreading on land have been shown to decrease NH_3 losses (Sommer and Hutchings, 2001). Among them, rapid incorporation of manures into arable land by ploughing is the most cost-effective (Webb et al., 2005). Incorporation should be as soon after application as possible, especially after slurry application, as loss rates are high in the first hours after application (Sommer and Hutchings, 2001). For slurry, the use of disk or tine cultivators may be as effective as ploughing (Webb et al., 2005). Incorporating manure was found to increase (Ferm et al., 1999) or decrease N_2O emissions by soils (Webb et al., 2004). It is therefore not possible to generalise the effects of manure incorporation into the soil on N_2O emission, most probably because the denitrification process in soils depends on many factors (see e.g. Oenema et al., 2005; Mathieu et al., 2006).

Spreading the Slurry in Bands

The application of slurry in narrow bands (i.e. band spreading), rather than over the entire soil or crop surface, by trailing hose or trailing shoe was also shown to be an effective technique to reduce NH_3 losses after liquid manure spreading (Webb et al., 2005). The effectiveness of band spreading increases if the slurry is placed below the canopy of a well-developed crop (Webb et al., 2005). Trail hoses or trail shoes have the advantage of limiting N_2O emissions in comparison with slurry injection, which reduces NH_3 emissions reliably but may greatly enhance N_2O emissions (Flessa and Beese, 2000). Injection of slurry also causes higher fuel consumption (Wulf et al., 2002a). Trail hoses are, in general, more effective on arable land than on grassland and when used with dilute than with more viscous slurries, whereas trail shoes are designed more for grasslands (Webb et al., 2005).

Investigating the effect of different application techniques on greenhouse gas and ammonia emissions from co-fermented slurry, Wulf et al. (2002a) found that the most effective methods to limit both GHG and ammonia emissions were trail hose application with immediate shallow incorporation for arable land, and trail shoe application for grassland.

One must also consider the indirect effects on GHG emissions of an improved application of manure in the field (Weiske et al., 2006). For instance, as NH_3 losses from the manure are reduced, there are less indirect emissions of N_2O due to deposition of NH_3 outside the field. But as more nitrogen is effectively applied to soil, nitrate leaching is likely to increase and hence the derived N_2O emissions. This can, however, be counterbalanced by increased crop yields resulting from the increased amount of nitrogen available for the crops.

The effects of timing applications to match crop demand are discussed later in the "crop production" section.

Solid Versus Liquid Manure

Depending on the amount of straw used for bedding, the manure handling chain will manage straw-based manure only, both solid (straw-based) and liquid (slurry-based) manures or even liquid manure only. We report here the results of studies or reviews comparing the emissions of GHG or NH_3 of straw-manure-based systems versus slurry-based ones. From different experimental studies, Monteny et al. (2006) concluded that animal housing and manure stores of straw-based systems (deep litter) result in greater N_2O emissions than the more anaerobic slurry-based systems. On the other hand, several studies reported substantially lower N_2O emissions following the application of solid manure compared with the application of liquid manure (Gregorich et al., 2005).

Concerning CH_4, Stolze et al. (2000) estimated that lower potential CH_4 emissions are likely for straw-based manure because stable manure has a significantly lower metabolic factor for methane than liquid manure. Conversely, Monteny et al. (2001) hypothesised that deep litter systems may emit larger quantities of CH_4 than slurry-based systems, due to the increased temperature in the deep litter bed and (partially) anoxic conditions resulting from compaction by the animals. On the other hand, Hansen et al. (2002) estimated that CH_4 emissions from deep litter were similar to those of slurry-based housing systems. The application of solid manure generally results in higher C storage than slurry (Foereid and Hogh-Jensen, 2004; Ceotto et al., 2006). Concerning NH_3, increased use of straw for bedding is likely to reduce NH_3 emissions from housing (Bussink and Oenema, 1998; Stockdale et al., 2001).

These sometimes conflicting results show the difficulty in drawing conclusions about the magnitude of GHG and NH_3 emissions from straw-based manures compared with slurry-based ones when the whole farming system is considered. It is all the more difficult to give a generalised conclusion, since GHG and NH_3 emissions closely depend on the techniques and practices used for livestock housing, manure storage and manure application. Besides, an efficient comparison of solid and liquid manure systems needs to take into account the emissions generated by the production of the straw. Finally, at the whole-farm level, a change in the manure management system has implications on the balance between grassland and arable areas, these latter determining the production of straw used for the bedding.

In conclusion, Table 3 summarises the effects on GHG and NH_3 emissions of the different mitigation options discussed for the manure handling chain. An overall reduction of GHG and NH_3 emissions at the manure handling chain requires that mitigation options are implemented on all its parts, from housing to application. For housing, the reduction of NH_3 and CH_4 emissions may be achieved by a judicious management of the bedding, whereas among the several techniques available at the storage level, anaerobic

Table 3 Effects on greenhouse gas and ammonia emissions of mitigation options reported for manure management

Mitigation options for manure management		CH_4	N_2O	CO_2	NH_3
Housing	Increasing the amounts of straw used for bedding	–	–	↘	–
	Avoiding anaerobic conditions in the bedding	–	–	–	↘
Storage	Emptying the slurry store before the increase in air temperature	–	–	↘	↘
	Cooling the manure tank	–	↗ (fossil energy)	↘	↘
	Favouring the formation of a surface crust	↗ ?	–	↘	↘
	Covering slurry tanks	↘ in cold conditions, ↗ in warm conditions	–	↘	↘
	Performing the anaerobic digestion of the slurry	at field application: ↘ or 0	–	at field application: 0, ↗ or ↘	↘
	Performing a mechanical separation	↗ ?	0	↗ ?	↘
	Lowering the pH of slurry	↘	–	–	↘
	Aerating the slurry	↗ ?	↗ (fossil energy)	↗	↘
	Composting solid manure	↘	↗ during composting ↘ at application	↗ during composting ↘ at application	↘
	Compacting and covering manure heaps	↘	–	↘ ?	↗ ?
	Adding straw to solid manure	↘ ?	–	↘	↘ ?
Application techniques	Spreading manure during the coolest part of the day	–	–	↘	–
	Incorporating manure (rapidly)	↗ or ↘	–	↘	–
	Spreading the slurry bands with trail hoses or trail shoes	↘ in comparison with slurry injection	↘ (fossil energy)	↘	–
Solid versus liquid manure		0?, ↗ ? or ↘ ?	↗ at housing and storage ↘ at application	↘ (higher carbon storage)	↘

digestion seems the most promising to reduce the overall GHG emissions from storage and land spreading, without increasing NH_3 emissions. At application, band spreading effectively reduces emissions, all the more since the manure is applied to arable land by trail hose and rapidly incorporated.

3.2 Crop Production

For the crop production sub-system, the mitigation options focus on limiting CO_2 and N_2O emissions, which are the main gases produced at this level. The limitation of net CO_2 emissions from cropping systems may be achieved either by increasing the C storage in soils or plants, or by slowing the return of stored C into the atmosphere via mineralisation (or fire). For instance, measures such as the incorporation of straw aim at enhancing the input of carbon to the soil, while measures such as reduced tillage aim at reducing the output of carbon from the soil. Mitigation options dealing with N_2O emissions are mainly based on an improvement of the N-use efficiency of crops, because N_2O is generated by soil microorganisms largely from surplus mineral N, which may also lead to nitrate leaching (Syväsalo et al., 2006; Kuikman et al., 2004; Smith et al., 2008). To a lesser extent, mitigation options aim to improve soil structure to limit anaerobic sites favouring N_2O emissions (Ball et al., 1999). We will present the main mitigation options reducing N_2O and CO_2 emissions of the crop production system by considering in turn crop rotation, fertilisation and soil tillage.

3.2.1 Crop Rotation

Increasing diversity in crop rotation is generally associated with a positive effect on C sequestration (West and Post, 2002), especially when legumes are introduced (INRA, 2002; Gregorich et al., 2005). Some crop species favour C storage in soils, such as crops with large deep root systems and perennial crops, which are generally regarded as allocating more C below-ground (Rees et al., 2005; Smith et al., 2008). On the other hand, silage maize, which is characterised by a low return of crop residues, is thought to decrease the C stock of soils (INRA, 2002). Perennial crops also have a beneficial effect on N_2O emissions during winter (Gregorich et al., 2005) and on nitrate leaching losses (Watson et al., 2005), which have generally been shown to be smaller from perennial than from annual crops following manure application.

Organic cropping systems are generally characterised by temporary leys which alternate with arable crops. Temporary leys have a potential for soil C storage intermediate between crops and permanent grassland (Soussana et al., 2004). Prolonging the lifespan of temporary leys permits one not only to maintain their C storage for a longer time, but also to increase their storage capacity (INRA, 2002). Part of the additional carbon stored in the soil during the ley phase is released when it is ploughed up (Soussana et al., 2004). Ploughing of the ley results in a rapid mineralisation of soil organic matter and thus in large CO_2 emissions. The release of large quantities of N from mineralisation of grass/clover residues may also favour nitrate leaching (Berntsen et al., 2006) and may significantly contribute to the total N_2O emission from cultivated soils (Flessa et al., 2002; Vellinga et al., 2004).

Nitrate losses following grassland cultivation may be controlled by good management practices, such as delaying ploughing until late winter or spring for spring crops (Watson et al., 2005). If spring incorporation of the ley is not possible, it is advised to establish the following crop early in autumn to maximise N uptake during autumn (Berry et al., 2002) or to use efficient catch crops after ploughing. For instance, Eriksen et al. (2004) showed a considerable decrease in nitrate leaching when replacing the rotation "winter wheat - bare soil", following the ploughing of the ley, by spring oats and ryegrass catch crops in the two winters. More generally, cover crops or "catch crops" may be planted when the period between two main crops is rather long, with the advantage of reducing nitrate leaching after harvest of the main crop in autumn and increasing the total duration of photosynthesis (INRA, 2002). The immobilised soil nitrogen is subsequently made available after incorporation by mineralisation. Therefore, careful attention to the timing and method of incorporation of the cover crop, to synchronise mineralisation with periods of high crop demand (Hu et al., 1997), is very important. However, there are still uncertainties on how decomposition and mineralisation of cover crop residues that are incorporated into soil will affect nitrate leaching over the long term (McNeill et al., 2005). For enhancing the C storage from cover crops it is preferable to incorporate them with reduced tillage.

3.2.2 Fertilisation

Mitigation options concerning fertilisation are mainly based on an improvement of the N efficiently used by crops to reduce mineral N accumulation in the soil, which may generate both N_2O emissions and nitrate leaching.

Improving N-Use Efficiency

Practices improving N-use efficiency consist of synchronising N inputs with crop growth and crop uptake, such as, for instance, adjusting application rates with crop needs or avoiding time delays between N application and plant N uptake (improved timing). However, these mitigation measures reviewed by Monteny et al. (2006) were mainly devised for farming systems using mineral fertilisers and not for organic systems where the supply of soil mineral N is the sum of biological fixation from legumes, and direct inputs of mineral N from atmospheric deposits and manure, plus mineralisation from soil organic matter and other organic materials (crop residues, manure).

N fixed by legumes represents the major source of N inputs into organic cropping systems but its quantification is very uncertain (Nicholas et al., 2004), because its magnitude is regulated by changes in soil inorganic N and competition from associated grasses (Ledgard and Steele, 1992). For instance, the percentage of legumes in a ley decreases with increasing manure application (Hansen, 1996). This feedback mechanism has, however, the advantage of limiting the N inputs to legume/grass soil and consequently regulating the potential for N losses to watercourses or air (Syväsalo et al., 2006). Besides, according to Rochette and Janzen (2005), much of the increase in soil N_2O emissions in legume crops would be attributable to the N released from root exudates during the growing season and from decomposition of crop residues after harvest, rather than from biological N fixation per se. Carter and Ambus (2006) also found that recently fixed N released via easily-degradable clover residues was a minor source of N_2O in a grass-clover grassland.

The soil mineral nitrogen coming from the mineralisation of soil organic matter is also hard to predict, since the actual amount and timing of the mineralisation is influenced by a number of factors including soil moisture, aeration and temperature, the nature and accessibility of the organic matter, previous fertilisation, intensity and timing of cultivation, and cropping patterns (Stockdale and Rees, 1995; Rasmussen et al., 1998; Berry et al., 2002). Matching nitrogen supply from the mineralisation of soil organic matter with crop demand is thus a difficult exercise.

In organic farming, improvements in N-use efficiency are particularly related to an effective use of manures as nutrient resources. This effective management of manure and slurry must take into account that nutrients in farmyard manure are available at a slower rate for plant uptake than from slurry (Watson et al., 2005) and that uncomposted manure contains more readily available N than composted manure (Berry et al., 2002). As manures vary widely in composition, depending on feed composition, quality and quantity of bedding material, length of storage and storage conditions (Shepherd, 2000; Watson et al., 2002), manure analysis may help to predict its available N. For instance, N content and the C/N ratio of animal wastes are highly correlated with the N mineralisation rate (Morvan et al., 2006) and they may be used to predict it. The use of agronomic models to provide decision support systems or appropriate management guidelines for organic N fertilisation is promising but still needs further development (David et al., 2005; Morvan et al., 2006). These models could help to improve manure and slurry use; for instance, by determining the type of manure most beneficial to the different crops of the rotation over longer periods than a single crop or growing season to ensure both short-term productivity and long-term sustainability (Watson et al., 2005). Future improvements in N utilisation within organic systems could also be made by breeding crops with traits that improve the capture of available N and the efficiency with which is it converted to yield (Berry et al., 2002).

Improved timing of manure application may also help to prevent nitrate leaching, which generally occurs when large amounts of nitrate in the soil profile coincide with a period of high drainage (Di and Cameron, 2002). In areas with light-textured soils, manures applied in spring pose a smaller risk for leaching than when they are applied to bare soil in autumn (McNeill et al., 2005). Splitting the annual N application rates was also shown to reduce nitrate leaching losses for pasture systems, by improving the synchrony between the pasture N demand and supply (Di and Cameron, 2002). Composting the manure may also be effective at reducing nitrate leaching losses, which were shown to be lower from the application of composted manure compared with its raw materials (Basso and Ritchie, 2005).

More generally, strategies reducing N leaching will also limit N_2O emissions. Besides, modelling studies showed that improving farm efficiency of N management would reduce overall GHG emissions from conventional as well as from organic dairy farms (Schils et al., 2006; Olesen et al., 2006). However, N budgets for organic farms are less reliable than those for conventional farms, particularly because N fixation represents the predominant N input source for organic farms (see e.g. Steinshamn et al., 2004) and its estimation remains highly uncertain even with the use of empirical models. Besides, Olesen et al. (2006) indicated that the N efficiency is strongly affected by the ratio of crop to animal products in the farm output. Further investigations are therefore necessary to confirm this relationship between farm N efficiency and GHG emissions for organic dairy farms.

Timing the Effluent Application with Soil Wetness

Another strategy, proposed by de Klein and Eckard (2008) to limit N_2O emissions resulting from fertilisation, is the timing of effluent application in relation to soil wetness. N_2O emissions were indeed shown to be higher when the slurry was applied to wet soil compared with drier soil (Saggar et al., 2004).

Sequestering C Through an Improved Fertilisation

Manure management also affects soil organic carbon sequestration in different ways, the two main ones being biomass production and N availability. Firstly, fertilisation increases the primary productivity of crops and therefore the quantities of above- and below-ground residues added into soil in unharvested plant parts (Rees et al., 2005). However, the decomposition rates of the crop residues (mineralised into CO_2) could also be increased for high fertilisation inputs (Rees et al., 2005). Secondly, soil N availability is one of the main factors affecting humus formation in agricultural soils (Christopher and Lal, 2007). Furthermore, manures are a source of C per se, and the fate of their C in soil (mineralised versus humified) depends particularly on the biochemical characteristics of their organic matter (e.g. Morvan et al., 2006), and therefore on the cattle's diet and on the straw characteristics. As stated by Christopher and Lal (2007): "the challenge of sequestering C in agricultural soils is to increase the concentration of humus while producing good crop yields and maintaining low concentrations of N in soil solution and discharging waters". The hypothesis put forward by Smith et al. (2000) that the application of farm manures to arable soils can increase the soil carbon stock to a greater extent than application to grasslands was questioned by Soussana et al. (2004).

3.2.3 Soil Tillage

Ploughing is an efficient practice for weed management in organic cropping systems. However, soil tillage stimulates the decomposition of soil organic matter, particularly by disrupting soil aggregates (Balesdent et al., 2000).

Limiting Soil Tillage

Limiting soil disturbance by reduced (shallow) tillage or no tillage therefore decreases the decomposition rate of soil organic carbon (Smith et al., 2000; Rees et al., 2005). Depending on the study concerned, reduced tillage was as effective as no tillage at storing C in soil (INRA, 2002) or it did not induce significant change in soil organic carbon (Kern and Johnson, 1993). Effectiveness of conversion to conservation tillage on soil organic carbon sequestration also depends on soil and climatic factors such as texture, temperature and water availability (Lal, 2004a; Rees et al., 2005). For instance, light-textured and well-drained soils in moist and cool climates would sequester more soil organic carbon than clayey and poorly-drained soils (Lal, 2004b). Reduced tillage would also be more effective where relatively high soil carbon contents occur simultaneously with relatively high decomposition rates (Vleeshouwers and Verhagen, 2002).

The permanence of the tillage practices is another determining factor in soil carbon sequestration, because soil carbon sequestered in arable soils is impermanent and is lost more rapidly than it accumulates (Freibauer et al., 2004). Agricultural soils that are tilled every few years may contain more carbon than the same soils cultivated every year (Smith et al., 1997), but even a single ploughing can drastically accentuate emissions of CO_2 from soil, because of an increase in the rate of mineralisation (Reicosky et al., 1999).

One must also take into account that the absence of tillage may lead to higher N_2O emissions than conventional tillage, due to larger soil aggregates, low gas diffusivity and greater water retention near the soil surface making the soil less aerobic (Ball et al., 1999; Smith et al., 2001). The results of studies comparing N_2O emissions from conventional versus reduced tillage are, however, conflicting (see e.g. Holland, 2004; Smith and Conen, 2004), like those of studies comparing manure incorporation into the soil (as previously reported).

Moreover, the differences in N_2O fluxes between the two tillage systems are likely to change over time. Six et al. (2004) showed increased N_2O fluxes in the first 10 years of adoption of no-tillage, but lower N_2O fluxes in humid climates in no-till than conventional till after 20 years. These results can be explained by the transient lower crop yields and the greater soil water content generally observed in recently established no-till systems (Six et al., 2004), or by an improvement of the soil structure with time (Holland, 2004). Conversely, methane uptake was reported to be significantly increased under no-tillage

(Six et al., 2004). Finally, considering the three GHG fluxes together, Six et al. (2004) found that no-tillage leads to an increase in net global warming potential during the first 10 years, but to a strong decrease after 20 years of adoption in humid climates.

West and Post (2002) and West and Six (2007) found greater C sequestration for a decrease in tillage intensity as compared with an increase in rotation complexity, but they emphasised that increase in soil C following a change in rotation complexity may occur over a slightly longer period of time. The sequestration of soil organic carbon resulting from a change in tillage may also depend on the crop species (Wright et al., 2007).

None of these results take into account the direct machinery energy consumption. The cultivation of soils by ploughing is the most fuel-consuming process in the production of arable crops (Holland, 2004). Conservation tillage has the additional benefit of saving fuel and therefore of limiting CO_2 emissions (see e.g. Filipovic et al., 2006), even if systems based upon conservation tillage may require additional operations such as the creation of a stale seedbed (Holland, 2004).

Avoiding Soil Compaction

Soil compaction by tractor wheels and tillage machinery may increase N_2O emission (Ball et al., 1999) by favouring the development of anaerobic zones within some of the soil structural units as a result of the loss of air-filled macropores (McNeill et al., 2005). Therefore, avoiding compaction by traffic and tillage may help to reduce N_2O emissions, especially since compaction has a great effect on yield (Hansen, 1996; Stockdale et al., 2001) and thus on C storage and nitrate leaching.

Incorporating Crop Residues

Incorporation of crop residues tends to increase soil organic matter, thus storing C in the soil, but N-rich crop residues may stimulate rapid denitrification and associated nitrous oxide emissions, even in coarse-textured soils (Velthof et al., 2002). From a N_2O mitigation point of view, incorporating residues with low N content is better than a homogeneous mixing of N-rich materials into the soil (Ambus et al., 2001). Incorporation of N-rich, low C/N ratio residues leads to rapid mineralisation and a large rise in soil mineral N, while residues low in N, such as cereal straw, can lead to net immobilisation of N in the short to medium term (Watson et al., 2002). On livestock farms, however, straw is more likely to be used for bedding. Moreover, based on a review of published Canadian studies, Gregorich et al. (2005) concluded that ploughing manure or crop stubble into the soil in the autumn led to higher levels of N_2O production ($2.41\ \mathrm{kg\ N\text{-}N_2O\ ha^{-1}\ year^{-1}}$) than if residues were left on the soil surface ($1.19\ \mathrm{kg\ N\text{-}N_2O\ ha^{-1}\ year^{-1}}$). Mineralisation of N from crop residues may also result in nitrate leaching, depending on the quantity of N in the plant material and its C/N ratio (Berry et al., 2002), on the synchrony between N release and plant demand (Eriksen et al., 2006), and on soil texture.

The N content of crop residues also influences soil C sequestration. Incorporation of crop species producing residues with high C/N ratios (such as wheat) was shown to favour C sequestration compared with residues with low C/N ratios, which stimulated decomposition of native soil organic matter (Wright et al., 2007). Nevertheless, even low C/N ratio materials are beneficial for C sequestration after their incorporation (Rees et al., 2005).

In any case, crop residues should not be burned to avoid emissions of aerosols (Hays et al., 2005) and of CO_2, resulting in C losses for the system (Lal, 2004b).

In conclusion, Table 4 summarises the effects on GHG and NH_3 emissions of the different mitigation options discussed for crop production. The main mitigation options concern the introduction of perennial crops or the longer duration of temporary leys, a reduction of the tillage or the improvement of crop N-use efficiency through effective management of manure and slurry, by growing catch crops or by delaying the ploughing of leys. The long-term effects of these mitigation options are still uncertain because of the difficulty of predicting and controlling the C and N dynamics of organic matter in soils.

3.3 Grasslands

In this section we will discuss conversion from arable to grassland and review mitigation options applicable to grazing management.

Table 4 Effects on greenhouse gas and ammonia emissions of mitigation options at the crop production stage

Mitigation options for crop production		CH_4	N_2O	CO_2	NH_3
Crop rotation	Increasing diversity in crop rotation	–	–	↘	–
	Introducing perennial crops	–	↘	↘	–
	Prolonging the lifespan of temporary leys	–	– ↗ after ploughing	↘ ↗ after ploughing	–
	Cultivating catch crops	–	↘ at short term ↗ ? at long term	↘ at short term ↗ ? at long term	–
Genetic selection	Breeding crops improving N use efficiency	–	↘ ?	–	–
Fertilisation	Synchronizing N inputs with crop uptake	–	↘	–	–
	Timing effluent application with soil wetness	–	↘	–	–
	Improving the fertilisation	–	↗ ?	↘ ? or ↗ ?	–
Soil tillage	Reducing tillage	↗ ?	↗ ? or ↘ ?	↘	–
	Avoiding soil compaction	–	↘	↘	–
	Incorporating crop residues	–	↗ ?	↘	–

3.3.1 Conversion from Arable to Grassland

Several studies comparing farmland management options in Europe have indicated that conversion from arable to grassland (and to a lesser extent from temporary grassland to permanent grassland) provides high soil C sequestration potential (INRA, 2002; Smith, 2004). The results of Ammann et al. (2007) suggest, however, that the conversion of arable land to managed grassland has a positive effect on the carbon balance during the first 3 years only if the system receives nitrogen inputs. A moderate increase in N supply to nutrient-poor permanent grasslands has also been shown in long-term surveys to increase grassland topsoil organic carbon stocks, except for nutrient-poor grasslands developed on organic soils (Soussana et al., 2004). To be effective, the conversion from arable to grassland should remain permanent, because the ploughing under of grassland results in large CO_2 and N_2O emissions (Vellinga et al., 2004). Also, carbon losses are much faster after returning grassland to arable use than the build-up of soil carbon when establishing grassland (Soussana et al., 2004).

Until recently, the general assumption was that soil carbon fluxes were in balance in permanent pasture, i.e. in the absence of changes in environmental factors and in land use and management, an equilibrium value was reached for all soil organic C pools (e.g. Freibauer et al., 2004; Lovett et al., 2006). However, the recent results of Soussana et al. (2007) do not confirm this concept of carbon sink saturation for permanent semi-natural grasslands, which displayed net carbon storage. Their study indicated that extensively managed (i.e. semi-natural) but N-rich grasslands may store more carbon than highly intensive (i.e. newly sown grass-clover mixtures) grasslands.

Conversion of annual fodder or cereal crops into temporary or permanent grasslands or conversion of temporary grassland into permanent grassland means an increasing proportion of grass in the diet of animals, which often corresponds with an extensification of the livestock production at the farm level.

3.3.2 Grazing Management

Livestock Density

We will consider here the possible mitigation options induced by the management of the livestock density with a constant herd size, and not the limitation of emissions resulting from a reduction of the number of animals on the farm. Under continuous grazing systems, the effect of stocking rate on methane production per animal is not consistent. For instance, McCaughey et al. (1997) observed a decrease in absolute CH_4 emissions (g d^{-1}) and CH_4 yield (CH_4 energy loss as a percentage of gross energy intake, % of GEI) with increasing stocking rates of steers (1.1 versus 2.2 steer ha^{-1}), while Pinares-Patino et al. (2007) found only a reduction in the CH_4 yields (% GEI) and not in the absolute CH_4 emissions per

Table 5 Effects on greenhouse gas and ammonia emissions of mitigation options for grasslands

Mitigation options for grasslands		CH_4	N_2O	CO_2	NH_3
Land use	Permanent conversion from arable to grassland (to a less extent from temporary to permanent grassland)	–	–	↘	–
Grazing management	Increasing livestock density	↘ ? or 0?	↗ ?	↗ ?	–
	Selective grazing associated with grass maturity	↘ ?	–	–	–

animal with increasing stocking rates of heifers (1.1. versus 2.2 LU ha^{-1}). When pastures were rotationally grazed, stocking rates had no effect on CH_4 production (McCaughey et al., 1997).

Increasing livestock density seems not to be an efficient mitigation option, since the nitrogen surplus increases with increasing stocking density (Stockdale et al., 2001; Dalgaard et al., 2002). It is therefore likely to enhance the potential for N_2O losses from urine and dung excreted during grazing, especially since the increased trampling of grazing animals causes soil compaction and thus an increase in the number of anaerobic sites on the soil, which in turn may favour N_2O emissions (Oenema et al., 1997). The surplus nitrogen may also be lost by nitrate leaching: Eriksen et al. (1999) observed a tendency towards increased nitrate leaching losses with increasing stocking density. Moreover, modelling results from Soussana et al. (2004) indicated that the magnitude of the C sink of grazed grasslands declined with the mean annual stocking rate. On the other hand, a drastic reduction in livestock density is also not recommended, since Allard et al. (2007) showed that grassland management methods that abruptly reduced grazing pressure (1.2 to 0.6 LU ha^{-1} $year^{-1}$) and fertiliser input (80 to 0 kg N ha^{-1} $year^{-1}$) gradually reduced the C storage potential of the grassland.

Grazing System

There is limited information with regard to the effects of rotational grazing versus continuous grazing on CH_4 production. For steers, McCaughey et al. (1997) observed that at low stocking rates (1.1 steer ha^{-1}), CH_4 production (L ha^{-1} d^{-1}) was 9% lower for rotational grazing than continuous grazing.

Selective grazing which is associated with grass maturity is likely to limit CH_4 emissions. Pinares-Patino et al. (2003) showed that the grazing of grass at early heading induced a decrease of 10% in CH_4 emissions compared with the grazing of the same grass at a later stage of maturity when digestibility was lower. Nevertheless, CH_4 emissions are generally more determined by feed intake rather than feed digestibility (McCaughey et al., 1999; Pinares-Patino et al., 2007), an increase in feeding level inducing lower CH_4 losses as a fraction of gross energy intake (Boadi et al., 2004).

As EU 1804/99 regulation specifies that the rearing systems for herbivores are to be based on maximum use of "pasturage", we will not discuss mitigation options affecting the length of the grazing period. This maximum use of grasslands should, however, be managed in order to prevent adverse effects on soil (compaction) or vegetation (through overgrazing), which affect N_2O and CH_4 emissions (soil compaction) or nitrate leaching (scarce vegetation), as discussed previously.

In conclusion, Table 5 summarises the effects on GHG and NH_3 emissions of the different mitigation options discussed for grasslands. The main mitigation option consists of converting arable to grassland, especially permanent grasslands which allow C sequestration. Concerning grazing management, a higher livestock density may lead to a decrease in CH_4 emissions by the grazing animals, but to an increase in N_2O emissions from their excreta and to a decrease in the C stored by the grasslands. In the same way, selective grazing associated with lower stocking density allows a decrease in CH_4 emissions through an increasing digestibility of intake compared with a more complete use of grass.

3.4 Other Prospects

In this paragraph we give some leads on other types of mitigation options that may apply to organic mixed crop-dairy systems. Their effects on GHG and NH_3 emissions are summarised in Table 6. Hedgerows

Table 6 Effects on greenhouse gas and ammonia emissions of mitigation options for other prospects

Mitigation options		CH_4	N_2O	CO_2	NH_3
Arable field	Introducing hedgerows or tree belts	–	–	↘	↘
Farm scale	Increasing energy efficiency	–	–	↘	–
Crop	Producing plant oil	–	–	↘	–

or tree belts may be introduced in arable field margins to store carbon, and to recapture part of the NH_3 emitted into the air by the farm (Theobald et al., 2002). Increasing the energy efficiency is also a way to reduce CO_2 emissions. Diesel savings may be achieved by technological solutions (e.g. better mechanical efficiency of tractors, use of wind or solar energy instead of fossil fuel) or by improved farm machinery management (e.g. optimised field operations and work planning, see e.g. Dyer and Desjardins 2003; Couvreur, 2006). In the context of organic mixed crop-dairy systems, the production of biomass for energy (bioenergy crops) is not feasible on a large scale. However, the production of small quantities of plant oil that can be used as fuel and which also provide oil-seed cakes for livestock feeding (see e.g. Brunschwig and Lamy, 2006) is worth studying, though these crops should not be grown in preference to pastures.

4 Discussion

As discussed at the beginning of this article, to judge the appropriateness of mitigation options, it is essential to assess their impact on the carbon and nitrogen cycles at the whole-farm level. However, as highlighted in this review, it is far from easy because each mitigation option involves trade-offs between gases, and because there are often interactions and feedbacks among mitigation options. The choice of a set of mitigation options will therefore rely on the careful assessment of the balance between their beneficial and adverse effects. The establishment of this balance should consider not only the overall emissions of GHG and NH_3 resulting from the implementation of the chosen mitigation options (including fuel carbon costs for farm operations), but also their environmental and agronomic side effects. GHG and NH_3 mitigation measures often have accompanying benefits on other environmental components than global warming. For instance, C storage in soils has several unintended beneficial effects on soil fertility and water quality (see e.g. Lal, 2004a) and hedgerows used to store C in vegetation have many other valuable functions (see e.g. Marshall and Moonen, 2002) such as windbreaks or habitat for wild plants and animals. In addition to the consideration of environmental side effects, it is important to consider the effects of mitigation measures on animal welfare, which is another objective of organic farming. Animal welfare may be affected in several ways: e.g. through the feeding, housing conditions (type of bedding), presence of shelters (trees) on the pasture, length of the grazing period, etc. We will not consider in this article the economic and social aspects of the implementation of mitigation options (see e.g. Smith et al., 2007).

In this article we have examined the effects of mitigation options at the farm level. But to draw conclusions about their positive effects on global change, it seems important to consider also the emissions generated by the production and transport of materials needed to implement the mitigation measures (e.g. purchased concentrates, biogas plant). Besides, mitigation options also have to be devised for the transport of dairy farm products (exported from the farm). To reduce fuel consumption, an off-farm strategy consists, for instance, of moving to a more regionally-based economy, in which the producer and consumer are brought closer together (Johnson et al., 2007).

Another factor to take into consideration is the period of time needed to establish the balance between beneficial and adverse effects of mitigation options. GHG (especially N_2O) and NH_3 emissions vary greatly over time, particularly because of the influence of meteorological conditions on physicochemical and biological reactions governing them. The effectiveness of a mitigation measure will thus show strong year-to-year variations. Furthermore, the delay between the implementation of a mitigation measure and the reduction of the emissions will greatly vary between mitigation options. For instance, an improvement in the method of applying manure will have an immediate effect on NH_3 emissions, whereas a change in land management or in soil tillage affecting the soil properties (and thus soil carbon sequestration and

N_2O emissions) will probably involve a time scale of several years. Also, the kinetics of carbon accumulation following change in land use or grassland management are non-linear, i.e. they are more rapid during the early years after adopting a practice which enhances accumulation (INRA, 2002). The balance of a mitigation measure may thus change with time. For instance, as discussed above, Six et al. (2004) found that zero tillage leads to an increase in net global warming potential during the first 10 years, but to a strong decrease after 20 years of adoption in humid climates.

The soil and climatic context of each mixed crop-dairy system will be important for determining the most effective set of mitigation options. The soil characteristics, particularly its hydromorphy, will play an important role in the N_2O emissions and in soil organic matter mineralisation; it will also affect the best time for manure application or soil tillage (trafficability), and even the balance between grassland and arable areas. This balance may also determine the amount of straw in the manure but it is not clear whether slurry or farmyard manure will be more beneficial for global warming.

Decision support tools would be useful to assess the balance of different sets of mitigation options by taking into consideration trade-offs, interaction and feed-back among practices for different time scales and at the farm level, and by evaluating their impact upon environmental and agronomic components. Simulation models already exist on the dairy farm scale (reviewed by Schils et al., 2007; Table 7) but they still need improvements to accurately evaluate mitigation options from organic dairy farms. In particular, GHG emissions resulting from legume incorporation and from deep litter mats need further research to be accurately modelled. Simulation modelling, combining biophysical and decisional models, would also help farmers with their management decisions at both strategic and tactical levels.

Besides, the assessment of mitigation options (and the validation of models) is only possible if accurate measurements of the GHG and NH_3 emissions or of the carbon storage are available. Measuring greenhouse gas emissions for the different farm components, however, presents serious difficulties (see e.g. the review of McGinn, 2006), particularly because emissions are characterised by a high spatial and temporal variability. Measurements are necessary, not only to assess the efficiency of mitigation options but also to identify all the major sources of GHG emissions of mixed crop-dairy systems, some of which may currently be underestimated, such as, for instance, ditches or fresh heaps of maize silage (Hensen et al., 2006).

5 Conclusion

The most promising mitigation options are firstly measures that increase energy efficiency or fuel savings, because they are beneficial in any case. Secondly, techniques improving efficiency of N management at field and farm levels should also be promoted because they affect not only N_2O and NH_3 emissions, but also nitrate leaching. A better use of N at the farm level is, however, confronted by difficulties in organic mixed crop-dairy systems, either to optimise the total N intake of the livestock or to improve the N-use efficiency of crop and grass production. For instance, the management of the N content of the diet has to cope with the varying and unpredictable proportion of legumes in pastures, and the mineral N content of

Table 7 General characteristics of whole-farm greenhouse gas models (adapted from Schils et al., 2007)

	DairyWise	FarmGHG	$\text{SIMS}_{\text{DAIRY}}$	FarmSim
Authors	Schils et al. (2006)	Olesen et al. (2006)	Del Prado et al. (2006)	Saggar et al. (2004)
Model type	Empirical	Empirical	Semi-mechanistic	Semi-mechanistic
CH_4 and N_2O emissions	x	x	x	x
CO_2 emissions	x	x		x
C sequestration				x
NH_3 and NO_3 emissions	x	x	x	x
Pre-chain emissions	x	x		x
Animal welfare			x	
Biodiversity			x	
Soil quality			x	

the soil is difficult to control through application of manures and incorporation of crop residues. Thirdly, biogas production through anaerobic digestion of manure seems a promising and efficient way to mitigate GHG emissions, but the profitability of this expensive investment needs to be studied in the local context before its implementation. Last but not least, the way the farmer will implement the mitigation options, i.e. his practices, will be a determining factor in the reduction of GHG and NH_3 emissions. Some techniques such as composting may or may not be considered as a mitigation option, depending on the farmer's practices.

Options aiming to reduce GHG and NH_3 emissions result in numerous trade-offs between air pollutant emissions and nutrient flows that may occur in all the components of the farming system, and that may involve time scales of decades or even more. Before its implementation, a mitigation option should therefore be assessed in the context of the whole farming system or even larger by including pre- and post-chains, at least on the time scale of the crop rotation and if possible, by considering other issues than global warming, e.g. water quality, soil fertility, animal welfare, biodiversity, etc. The interactions and feedback that can take place among a set of mitigation options also have to be considered. The determination of the most effective set of mitigation options would thus benefit from the development of decision support tools based on dynamic models of the C and N cycles of the whole farming system.

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Water Deficit and Nitrogen Nutrition of Crops

Victoria Gonzalez-Dugo, Jean-Louis Durand, and François Gastal

Contents

Abstract Among the environmental factors that can be modified by farmers, water and nitrogen are the main ones controlling plant growth. Irrigation and fertilizer application overcome this effect, if adequately used. Agriculture thus consumes about 85% of the total fresh water used worldwide. While only 18% of the world's cultivated areas are devoted to irrigated agriculture, this total surface represents more than 45% of total agricultural production. These data highlight the importance of irrigated agriculture in a framework where the growing population demands greater food production. In addition, tighter water restrictions and competition with other sectors of society is increasing pressure to diminish the share of fresh water for irrigation, thus resulting in the decrease in water diverted for agriculture.The effect of water and nutrient application on yield has led to the overuse of these practices in the last decades. This misuse of irrigation and fertilizers is no longer sustainable, given the economic and environmental costs. Sustainable agriculture requires a correct balance between the agronomic, economic and environmental aspects of nutrient management. The major advances shown in this review are the following: (1) the measurement of the intensity of drought and N deficiency is a prerequisite for quantitative assessment of crop needs and management of both irrigation and fertilizer application. The N concentration of leaves exposed to direct irradiance allows both a reliable and high-resolution measurement of the status and the assessment of N nutrition at the plant level. (2) Two experiments on sunflower and on tall fescue are used to relate the changes in time and irrigation intensity to the crop N status, and to introduce the complex relationships between N demand and supply in crops. (3) Effects of water deficits on N demand are reviewed,

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_25,

from Gonzalez-Dugo et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2009059

pointing out the high sensitivity of N-rich organs versus the relative lesser sensitivity of organs that are poorer in N compounds. (4) The generally equal sensitivities of nitrifying and denitrifying microbes are likely to explain many conflicting results on the impact of water deficits on soil mineral N availability for crops. (5) The transpiration stream largely determines the availability of mineral N in the rhizosphere. This makes our poor estimate of root densities a major obstacle to any precise assessment of N availability in fertilized crops. (6) The mineral N fluxes in the xylem are generally reduced under water deficit and assimilation is generally known to be more sensitive to water scarcity. (7) High osmotic pressures are maintained during grain filling, which enables the plant to recycle large amounts of previously assimilated N. Its part in the total grain N yield is therefore generally higher under water deficits. (8) Most crop models currently used in agronomy use N and water efficiently but exhibit different views on their interaction.

Keywords Drought • Nitrogen nutrition status • Supply • Demand • Balance

1 Introduction

Water and N are the most limiting factors in agricultural production in most parts of the world, especially in arid and semiarid zones. Given current and future needs for high yields (Cassman, 2001; Sheehy, 2001), concerns are now being raised regarding the soil resources necessary to meet human needs. Traditionally, water and N have been overused in agriculture for decades, but this is no longer sustainable, considering the economic and environmental costs of these practices (Addiscott et al., 1991; Jury and Vaux, 2005). More than 55% of the increase in crop production, especially in emerging countries, comes from the use of chemical fertilizers, with N fertilizers being dominant (Li et al., 2009). However, N is also a significant pollutant that has a great impact on ecosystem deterioration (Galloway et al., 2008) and biodiversity (Stevens et al., 2004). Water scarcity, pollution and energy consumption are driving the maximization of water and N use in agriculture, in order to meet current and future demand while reducing resource requirements.

Climate change is an additional factor that increases uncertainty over the supply of resources (Asseng et al., 2009). Rainfall patterns are expected to change across the world (Giorgi and Bi, 2005), and for some regions, including most of Europe, droughts will be more frequent and severe (Easterling et al., 2000). This means that irrigation water will be more necessary than before to maintain production levels, and that crops will face water scarcity more often.

In situations where water availability cannot be assured, a plant can be subjected to a water deficit that may diminish growth. There are many physiological processes that are affected by water availability, mineral nutrition among others (Lemaire and Denoix, 1987; Onillon et al., 1995; Gonzalez-Dugo et al., 2005). As the major transport agent, and given its central role in biology, water determines the entire N biogeochemical cycle and, ultimately, its availability for plant production. The purpose of this work is to review the major advances in the assessment of the effect of water deficit on N nutrition.

The effect of water deficit on N nutrition has been the subject of considerable research at both plant (Morgan, 1984; Nicolas et al., 1985; Larsson, 1992; Matzner and Richards, 1996) and canopy (among others, Lemaire and Denoix, 1987; Cantero-Martinez et al., 1995; Onillon et al., 1995; Gonzalez-Dugo et al., 2005, Mistele and Schmidhalter, 2008) levels, but its involvement in a large number of interconnected metabolic processes makes it difficult to predict the effect of water deficit on plant N nutrition status. It is therefore necessary to consider the series of biophysical and chemical processes to which N is subjected, in different chemical forms and through transfers between living organisms and abiotic components in an ecosystem.

N_2 fixation in leguminous plants is also known to be affected by water scarcity. Symbiotic fixation will not be considered within this work. For more information, see Streeter (2003) and Thomas et al. (2004).

During its life cycle, a plant may be subjected to a water deficit, a N deficit or a combination of both, thus co-limiting (Sadras, 2005) its productivity. The effects of water and/or N deficit on plant production therefore depend on their timing and intensity (Bradford and Hsiao, 1982; Nielsen and Halvorson, 1991). Irrigation and fertilizer application increase yield when soil water and N levels are limited. Due to increasing water shortages at the global level and groundwater

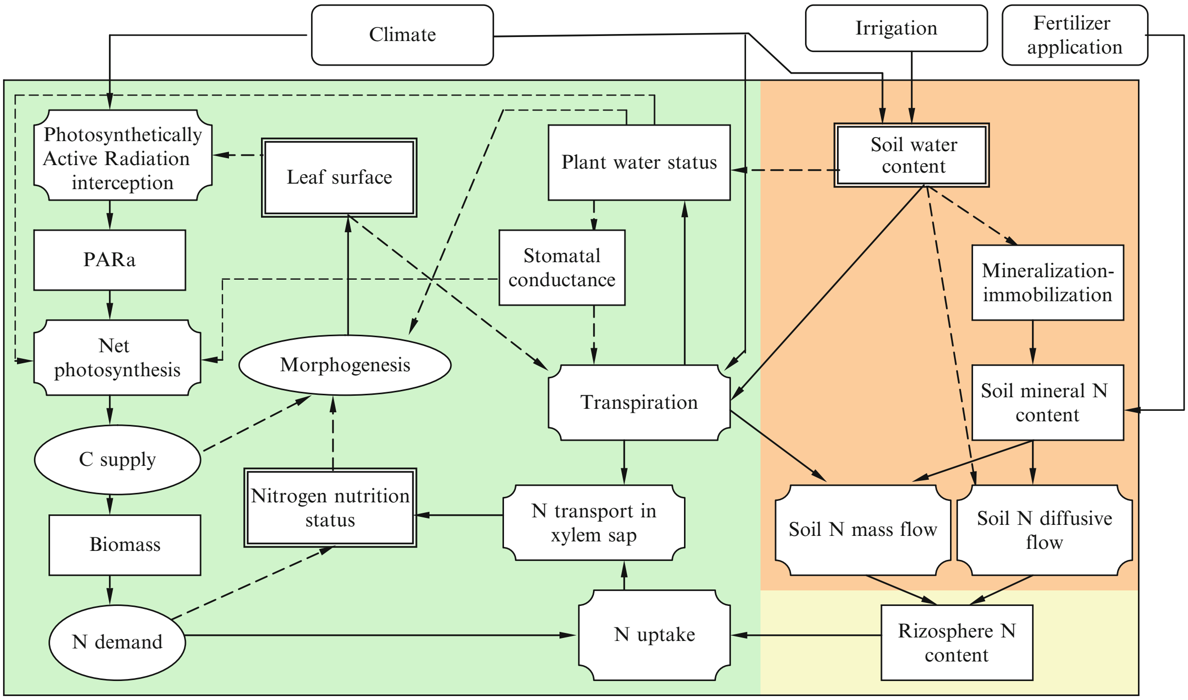

Fig. 1 Conceptual framework of the principal processes involved in the response of crop N nutrition to water deficit. Plain arrows indicate fluxes (mass or energy), while dotted arrows indicate direct causal relationships

pollution, while demand for food grows, it is essential to maximize the yield per unit water and N applied.

Crop N uptake has often been considered in relation either to soil availability (soil N supply approach) or to crop growth (N demand approach). It actually results from both (Gastal and Lemaire, 2002). Nitrogen absorption by crops is automatically reduced under dry conditions, even when mineral N is present in the soil colonized by roots (Gonzalez-Dugo et al., 2005). The absorption of N by roots requires the presence of water in the soil, as it is the agent that transports solutes to the soil-root interface (Garwood and Williams, 1967). Under water scarcity, N demand by plants is reduced, as growth rate is diminished. If the effect on N supply is greater than that on plant growth, the result will be a N deficiency. The implications of this demand/supply duality can be observed when different species exhibiting contrasted growth rates are compared under similar conditions (Gonzalez-Dugo et al., 2005). This deterioration of N nutrition also implies a reduction in growth in addition to water deficit–induced reduction. This series of direct and indirect (via N limitation) effects of water shortage on plant growth makes any quantification of the effect of water deficit on N nutrition a complex task. The correct assessment of nutrition status by indices and the use of physiology-based simulation models are therefore valuable tools for the quantitative assessment of direct and N-induced water effects on growth.

The influence of water deficit on N nutrition status is at play at the level of the soil (through the availability of mineral N for root uptake) as well as at plant level. However, it is difficult to ascribe a ranking to any of these levels in terms of their effects because water availability for transpiration, carbon supply and growth potential all determine N demand, assimilation and distribution within the plant. These complex relationships are summarized in Fig. 1, where the most important processes located in the soil and plant are shown. This review focuses on the principal processes affected. It first describes the main features of an operational definition of plant N nutrition status, in order to assess plant N demand correctly. It then analyzes the N biogeochemical cycle from the soil to its incorporation in the plant structure. Finally, some of the most used physiology-based simulation models are reviewed in order to analyze how the effect of water and N interaction on plant growth is modeled.

2 Quantification of Plant Water and Nitrogen Nutrition Status

2.1 Plant Water Status

The measurement of plant water status in terms of the water potential of leaves (ψ; MPa) directly generates a variable that is relevant to both water absorption and plant functioning, related specifically to the turgor pressure of cells (P; MPa):

$$\psi = \mathrm{P} - \pi \qquad (1)$$

where π is the osmotic pressure of leaf tissues (MPa). The absorption of water into the plant, and its transpiration, are directly proportional to the fall in ψ values throughout the *soil plant atmosphere continuum*. This approach (Van Der Honert, 1948) has been widely validated and has proved its ability to explain most changes in plant water status in the field, because the main factors involved in water movements are fundamentally physical (Dixon and Joly, 1895; Phillip, 1966; Wei et al., 1999; Tyree and Cochard, 2003). Furthermore, physiological variables relevant to the plant (π and P) are directly related to the ratio between the actual water volume of the plant and its maximum volume (at full turgor and $\psi = 0$). The plant water status is hence best measured in most cases by the mean leaf water potential.

One of the main limitations for the assessment of water status using leaf water potential is that it is very time-consuming. Remote sensing-derived techniques are opening new avenues in assessing plant nutritional status (Berni et al., 2009). Thermal and multispectral information acquired by sensors onboard satellite or airborne platforms provides a sound basis for developing indices to assess plant water status, such as the Crop Water Stress Index (Idso et al., 1981; Jackson et al., 1981), which is closely related to the canopy temperature and stomatal conductance (Berni et al., 2009). Other indices have been developed from spectral information. The Photochemical Reflectance Index (Gamon et al., 1992), which is related to the xanthophyll cycle, has demonstrated a strong relationship with water status and canopy temperature (Suarez et al., 2009). A major reason for the interest in these techniques is the possibility of remote measurement, which helps avoid time-consuming techniques, such as water potential or stomatal conductance, used for assessing water status at the field or farm level.

2.2 Crop and Plant Nitrogen Status

The assessment of plant N nutrition status is far more complex than that of plant water status, because both in-soil and in-plant biological processes directly interfere with N availability and uptake, and even under optimum conditions, N concentration in the plant diminishes continuously during crop development (Van Dobben, 1962; Angus and Mancur, 1985; Van Keulen and Seligman, 1987). The cause of this decline is linked firstly to N dilution within the plant volume. Plant N is mainly located in metabolically active aerial plant parts, and is thus related more directly to plant surface than to plant volume. As a plant grows, the proportion (in weight) of structural and storage tissues, which are relatively poor in N, increases. As a consequence, the average plant N concentration diminishes (Greenwood et al., 1990; Lemaire and Gastal, 1997). At the crop level, the relationship between plant N content and its surface also results from the close relationship between the shoot N concentration and the incident solar irradiance (Hardwick, 1987; Gastal and Lemaire, 2002). Within the canopy, leaves exposed directly to solar radiation have the highest N concentration, which optimizes N use relative to carbon assimilation (Hirose and Werger, 1987; Hikosaka et al., 1994). Three-quarters of total reduced N in the leaf may be connected with photosynthesis (Field and Mooney, 1986), mostly in the form of RuBP carboxylase and chlorophyll. In order to measure plant N nutrition status, several indices have been designed in recent decades. Overman et al.. (1995) developed a model where growth was related to fertilizer application. However, the actual amount of N available for the plant, including mineralized soil organic N, is not considered, making that approach difficult to extrapolate to different scenarios (Jeuffroy et al., 2002). In spite of their wide range of possible applications, indices considering soil N content only do not seem suited for assessing plant N status. Nitrogen concentration or uptake could not represent by itself the plant's N nutrition status, as it is directly related to plant biomass and growth rate (Lemaire and Gastal, 2009). In a controlled environment, it was shown that the relationship between N uptake (NO_3^- or NH_4^+) and the ionic concentration in soil solution followed a hyperbolic-type law with several kinetic phases (Rao and Rains, 1976; Macduff et al., 1989; Tischner, 2000; Glass et al., 2002). Devienne-Barret et al. (2000) showed that such relationships also held true in the field. Meanwhile, Lemaire and Meynard (1997) pointed out that the N available for plant uptake is not only defined by the amount of mineral N contained in the rooting zone, but also by soil conditions defining root growth and activity.

At the crop level, Lemaire and Salette (1984) studied the relationship between shoot N content (in %) and dry matter content in tall fescue and cocksfoot plots cut to a stubble height of 5 cm. They defined a "critical N concentration" as the minimal N concentration required to achieve a maximal growth rate. Using the data obtained at several N fertilizer application rates, they showed that the critical N concentration was a function of aboveground biomass, which was statistically fitted to an allometric function:

$$N_c = a \cdot W^{-b} \tag{2}$$

where N_c is the critical N concentration (%) in the dry matter yield (W, in T ha^{-1}), and a and b are two parameters (equal to 4.8 and 0.36, respectively) for a tall fescue crop. Similar values were found in a number of other crop species (e.g. wheat: Justes et al., 1994; Mistele and Schmidhalter, 2008; pea: Ney et al., 1997; rape: Colnenne et al., 1998; potato: Bélanger et al., 2001; maize and sorghum: Greenwood et al., 1990; maize: Plénet and Lemaire, 1999). In all these species, the main difference was clearly related to the two metabolic pathways, C3 and C4 (Greenwood et al., 1990). According to this, the Nitrogen Nutrition Index proposed (I_{NN}, Eq. (3)) is the ratio between the actual shoot N concentration (N_o) and the critical value given for its shoot dry weight (Lemaire and Gastal, 1997).

$$I_{NN} = \frac{N_o}{N_c} \tag{3}$$

where N is non-limiting for growth when the Nitrogen Nutrition Index is larger than 1. The N Nutrition Index is subject to considerable inertia as it requires a significant quantity of biomass and it is difficult to keep accurate records over time, as the surface area required to measure yield is relatively large. This is a major drawback when trying to link rapid changes in water status to crop N status. Another limitation when determining the Nitrogen Nutrition Index is that it only applies to crop level and does not provide a direct and accurate estimate of the N status of individual plants.

At the plant level, it will be necessary to distinguish between the N nutrition status of different elements (species, populations or individual plants with different water status) within a complex crop, as in intercropping systems (e.g. in vine-grass systems, Celette et al., 2005) or in forage production. A solution to this problem was recently proposed by Farruggia et al. (2004), who found a linear relationship between the Nitrogen Nutrition Index (I_{NN}) and the N concentration of leaves directly exposed to incident radiation (N_{sup}), i.e., lamina at the top of the canopy:

$$N_{sup} = 3 \times I_{NN} + 0.97. \tag{4}$$

Duru (2004) fully confirmed this relationship in cocksfoot. With this method, the Nitrogen Nutrition Index can be determined rapidly, and it allows separate analysis of the N status of species growing together on the same plot, provided they have some leaves in the top layer of the canopy. It also increases the possibility of assessing the N status of a crop in situations where its growth is very limited, as is often the case with water deficits. Other authors recently found a relationship between the Nitrogen Nutrition Index and the chlorophyll and polyphenol contents in wheat, which can be used as an indicator of the Nitrogen Nutrition Index (Cartelat et al., 2005; Rodriguez et al., 2009). Accurate tools are thus already available for the assessment of water and N nutrition status, which is a sine qua non condition for elucidating their interaction and its final effect on growth and development.

3 Effect of Water Deficit on Nitrogen Nutrition Dynamic

There is some evidence in the literature that shows how N status is altered by water deficit, both in the shorter and the longer term. Using the tabulated data generated by Alvarez de Toro (1987) during an irrigation experiment on sunflower, the relationship between shoot

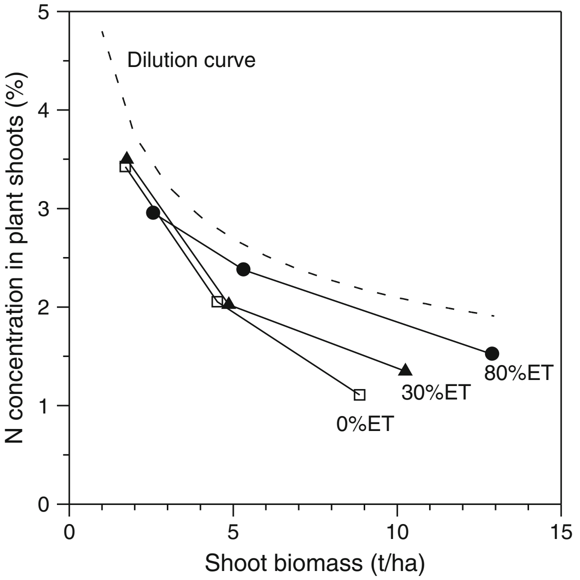

Fig. 2 Relationship between shoot biomass and N concentration during the pre-anthesis period under four water treatments, ranging from 0 to 80% of the fully irrigated level (FI), with a N application rate of 210 kg N·ha^{-1}. The dotted line corresponds to the dilution curve of equation (2) for tall fescue and cocksfoot (Lemaire and Salette, 1984). Based on tabulated data from Alvarez de Toro (1987)

growth and N content during the pre-anthesis period was established (Fig. 3). From this work, we have selected three watering treatments (applying 0, 30 and 80% of evapotranspiration requirements, ET) receiving 210 kg N/ha. Despite the lack of any published references concerning the suitability of the critical N curve in sunflower, this figure clearly shows that for the same quantity of N applied to the soil, and with lower shoot biomass, especially on the last measurement date, N nutrition status declined under drier treatments (0% ET and 30% ET). A similar response was found with less fertilizer application, but the differences between irrigation regimes were smaller (not shown).

In tall fescue, Onillon et al. (1995) analyzed the effect of drought on the relationship between shoot growth and shoot N content from which the Nitrogen Nutrition Index could be computed (Fig. 2). Dry treatments generally exhibited lower Nitrogen Nutrition Index values than irrigated treatments. This effect was more pronounced when the amount of available N was higher. The authors found that in most cases, rewatering and the recovery of crop water status increased N status to values that were similar to those measured on irrigated plots. Gonzalez-Dugo et al. (2005) showed the variability in the resulting N nutrition status (established by means of the Nitrogen Nutrition Index) in two forage species displaying contrasting growth rate and root architecture (and sown on the same soil) when water and N were both limited. Italian ryegrass had a slower growth rate with a high percentage of fine roots in upper layers, while tall fescue showed a higher growth rate, but with thicker and deeper roots. Tall fescue was found to be more sensitive to water shortage-induced N deficiency, as its growth rate was higher and its root system performed less well in N acquisition. The importance of biomass and plant N remobilization in the inertia of N nutrition status as well as of the root system distribution was highlighted. The recovery capacity of Italian ryegrass (determined as the rate of increase in the Nitrogen Nutrition Index during a rewatering period) was thrice that of tall fescue.

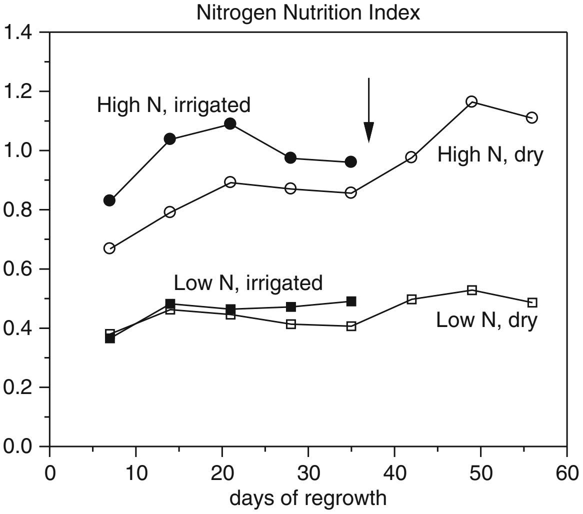

Fig. 3 Changes over time in the Nitrogen Nutrition Index during summer regrowth of a tall fescue sward. Four treatments were studied: high (circles) and low (squares) N applications. Black and white symbols show the irrigated and dry treatments, respectively (Onillon et al., 1995)

Nitrogen-use efficiency (biomass produced per unit of N absorbed), which is related to the final Nitrogen Nutrition Index obtained at harvest and which may reflect the integrated impact, is also affected by water shortage. In wheat, Fan and Li (2001) found that N-use efficiency was increased by water deficit and diminished by the dose of N applied. Pirmoradian et al. (2004) reached the same conclusions in rice, but the effect was only significant at high fertilizer application rates. In that case, and with a N application of 80 kg N · ha^{-1}, physiological efficiency (kg grain produced · kg^{-1} N removed) doubled when 50% more water was applied. Gajri et al. (1993) found a marked interaction between N and water for yield, and dependence of water-use efficiency on the N rate and N-use efficiency on the water supply. The effect of water deficit on plant N recovery capacity and on N-use efficiency was reviewed by Aulakh and Malhi (2005).

4 Effect of Water Deficit on Plant Nitrogen Demand

4.1 Growth of the Whole-Plant Biomass

It has been well established that water deficit reduces plant growth, primarily due to a reduction of the stomatal conductance that inhibits the C assimilation (Garwood et al., 1979; Bradford and Hsiao, 1982). Concerning yield, the stage at which drought occurs is critical; its effects are most pronounced if it takes place at an early stage. The first process affected by water deficit is foliar development and expansion (Fischer and Hagan, 1965; Bradford and Hsiao, 1982). As leaves are the vegetative organs that display higher N content during early developmental stages, N demand is also drastically reduced in early water deficits. Furthermore, during exponential growth, when the soil is not fully covered, drought restricts the active photosynthetic surface area, which causes a reduction in carbon assimilation and transpiration (Cowan, 1982; Durand et al., 1989) (see Fig. 1). When the leaf area index is below a value of approximately 3, the percentage of PAR absorbed to irradiated is below its potential maximum (Gosse et al., 1982; Durand et al., 1991; Akmal and Janssens, 2004). This also diminishes N needs (Nielsen and Halvorson, 1991), and may lead to a somewhat paradoxical situation where, for the same N uptake, an irrigated plant might experience N deficiency while a rainfed (and smaller) plant will maintain optimum nutrition status (Gonzalez-Dugo et al., 2005).

One source of complexity is that both N and water can affect leaf expansion and leaf photosynthesis simultaneously (Gastal and Saugier, 1989; Ghashghaie and Saugier, 1989, Durand et al., 1995; Jeuffroy et al., 2002) (Fig. 1), and their effects are intermingled. In tall fescue, experimental evidence suggested that water deficit did not alter the response of LER to N stress and vice versa (Durand, 1994). Under normal field conditions, such a reduction in photosynthesis is primarily a consequence of stomatal closure (Ghashghaie and Saugier, 1989). In the longer term, water deficit also induces N deficiency, that may further limit photosynthesis (Morgan, 1984; Lawlor et al., 1987; Ciompi et al., 1996; Arora et al., 2001). Photosynthetic rate is largely determined by the presence of RuBP carboxylase and chlorophyll content, both linearly related to leaf N content (Evans, 1989). Furthermore, Radin and Parker (1979) studying cotton plants and Broadley et al. (2001) studying lettuce demonstrated that soil N deficiency increased the sensitivity of stomata to water deficit, inducing lower leaf water potentials in a high transpiration regime, as measured in tall fescue (Onillon et al., 1995). Jacob et al. (1995) stated that the most important effect of N deficiency on plant water conductance occurs at the mesophyll level.

4.2 Root Shoot Ratio and Nitrogen Demand

The root/shoot ratio tends to increase with drought, largely due to a stronger effect of water deficit on shoot growth than on root growth (Sharp et al., 1988; Durand et al., 1989; Saab et al., 1990; Engels et al., 1994). In some cases, the root system may even continue to expand at very low water potential, whereas aboveground growth is completely halted (Wu et al., 1996). Because roots contain much lower concentrations of N than aboveground biomass, this change in the allocation pathway diminishes N needs. Measurements on roots are prone to major errors in the field (Pierret et al., 2005). Root mass changes under water deficit may be overestimated because of the restriction of root fragmentation and destruction by soil organisms, which depend on soil water content. However, some authors have found that the root growth of rice increased under water deficit (O'Toole, 1982; Ingram et al., 1994). An increase in root depth penetration has also been reported in rice (Mambani and Lal, 1983). By contrast, Yamauchi et al. (1996) observed a reduction in root growth as a result of drought. Engels et al. (1994) found that drought reduced root growth near the soil surface but enhanced it at lower levels. Onillon (1993) stated that the effect of water deficit on root dry weight was dependent on N fertilizer application. In any case, such positive effects on root growth may not necessarily ensure improved conditions for N nutrition because newly colonized root zones are often deeper than N-rich topsoil horizons.

5 Effect of Water Deficit on Nitrogen Supply

5.1 Effect on Soil N Transformation Processes

Nitrogen transformation processes closely depend on water and its mobility in the soil. Among all the factors affecting soil nutrition, storage capacity and accessibility, soil texture, root depth and organic matter concentration deserve particular attention (Keller, 2005).

The size of soil NO_3^- and NH_4^+ pools reflects the balance between various fluxes which result from several soil processes (Fig. 4): plant N uptake, mineralization, immobilization, nitrification and losses to the atmosphere following denitrification and volatilization (Scholes et al., 1997, Lemaire et al., 2004). Mineralization, immobilization and nitrification are affected by temperature and soil water content (Pastor and Post, 1985; Gorissen et al., 2004; Lemaire et al., 2004). Smolander et al. (2005) showed that a drought lasting less than two months was not sufficient to destroy microbial biomass; its activity was diminished but recovered completely soon after rewetting. Although sensitive to water deficits, N mineralization by soil micro-organisms may indeed be more resistant than is sometimes suggested, partly because immobilization could be more sensitive to water deficits (Valé et al., 2007).

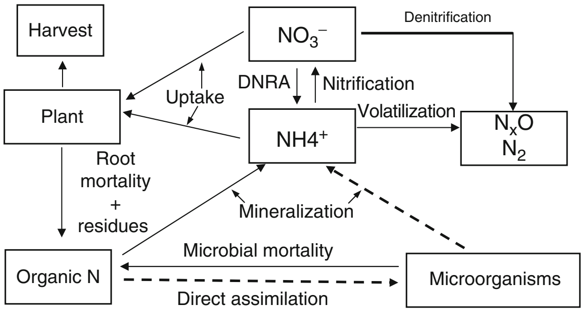

Fig. 4 N cycle in the soil-plant-atmosphere system. Each process can be altered by the soil water regime. Dashed arrows show the processes that comprise net mineralization (After Lemaire et al., 2004)

The response of microbial biomass, nitrification and mineralization during dry periods has been studied in various types of vegetation (Mazzarino et al., 1998 in the Patagonian steppe; Pulleman and Tietema, 1999 in *Pseudotsuga mensiezzii*; Schimel et al., 1999 in the Alaskan taiga; Fierer and Schimel, 2002 in perennial oak and *Bromus*; Kelliher et al., 2004 in pine forest, Smolander et al., 2005 in a Norwegian Picea forest). Westerman and Tucker (1978) showed that during a wet period, the soil mineral N content decreased because of the increase in immobilization by microorganisms and losses to the atmosphere. Rain pulses can thus enhance microbial activity without exerting any significant effect on plant growth and hence on N demand by plants (White et al., 2004). During dry periods, root death increases (Huang and Gao, 2000); the soil organic labile N pool may do so as well (White et al., 2004). Austin et al. (2004) demonstrated that mineralization was activated by a water pulse after a drought period. However, following a dry period, during which root and bacterial death increased the amount of soil organic matter, both the immobilization rate and nitrification increased after soil rewetting (Smolander et al., 2005). No general effect of drought on long-term soil mineral N availability can thus be expected from such a complex network. Furthermore, because of the large error inherent in the measurements of root compartments, and given the importance of the disturbances caused by drought in cropping systems, this topic continues to be a major area for research.

5.2 Soil Fluxes: Transport of Nitrogen to the Roots, Mass Flow and Diffusion

Luxmoore and Millington (1971) indicated that the uptake of N and water may not occur throughout the entire root system. Using a system to inject nutrients at controlled depths in soil, the experiments carried out by Garwood and Williams (1967) proved that, as the soil became drier, N could be removed by a grass crop only when it was made available in deeper, moist soil horizons. Although other mechanisms might have been involved, their experiment was pioneering in suggesting that water flow influenced the local availability of N to roots. Indeed, insofar as N is dissolved in the soil solution, N uptake clearly depends on (i) water flows from the soil to the root system (Keller, 2005), and distribution of N and roots within the soil profile, and (ii) ion diffusion fluxes in the rhizosphere.

Water in the soil solution is loaded with anions, cations and soluble organic molecules, transported to the roots by the stream produced by the transpiration demand, creating convective flow or "mass flow" (Fig. 1). Thus, the amount of N solutes reaching the root surface is dependent on the water flux and the N concentration of the soil solution. Any trait in the soil or root system that alters water extraction from the soil will thus directly determine the associated flow of ions to the root surface. Kovacs (2005) demonstrated the importance of mass flow to the simulation of plant N uptake, and its effect on maize production.

Because root N uptake is an active process, if the plant N uptake capacity is higher than the amount of N reaching the root, a N concentration gradient can be built up in the rhizosphere, with lower concentrations at the root surface (Porporato et al., 2003). This causes a diffusive flow, expressed as follows:

$$J = -D \cdot A \cdot \nabla c \tag{5}$$

where J ($mol{\cdot}s^{-1}$) is the flux, and ∇c ($mol{\cdot}cm^{-3}{\cdot}cm^{-1}$) the concentration gradient, D ($cm^2\ s^{-1}$) the diffusion coefficient and A (cm^2) the area for diffusion. As already noted, measurements of A are known to display large errors (Pierret et al., 2005). A is generally estimated after root length density. The D value also (empirically) reflects the reduction in the diffusion rate due to chemical reactions, and the tortuousness of the trajectory, the latter being related to the spatial distribution of water-filled soil pores (Barber, 1974). Diffusion is thus strongly dependent on soil properties and humidity. It is also sensitive to dispersion, which depends on the shape and orientation of soil pores. This is generally expressed using an additional dispersion coefficient. In order to simulate the diffusive flow, the root can be considered as a cylinder of a given radius surrounded by a concentric soil volume (Gardner, 1960). Diffusion to a single root depends, in the first instance, on the radius of the concentric soil cylinder that is explored by the root, which is a function of the distance to the next root. This can be related to the root length density (Van Keulen, 1981; Klepper and Rickman, 1990).

When water flow is weak, or if the solution concentration is low, diffusion increases in relation to mass flow (Passioura, 1963; Raynaud, 2004). This is thus the case in low fertility media (Williams and Yanai, 1996) or with low water availability. Using a theoretical model (de Wit and van Keulen, 1972), Van Keulen (1981) performed a series of simulations to compare the effects of mass flow rate, dispersion and root density on anion extraction from the soil. He showed that the anion extraction rate increased markedly when root density was doubled or when dispersion was set at nil.

The theoretical framework was hence well established by all these authors. However, the leap to accurate determination of N flow under field conditions is difficult due to our limited knowledge of functional root architecture (Pierret et al., 2005). However, there have been some promising advances in the analysis of root architecture (Doussan et al., 2003; Pierret et al., 2007).

5.3 Plant Physiological Processes Involved in the Definition of Nitrogen Supply

To date, simulation models and experiments performed in nutrient solutions with labeled N have been the only techniques available to analyze N uptake independently of soil water dynamics. Caution should thus be adopted when drawing any quantitative conclusions using these approaches. There is, however, evidence of the direct effects of water deficit on N uptake. Nitrogen uptake is an active process. Based on an analysis of root respiration, Bloom et al. (1992) showed that the amount of energy required to absorb 1 mol NO_3^- was equivalent to 1 to 2 adenosine triphosphates (ATP), or 0.16 to 0.32 mol CO_2. As for NH_4^+, Bloom et al. (1992) estimated that 0.33 mol CO_2 were needed to absorb and assimilate one mol of NH_4^+. An independent assessment of nitrate influx and efflux has been possible since the 1980s through the use of stable isotope labeling. Several transport systems for nitrate uptake have been found, operating at two different levels of soil concentration (Siddiqi et al., 1990) and with different affinities for nitrate. The high-affinity transport system is a low-concentration, saturable system. It reflects a strong ability for nitrate uptake at low concentrations in the soil solution, and it saturates between 0.2 and 0.5 mmol.m^{-3} [NO_3^-]. This high-affinity system is subject to negative feedback regulation by root NO_3^- concentrations or certain products of NO_3^- assimilation (Siddiqi et al., 1990). The low-affinity and nonsaturable transport system operates exclusively when the soil ion concentration is high, which may be a frequent occurrence under water deficit. This system has a linear relationship with soil nitrate concentrations and is only subject to negative feedback regulation after prolonged exposure to NO_3^- (Siddiqi et al., 1990). It may be related to a passive movement through specific NO_3^- channels, following an electrochemical potential gradient. Ammonium transport systems are similar to those of nitrate, as they also involve a low-capacity, high-affinity system and a passive transport system with high capacity and low affinity.

Studies of the effects of plant-soil water relationships on root N uptake have produced contrasting results (BassiriRad et al., 1999). Nitrogen uptake can be considered as the minimum between N supply from soil and N demanded for growth. Some authors concluded that N uptake was independent of transpiration (Grubb, 1977; Schulze and Bloom, 1984; Gastal and Saugier, 1989; Hopmans and Bristow, 2002). Experiments analyzing the effect of the transpiration stream on N uptake during diurnal cycles reached similar conclusions, i.e., N uptake was found to be independent of transpiration (Triboi-Blondel, 1979; Bhat, 1982). During experiments carried out in nutrient solution with polyethylene glycol as an osmotic agent, Talouizite and Champigny (1988) indeed found an increase in nitrate uptake with short-term water deficit. Using ^{15}N-labeling, Matzner and Richards (1996) demonstrated that root capacity for N uptake diminished with a mild water deficit in *Artemisia tridentata*, but no further decrease was found when the drought stress was more severe. They stated that variations in the net effect of water deficit on N uptake might be correlated with the differing sensitivity of each transport system to water status. Buljovcic and Engels (2001) found a similar response in maize. In their experiment, the nitrate uptake capacity of excised maize roots fell to about 20% only when the soil water content decreased to 5% (w/w), corresponding to a soil water potential of about –3 MPa. During the same experiment, N uptake capacity was fully recovered after rewatering. These authors concluded that nutrient uptake from dry soil was primarily regulated by nutrient transport in the soil to the root surface. Using split-root experiments with polyethylene glycol and isotopic labeling, Larsson (1992) showed that N uptake in wheat was strikingly reduced with osmotic stress. This effect seemed to be more closely related to the water status of the shoot than to the root osmotic environment, which may have been the reason for the different results of all these experiments. N accumulation in the roots may also cause the inhibition of nitrate uptake (Larsson, 1992; Gonzalez-Dugo, 2006).

Most of the physiological studies concerning the N uptake rate during water shortage periods have been done under controlled environments. Diouf et al. (2004) analyzed the effect of water deficit cycles on N uptake and N assimilation enzymes in the field by means of a plant N balance. There have been some attempts to assess the N uptake and allocation in the field, generally through the use of ^{15}N (Malagoli et al., 2005). However, the uncertainty about the size of the actual soil N pool due to N transformations under field conditions makes it difficult to make a quantitative assessment of N uptake by the plant, although it is a valuable tool for the analysis of the fertilizer recovery and the allocation pattern during plant ontogeny.

Thus, the processes that directly control N uptake under water deficit still need to be established, both at the molecular level (effect on transport system) and at the level of the whole plant.

6 Response of Crop Physiological Functions Involved in Nitrogen Nutrition to Water Deficit

6.1 Nitrogen Assimilation in Plant Tissues

Depending on the species, the assimilation of mineral N takes place in roots or shoots (Andrews, 1986), both exhibiting different energy costs. Gojon et al. (1994) suggested that the site of assimilation depended on the plant growth rate, with slow-growing species mainly assimilating via their roots, while fast-growing species assimilate nitrate in leaves. However, within a sample of eight herbaceous species exhibiting contrasted growth and assimilation rates, Scheurwater et al. (2002) demonstrated that assimilation systematically occurred in the leaves, irrespective of their growth rate.

The site of nitrate reduction may have an important effect on a plant's carbon budget. Plants that reduce nitrate in leaves could use the excess reducing power from photosynthesis, while those that reduce nitrate mainly in roots should obtain their reducing power from glycolysis and the oxidative pentose phosphate pathway (Scheurwater et al., 2002). As the energy cost of reducing nitrate in leaves is smaller than that in roots (Raven, 1985), in water deficit situations, plants that reduce nitrate in leaves are more efficient than those where the reduction takes place in roots.

Lawlor and Cornic (2002) stated that a water deficit could have a more marked effect on N assimilation than on the uptake process, as nitrate reductase activity diminished sharply, albeit in a reversible fashion, in line with low relative water content values. Other authors have also pointed out the particular sensitivity of nitrate reductase activity (Triboi-Blondel, 1978; Larsson et al., 1989; Larsson, 1992; Azedo-Silva et al., 2004; Correia et al., 2005).

6.2 Effect on Nitrogen Movement Through the Xylem

Dynamic variations in xylem sap composition result from differences in the transpiration rate and plant-internal nutrient relationships, which control the production of N compounds present in the xylem (Herdel et al., 2001; Peuke et al., 2001). Water shortage may thus have two different and opposite consequences regarding N flow. In the field, Bahrun et al. (2002) demonstrated that nitrate concentration in xylem sap in non-irrigated maize plants fell by more than 50% compared with irrigated control plants. Total nitrate flow was not measured in that experiment, but given the reduction in nitrate concentration and the decrease in stomatal conductance as a consequence of drought, it could be concluded that the net delivery of N to shoots was diminished. It is worth noting that in such a field experiment, the effect of water deficit on nitrate concentration is the result of cumulative effects on soil nitrate flow, nitrate uptake and the ratio between the decrease in nitrate and water uptake. The relationship between xylem flow and composition appears to be even more complex. It has been established that an increase in the solute concentration in xylem sap can reduce xylem hydraulic resistance (Zwieniecki et al., 2001). This would mean that for a given transpiration demand, water flow in the xylem may be enhanced if the solute concentration is increased. This is certainly valid when water availability is not limiting, but if plants are subjected to a water deficit and if an increase in solute concentration was the only consequence of a reduction in solvent volume, xylem sap flow would be diminished. In experiments performed in wheat, Larsson et al. (1989) showed that an increase in the osmotic pressure of nutrient solution increased xylem N concentration, although this increase did not compensate for the reduction in the transpiration rate. As a consequence, the delivery of nitrate to shoots decreased. By contrast, Nicolas et al. (1985) found in rain-fed wheat plants that the increase in N concentration in xylem sap compensated for the lower sap flux and that the amount of N reaching the shoots was maintained.

In sunflower plants grown under controlled conditions and in sandy loam-filled pots, Schurr and Schulze (1996) found that water deficit had no effect on xylem nitrate concentration. Indeed, under similar conditions, these levels even fell as the soil water content declined (Gollan et al., 1992). In that case, a restriction of N flow in the soil may have been involved. In another experiment in a controlled environment where transpiration and the nutrient supply were dissociated, Tanner and Beevers (2001) concluded that nutrient transport from roots to shoots was independent of transpiration. The two transpiration-independent water flows, i.e., the flux of water associated with volume expansion and Münch's counterflow in the phloem were found to ensure nutrient transport throughout the plant.

6.3 Remobilization of Nitrogen to Grains

For grain crops, N remobilization must also be taken into account, as most of the N that is present in grain has been taken up during the pre-anthesis period and thereafter remobilized from reserves or other organs. Grain number is set during flowering, so any limitation of crop growth rate during this period decreases grain number and yield in maize (Uhart and Andrade, 1995). Once grain number, and hence potential production, is established in the period from planting to seed set, carbon assimilation during seed filling and translocation of assimilates from reserves and senescing organs will set the final yield (Schnyder, 1993).

Nitrogen assimilated during the pre-anthesis period is the main source of nitrogenous compounds for grain filling; the contribution of reserves to the final grain N ranged from 63 to 100% in wheat, 11 to 100% in soybean, 49 to 64% in sorghum and 41 to 69% in maize (Egli, 2004; Barbottin et al., 2005), depending on their capacity to store large amounts of C and N compounds in their vegetative organs before anthesis. The amount of N remobilized depends on the amount of N available and on the N remobilization efficiency. The N remobilization efficiency is high in situations of low N supply or low N availability (Barbottin et al., 2005).

Water deficit hastens leaf senescence (Palta et al., 1994); maintenance of green leaf area is therefore essential to grain filling and yield under drought. This should also alter the C flow to roots in order to maintain N absorption (Dreccer, 2005). Palta et al. (1994) showed that N remobilization efficiency was high in Mediterranean-like conditions, where plants are subjected to drought stress during the grain-filling period.

7 Concepts Required for Simulation Models

Conceptual frameworks similar to that represented in Fig. 1 have been converted into a series of quantitative equations linked together to build crop models. Those simulation models are valuable tools to gather the knowledge acquired by experimentation. Mechanistic models enable the synthesis of many experimental results in order to achieve a global knowledge of plant function. They also allow the extrapolation of knowledge to different and future scenarios, a major requirement in the context of a continuously changing climate. Given the complexity of the interrelated processes considered here, models are relevant tools to discriminate between water- and N-related reductions in crop growth and development.

There is a large number of simulation models, appropriate for very different scales. Only some of the most used crop simulation models operating on a daily time step will be considered here to illustrate the different approaches to the effect of water and N interactions on crop growth and development.

In modeling, deficits are generally defined by means of reducing indices that go from 0 to 1. The definition of these indices results from empirical equations relating state variables that are affected by the deficit. Each model thus exhibits a choice of significant state variables and equations defining the indices.

In the models considered here, the first effect of soil or plant water status on N nutrition is found at the soil level (Stockle et al., 1994; O'Leary and Connor, 1996; Van Ittersum et al., 2003; Singh et al., 2008). The amount of N that is available for plant uptake is calculated using the layers' water content and their contribution to total transpiration (Van Ittersum et al., 2003).

For most models, more than one index related to a deficit are used for different processes with a contrasted sensitivity to constraints. CROPSIM-WHEAT, CROPSYST and the model developed by O'Leary and Connor define the water deficit using the ratio between actual and potential transpiration (Hunt and Pararajasingham, 1995; Stockle et al., 1994; O'Leary and Connor, 1996). CERES-Maize and AFRCWHEAT2 determine water deficit using a soil water balance (Jones and Kiriny, 1986; Porter, 1993). STICS considers the water available to roots above the permanent wilting point (Brisson et al., 2009).

Indices defining N deficit are generally calculated according to actual, minimum and maximum N content, in leaves, as in CROPSIM-WHEAT (Hunt and Pararajasingham, 1995) or in the aboveground biomass, as in AFRCWHEAT2 (Porter, 1993). STICS uses the Nitrogen Nutrition Index to assess N deficit (Brisson et al., 2009).

In general, biomass accumulation and leaf area formation are the processes that are typically noted as the most sensitive to these stresses. Some models use the most limiting factor, and therefore, the minimum between water and N stress indicator is used, underestimating the effect of the second stress. Other models consider that both processes interact in growth and development. Hence, both indices are considered. A summary of the most common models used, and how they account for water and N interaction in biomass development and leaf area formation, is given in Table 1. Singh et al. (2008) demonstrated that CropSyst was better at predicting the influence of water and N interaction on biomass and yield formation, compared with CERES-Wheat.

The relative difference between the two approaches depends on the values of both indices. Let us consider

Table 1 Revision of some daily time-step models and their interpretation of the effect of water and nitrogen interaction on biomass accumulation and leaf area formation

Model	References	Biomass accumulation	LAI formation
Afrcwheat2	Porter, 1993	Water	Min (N, Water)
CERES-Maize	Jones and Kiriny, 1986	Min (N, Water)	Water
CropSim Wheat	Hunt and Pararajasingham, 1995	Min (N, Water)	Min (N, Water)
CropSyst	Stockle et al., 2003	Min (N, Water)	Water
Soygro	Sinclair, 1986	N*Water	N and water
STICS	Brisson et al., 2003	N*Water	Min (N, Water)
O'Leary and Connor	O'Leary and Connor, 1996	N*Water	N*Water

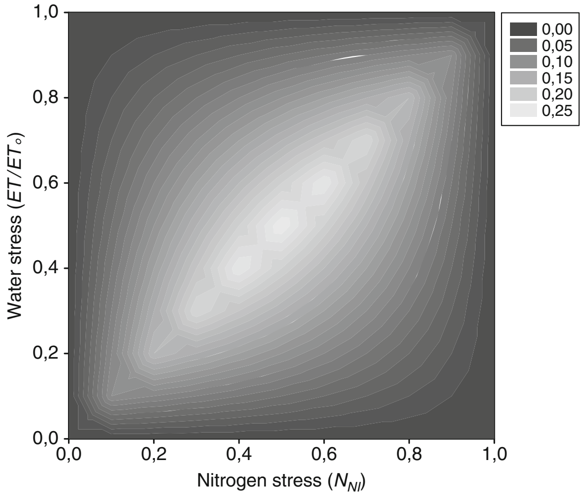

Fig. 5 Difference (expressed by grayscale) between the minimum of water and nitrogen stress indices and the multiplication of both indices [Min (Water, N) – Water*N] in the space defined by both indices

the result of the difference ε between the minimum of these indices and their multiplication:

$$\varepsilon = \mathrm{Min}\left(\mathrm{w_{ater}}, \mathrm{n_{itrogen}}\right) - \mathrm{w_{ater}}^{*}\mathrm{n_{itrogen}}$$

where $\mathrm{w_{ater}}$ and $\mathrm{n_{itrogen}}$ are stress factors of water and N, respectively. ε is largest when both indices are close to 0.5 (Fig. 5). When one of these indices is close to 0 (very limiting factor), the relative weight of this index becomes important and hence the effect of the second factor is weak. On the contrary, when one index is close to 1, the main effect on the variable considered will be mainly ascribed to the second factor. The maximal difference occurs in the intermediary region, i.e. when both indices are close to 0.5. Under natural conditions and because of its intrinsic inertia, N stress could be maintained close to this value. But in dry seasons, drought stress often remains at low values, reaching optimal conditions only following rainfalls. It is therefore maintained within these values for short periods of time only. As a result, the difference in the simulation results obtained using one or the other approach rarely appears significant.

8 Conclusion

The analysis of the effect of water deficit on N nutrition is complex and requires a multiscale approach, from the membrane to the crop, and different media and their interfaces. The first and most important need is to separate growth-induced changes from the direct effects of water deficits. The main effect of water restriction is certainly a reduction in N demand due to the marked sensitivity of leaf area expansion. Nitrogen nutrition indices can reveal the processes that alter plant nutrition independently of its actual demand. Process-based crop growth simulation models must currently incorporate these concepts, either implicitly or explicitly. It is noteworthy, however, that they still do not take similar account of the interaction between N and water stress, thus reflecting the ongoing discussion concerning co-limitation analysis in crops. Indeed, some aspects remain poorly understood, especially concerning the establishment of the supply of N to meet plant needs.

Two areas in particular deserve further investigative efforts. Firstly, our lack of knowledge of the true extension of active root surface area is strongly detrimental to any attempt to clarify the mechanisms of N absorption. Secondly, our inability to determine the mineral N absorption rate in the field still remains a major obstacle. To clarify these points, and render fertilizer application methods more precise, greater efforts should be made to achieve a methodological breakthrough in these two areas.

Acknowledgements The authors would like to thank Dr. Elías Fereres Castiel for his collaboration in the compilation of this paper. We also thank Dr. Jorge Alvarez de Toro. The anonymous reviewers are also acknowledged for their helpful comments that have improved this work. This research was supported by INRA, the Poitou-Charentes Region and the CNRS "ECosphère COntinentale" program, N#03CV114.

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Validation of Biophysical Models: Issues and Methodologies

Gianni Bellocchi, Mike Rivington, Marcello Donatelli, and Keith Matthews

Contents

Abstract The potential of mathematical models is widely acknowledged for examining components and interactions of natural systems, estimating the changes and uncertainties on outcomes, and fostering communication between scientists with different backgrounds and between scientists, managers and the community. For favourable reception of models, a systematic accrual of a good knowledge base is crucial for both science and decision-making. As the roles of models grow in importance, there is an increase in the need for appropriate methods with which to test their quality and performance. For biophysical models, the heterogeneity of data and the range of factors influencing usefulness of their outputs often make it difficult for full analysis and assessment. As a result, modelling studies in the domain of natural sciences often lack elements of good modelling practice related to model validation, that is correspondence of models to its intended purpose. Here we review validation issues and methods currently available for assessing the quality of biophysical models. The review covers issues of validation purpose, the robustness of model results, data quality, model prediction and model complexity. The importance of assessing input data quality and interpretation of phenomena is also addressed. Details are then provided on the range of measures commonly used for validation. Requirements for a methodology for assessment during the entire model-cycle are synthesised. Examples are used from a variety of modelling studies which mainly include agronomic modelling, e.g. crop growth and development, climatic modelling, e.g. climate scenarios, and hydrological modelling, e.g. soil hydrology, but the principles are essentially applicable to any area. It is shown that conducting detailed validation requires multi-faceted knowledge, and poses

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_26,

from Bellocchi et al., Agron. Sustain. Dev. 30 (2010) 109–130. DOI: 10.1051/agro:2009001

substantial scientific and technical challenges. Special emphasis is placed on using combined multiple statistics to expand our horizons in validation whilst also tailoring the validation requirements to the specific objectives of the application.

Keywords Accuracy • Modelling • Multiple statistics • Validation

Abbreviations

AIC	Akaike's information criterion
BIC	Bayesian information criterion
CD	Coefficient of determination
C_p	Mallows' statistic
CRM	Coefficient of residual mass
d	Willmott's index of agreement
D	Kolmogorov-Smirnov's statistic
E	Mean relative error
EF	Modelling efficiency
EF1	Modified modelling efficiency
Fa_2	Factor of two
FB	Fractional bias
E_f	Fractional gross error
LC	Lack of correlation
LCS	Lack of positive correlation weighted by the standard deviations
LOFIT	Lack of statistical fit
MAE	Mean absolute error
MaxE	Maximum error
MB	Mean bias
MBE	Mean bias error
MdAE	Median absolute error
MG	Geometric mean bias
MSE	Mean square error
NMSE	Normalized mean square error
NU	Non-unity slope
PI	Range-based pattern Index
PI-F	F-based pattern index
PI_{doy}	Range-based pattern index versus day of year
PI_{Tmin}	Range-based pattern index versus minimum air temperature
$P(t)$	Student's t-test probability
r	Pearson's correlation coefficient
r^2	Least-square regression coefficient of determination
REF	Relative modelling efficiency
RMA	Reduced major axis
RMdAE	Relative median absolute error
RMSE	Root mean square error
ROC	Receiver-operator characteristic curve
RRMSE	Relative root mean square error
RMSV	Root mean square variation
SB	Simulation bias
SDSD	Square differences of the standard deviation
U	Theil's inequality coefficient
U_B	Systematic error proportion of Theil's inequality coefficient
U_S	Variance proportion of Theil's inequality coefficient
U_C	Covariance proportion of Theil's inequality coefficient
VG	Geometric mean variance

1 Introduction

The mathematical modelling of natural processes has undergone a large development during the last decades and, due to the complexity of the processes involved, this development is expected to pursue for a long time. The development of quantitative models to support the description of natural and semi-natural systems and decision-making in natural resource management is indeed considered to be of high priority. This is because models have a multitude of uses for scientists, managers and policy-makers investigating and governing natural processes. A major strength of models is in exploring interactions and feedback (e.g. Wainwright and Mulligan, 2004), helping to identify uncertainties and areas were we lack knowledge. They are also important supportive tools for the communication of complex issues to stakeholders of a non-scientific background. It is therefore important to demonstrate that a model has been tested using the most appropriate methods in order to achieve credibility with users of the estimates the model makes.

A mathematical model is, by definition, an approximate reconstruction of actual phenomena and an integration of natural processes into mathematical formulae. For agricultural and ecological systems and resource use (climate, land, vegetation…), a multitude of different theories coexist, not only amongst disciplines (plant physiology, hydrology, climatology…), but also within disciplines (Argent, 2004; Beven, 2007; Arnold et al., 2008). Ecological, soil, meteorological and hydrological conditions in the actual systems are indeed the product of multiple concurrent processes, where multiple factors interact at different scales, each examined by different disciplines (Parker et al., 2003). This produces an abundance of theories and alternative explanations and, consequently, alternative models. One of the challenges is the process of bringing data and models together. It is required that numerical models should be preceded by thorough evaluation before use in practical applications, because the approximations used for the synthesis of a model often lead to discrepancies and deviations of the model results from nature.

Model evaluation is an essential step in the modelling process because it indicates if the implementation of the calculations involved reproduces the conceptual model of the system to be simulated (model reliability) and the level of accuracy of the model in reproducing the actual system (model usefulness) (Huth and Holzworth, 2005). Model evaluation includes any action in which the quality of a mathematical model is established (e.g., Metselaar, 1999; Jakeman et al., 2006). The topic of model evaluation has long attracted considerable debate amongst members of the scientific community. Much debate has stressed over the meaning of terms such as “testing”, “validation”, “verification” and “calibration” as part of the process collectively referred to as “evaluation” (Prisley and Mortimer, 2004).

The procedures to perform the evaluation task are also not widely accepted (Cheng et al., 1991) and appear in several forms, depending on data availability, system characteristics and researchers’ opinion (Hsu et al., 1999). Biophysical, process-based models (unlike statistical models) are made up of mixtures of rate equations, comprise approaches with different levels of empiricism, aim at simulating systems which show a non-linear behaviour and often require numerical rather than analytical solutions. Figure 1 identifies two types of models: a conceptual model and a computerized model. The conceptual model is composed of information (input data, parameters and equations) that describes the physical system or process of interest. The computer program includes technical issues and possible errors. In practice, the computer program is tested, rather than the mathematical model representing the system (Leffelaar et al., 2003). Subjects such as system representation (model structure) and program verification play a role besides numerical evaluation (structural assessment and program testing, not discussed in this paper) in assessing model accuracy (Donatelli et al., 2002b). Shaeffer (1980) developed a methodological approach to evaluate models that consisted of six tasks: (a) model examination, (b) algorithm examination, (c) data evaluation, (d) sensitivity analysis, (e) validation studies, and (f) code comparison studies.

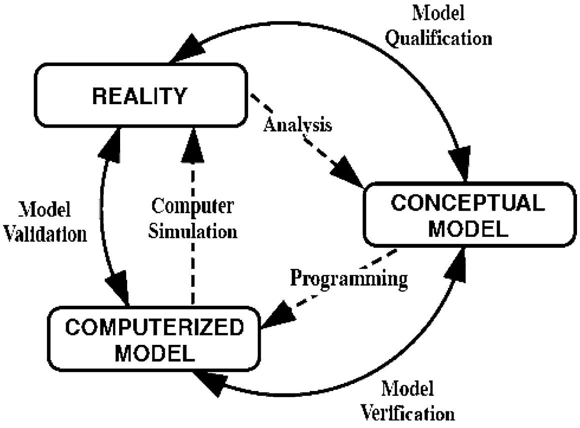


Fig. 1 Phases of modelling and simulation and the role of validation and verification (after Schlesinger, 1979)

This paper focuses on task (e) of Shaeffer’s methodology, where the term validation (Sage, 1987) is used for the process of comparing model outputs with measurements, although terminology is not standardized. A comprehensive list of publications regarding model validation was compiled by Hamilton (1991) but the connotation “valid” is rather controversial. Validation implies that the model is correct, whereas models (in the same way as hypotheses) can only be falsified rather than proven (Bair, 1994; Oreskes, 1998). This is why the meaning of the terms is not entirely consistent across fields with some eschewing the use of the term validation (e.g., Anderson and Bates, 2001), others noting the problems implied by the term while acknowledging it is widespread use (e.g., Oreskes and Belitz, 2001), and other distinguishing numerous kinds of validation including operational, conceptual, data, and even processes (e.g., Rykiel Jr., 1996). It is also acknowledged in this paper (and conveniently adopted) that the term validation is widely reported in the literature and generally used as a universal term to denote model assessment.

Definitions of validation in relation to computer software and modelling have changed little over the

Table 1 Alternative definitions of model (software) validation

Definition	Source
"It is a valid and sound model if it accomplishes what is expected of it"	Forrester (1961)
"The adequacy of the model as a mimic of the system which it is intended to represent"	Mihram (1972)
"Substantiation that a computerized model within its domain of applicability possesses a satisfactory range of accuracy consistent with the intended application of the model"	Schlesinger (1979)
"Comparison of model results with numerical data independently derived from experience or observations of the environment"	American Society for Testing and Material (1984)
"Validation means building the right system"	O'Keefe et al. (1987)
"The validation of a model package refers to the overall process of defining the range of circumstances or situations for which the package's behaviour and predictions are satisfactory"	Versar Inc. (1988)
"To determine that it [the software] performs its intended functions correctly, to ensure that it performs no unintended functions, and to measure its quality and reliability"	Wallace and Fujii (1989)
"The determination of the correctness of a model with respect to the user's needs and requirements"	National Acid Precipitation Assessment Program (1990)
"The process of testing a computer program and evaluating the results to ensure compliance with specific requirements"	Institute of Electrical and Electronics Engineers (1991)
"The process of determining the degree to which a model is an accurate representation of the real world from the perspective of the intended uses of the model"	American Institute of Aeronautics and Astronautics (1998)
"A process of adding strength to our belief in the predictiveness of a model by repeatedly showing that it is not blatantly wrong in specific applications"	Marcus and Elias (1998)
"Having a conclusion correctly derived from premises"	Sterman (2000)
"The process of assessing the prediction ability"	Li et al. (2003)
"Substantiation that a model within its domain of applicability possesses a satisfactory range of accuracy consistent with the intended application of the model"	Refsgaard and Henriksen (2004)
"Examining whether the system achieved the project's stated purpose related to helping the user(s) reach a decision(s)"	Sojda (2004)
"To gauge how well a model performs against observed field and laboratory measurements"	Huth and Holzworth (2005)
"A procedure consisting in comparing the model output with field or laboratory data to prove the model efficiency"	Dias and Lopes (2006)
"Substantiating that the behavior of the model "mimics" the behaviour of the system with sufficient accuracy so that it is impossible to distinguish the behaviors of both systems in the experimental frames"	Aumann (2008)
"A procedure consisting in verifying if the model is able to reproduce data, independently of those involved in its calibration"	Cardoso and Lopes (2008)
"The assessment of the performance of a model against an independently collected dataset"	Matthews et al. (2008)

years. A summary of definitions is reported in Table 1, which though not absolute, are becoming more definite over time. It is apparent that the most recent definitions tend to mirror the use of the concept in 1960s–1970s, whereas 1980s' definitions were more computer-oriented. The most recent definitions are by and large adopted by this paper. Whilst such relatively simple definitions of all the issues pertaining to validation can have their problems, they allow model community to communicate adequately enough in order to leave the semantic debate behind. It is worth noting that the understanding of validation is context dependant.

The overall model validation covers different areas, but the essence of it consists in defining criteria that will be taken into consideration in the choice of an "acceptable" model, and then testing the model performance according to those criteria. To assess the agreement between model results and observed data points, graphical plots are often made and judged qualitatively. It is acknowledged (e.g., Kuhnert et al., 2005) that if model output can be presented in appropriate formats, direct visual comparisons of models with data can yield significant insights about model performance. Statistical analysis by indices and test statistics play an important role to make comparisons reproducible, objective and quantitative. In general, however, the methodological basis for the validation of models to find the most suitable for specific applications is rudimentary, due to a lack of standardized terminology and procedures. Whilst statistical tools are easily applied for testing some empirical models, they might be of limited use with mechanistic (process-based) models whose primary aim is to represent system behaviour based on underlying principles (Berk et al., 2002). As modelling studies become more complex, models are used by parties less familiar with their peculiarities (e.g. structure, assumptions, data requirements and operability), and who may also lack understanding of the complexity of the entity being represented by the model. Some authors (e.g. Robinson and Ek, 2000) take the view that validation is the responsibility of the model user but improved accessibility to models by independent assessors and the ease with which they can be linked may increase their improper use. Hence, validation must not be seen as a one-off event or a "once-and-for-all" activity (Janssen and Heuberger, 1995), but as an on-going process to check for model compatibility to current evidence and variations (e.g. in spatial, climatic and hydrological conditions). Moreover, according to Sinclair and Seligman (2000), demonstration that model output more or less fits a set of data is a necessary but not sufficient indication of validity because model validity is rather the capability to analyzing, clarifying, and solving empirical and conceptual problems. Empirical problems in a domain are, in general, about the observable world in need of explanation because not adequately solved by a model, solved in different ways by rival models, or solved/unsolved depending on the model. Conceptual problems arise when the concepts within a model appear to be logically inconsistent, vague and unclear, or circularly defined, and when the definition of some phenomenon in a model is hard to harmonize with an ordinary language or definition (e.g. Parker, 2001). This raises the issue of widening beyond numerical testing by also including stakeholder evaluation and expert interpretation through soft systems approaches (Matthews et al., 2008). Working all this out would extend much further than the scope of this paper that is principally meant to recognise the limitations of numerical testing in achieving salience, legitimacy and credibility of models. Issues are initially discussed on the difficulties encountered when performing validation tests. Secondly, a review is given on how models are currently evaluated using qualitative and quantitative statistical techniques. Details are then provided on recent developments in validation criteria, decomposition of statistics and on how to combine validation statistics into single indicators of model performance. Principles and rules of general application are set forth with cited examples from the literature on ecology, agronomy, soil and climate modelling.

2 Issues on Model Validation

Before discussing specific approaches to validation, it is important to recognize a number of questions that arise in trying to validate a model. The following is a discussion of the main issues and factors as summarized in Table 2.

2.1 Validation Purpose

There are many purposes for validation, including establishment of overall credibility in the model, assessment of how "right" or "wrong" a model is in a given application, along with production of evidence to support that a specific configuration of input data, parameter sets and model structure are appropriate for a particular application.

Table 2 Key validation issues and relative modelling features

	Major factors to investigate				
Key validation issues	Modelling objective	Model inputs	Model outputs	Model structure	Modelling conditions
Validation purpose	X		X		X
Robustness of results			X		X
Interpretation of phenomena		X	X	X	
Model comparison				X	
Model predictions	X		X		X
Model complexity		X	X	X	
Data accuracy		X	X		
Time histories			X		

A model may be appropriate in one context and not in another. The validation tests employed, therefore, also have to reflect the differing contexts of model application. Similarly, there is a need for model developers to understand the things that would make a model valuable to a model user. Hence, validation purpose is somewhat related to the purpose for which the model was created and used. Feedback from validation should provide valuable information to both the developers on how their model may be improved, but also to end users who need to know how confident they can be in the quality of outputs. The type of validation to be executed depends on the objective for which the model was developed or purpose to which the output is to be used. It cannot be assumed that a model that is valid for one purpose is also valid for another (Sargent, 2001). This dependence on purpose may explain why common criteria, standard terminology and formalized protocols are missing, and why subjective judgement is included in the validation process (Hamilton, 1991; Landry and Oral, 1993; Rykiel Jr., 1996). In crop modelling, it emerged out of the work of various scientific research teams that early focus was on providing a simulation capability for scientists to use in distinct agricultural situations. In recent years, however, the models have been increasingly used for informing policy development and even for real-time information support for land-managers (e.g. Hochman et al., 2005). This change in model application has led to a change in the focus in model testing. Huth and Holzworth (2005) appeal to how a growing user-base for a model (including users making real-time decisions) can place a greater importance on the need for testing for model reliability (ability of the calculations involved to reproduce the conceptual model) than for model usefulness (ability to reflect the behaviour of actual systems).

2.2 Interpretation of Phenomena

Different interpretations of the real world (Checkland, 1981) may present problems for validation. It is essential that model variables have the same definition as the actual data meant to be represented by the model itself. In simulating the developmental response of photosensitive plants, for instance, the ability to compute day-length is essential, but day-length can be defined in different ways depending upon the angle of the sun with the horizon (Forsythe et al., 1995). In modelling leaf area expansion it is not always clear if both simulation representation and data collection target the expanded part of a leaf (i.e. lamina expansion and growth) only, or account for the stem-like structure of a leaf that is attached to the stem (i.e. base and petiole expansion and growth) as well (e.g. Cornelissen et al., 2003). Measurements of plant development present a series of challenges as differences in assessing development can be due to the subjectivity of an observer or to a definition that is not unambiguously applied in the field (Hanft and Wych, 1982). Similarly, methods of determining kernel number, kernel mass, and yield can vary among researchers, which can add errors to comparisons between experimental results and simulated values (e.g. Anonymous, 2003). Such examples emphasise the importance of meta-data associated with original observations and development of model parameters (Medlyn and Jarvis, 1999).

Process-based models are also moving targets: if, for instance, plant model version 1 is considered to be incorrect, even a small change in a sub-model introduced to correct its functionality may produce a different interpretation on simulated processes (similar to the problem of “regression” in software development jargon). The reason for these unwanted changes lies in the lack of independence/wrong dependencies of parts of code, which is not completely avoidable. This aspect might go beyond a simple evaluation by once again comparing against previously acceptable results (Huth and Holzworth, 2005) and poses the need for formal model validation against observed data at each published stage of model development (Van Oijen, 2002).

2.3 Model Comparison

Model comparison can be useful as a complement to model validation (Meehl et al., 2005). When two or more models are constructed for the same system or purpose, it is possible to make comparisons between them in order to select which is the best.

The Global Climate and Terrestrial Ecosystems group (GCTE) recognized in 1992 (GCTE, 1992) that there were at least 14 models of physiological processes that govern wheat growth and development. Landau et al. (1998, 1999, 2000) and Jamieson et al. (1999) reported on validation of three of such models against observed grain yields in the United Kingdom.

Diekkrüger et al. (1995) illustrated the simulation results from using simple as well as complex models against a common dataset covering the processes of water, nitrogen, plant growth and pesticide dynamics. In general, the models reproduced the measured dynamic only in part, with different response for different models. The study also made it clear that the experience of a scientist applying a model is as important as the difference between various model approaches.

When either field or reference modelled data are not available, attempts can be made to determine the proximity of one model to the other, also known as co-validation (Wright, 2001). Co-validation requires the assessment of the difference between models with respect to the values of their common output. The most likely case is that competing models do not share the same form. Typically, dissimilarly structured models not only have different inputs (including both variable and parameter sets), but they also have different levels of aggregation and capabilities in terms of modelling the actual system. In absence of actual data, the fact that several models show the same behaviour does not really give more confidence in each of them but only demonstrates that the models are capable of reproducing similar results for the observed system. This concept is often referred as equifinality (Beven, 1993; Franks et al., 1997; Beven and Freer, 2001; Medlyn et al., 2005). In general, potential "extra" capabilities of one model compared to another should not be used in co-validation. For example, nitrogen stress effects to plant growth should not be part of the comparison between two models where only one model includes nitrogen processes.

When statistical regression models (e.g. generalized linear models, generalized additive models) are compared, artificial data based on explicit theory can be used as "truth" (Austin et al., 2006).

2.4 Model Predictions

In model fitting, the model under investigation is evaluated for its adequacy in describing the observed data (Myung and Pitt, 2003). This is achieved by determining values for parameters in the model that will best describe the observations. Model fitting in this manner yields valuable estimates of quantities which might be used to differentiate between or explain process/system behaviours. However, papers on modelling often state that they aim to produce an instrument for prediction (Van Oijen, 2002). The issue of model prediction has been accompanied by some debate on the terminology to be used to describe model validation (see definitions by Marcus and Elias, 1998 and Li et al., 2003 in Table 1). In this case, a fundamental issue is to quantify the degree to which a model captures an underlying reality and predicts future cases.

According to Van Oijen (2002), model-based predictions only contribute to science if the underlying model mechanisms are described, are innovative, are compared to other approaches, and if the predictions can be checked and used as a test of the model. Predictions pose special problems for testing, especially if prediction focuses on events in the far future. Predictive models can be accepted if they explain past events (ex-post validation). However, the probability of making reasonable projections decreases with the length of time looked forward. A continuous exchange of validation data among developers and test teams should either ensure a progressive validation of the models by time, or highlight the need for updated interpretations of the changed system.

The problem of global change has generated much interest in the development of predictive models for crops and ecosystems (e.g. Intergovernmental Panel on

Climate Change, http://www.ipcc.ch) because model estimates are increasingly being used for decision support and strategic planning (e.g. Matthews et al., 1999; Rivington et al., 2007; Tingem et al., 2009). This requires that the quality of model estimates is assessed in advance, or that the decision support outcomes be made insensitive to the estimation uncertainty (Norton, 2003). Model quality may also show variability over geographical locations. For example, Moberg and Jones (2004), in testing hindcast estimates produced by the Hadley Centre Regional Climate Model (http://www.metoffice.com/research/hadleycentre) at 185 sites in Europe, found only some sites well represented. Responses like this restrict the geographical location to which the predicted climate change data could be used in model-based impact studies. Daily forecasts in hydrology largely use the previous week's real-time monitoring data as a training hindcast period, which permits validation and then project ahead for a period of two weeks (Quinn, 2008). In plant breeding, the issue of model-based prediction is dealt with when models are used as decision support tools to predict yield components of new cultivars and/or over new environments (Barbottin et al., 2006).

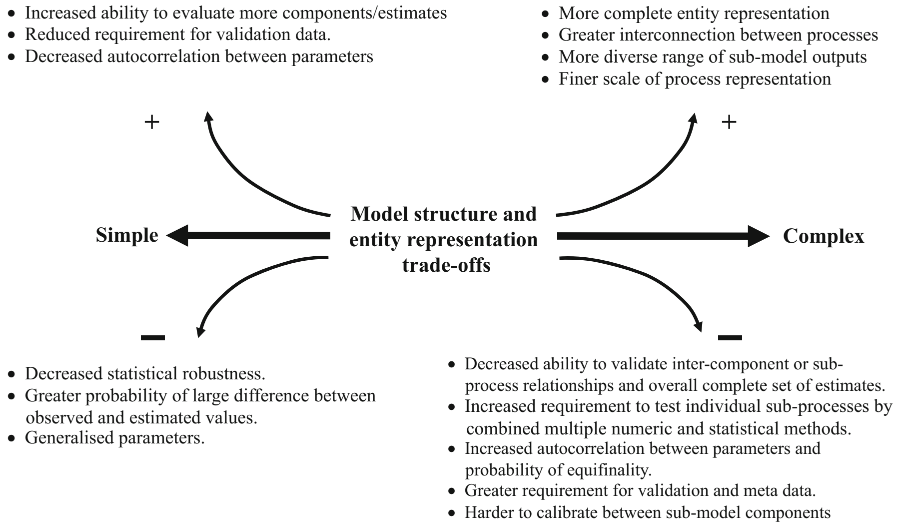
Like models which extrapolate in time, models used to extrapolate from small (e.g. leaf photosynthesis) to large spatial scales, such as regions, continents, or the global biosphere, are difficult to evaluate (e.g. Bolte et al., 2004; Chen and Coughenour, 2004). The heterogeneities in distributions of processes and non-linearity in their functional responses may make it infeasible to apply models representing these small scale processes over large areas and vice versa. There are several steps in performing spatial predictions where the variography, a well known geostatistical tool to analyse and to model anisotropic spatial correlations, is proposed to be used in the assessment of modelling results in addition to the traditional statistical analysis to demonstrate presence/absence of spatial structures in datasets (Kanevski et al., 2008).

2.5 Model Complexity

Process-based models reflect the same complexity as the system being represented (Bolte et al., 2004) and there are authors (e.g. Pilgram et al., 2002) who emphasize the need for models to accurately reflect the dynamics of the actual system. The comparison of model results with measured data is usually done at the system level and is thus the result of both the feedbacks within and between each of the sub-models making up the whole. Since possible counter-interaction between model components may go unnoticed, ideal validation should take place both at the level of sub-models and of the complete model (Leffelaar, 1990). As modelling projects progress, the model grows and it becomes harder to validate every part of it in detail. However, the independent testing of sub-units increases the possibility of building a complex system model as the assembly of interacting processes, rather than degrading a process-based model into a fitting exercise.

Systems with small timescale, spatial extension and complexity can be isolated, thus models of these systems are more readily accessible to validation. In complex models, specific sub-processes cannot always be tested at the level of the model output (Klepper, 1989). For systems with increasing time and spatial scales with increasing complexity, validation of system models requires increasingly more effort (Fig. 2). This increase in effort can be illustrated if one considers, for instance, validation requirements in terms of data, manpower, and organizational continuity for a model of an annual crop, for a model describing a crop rotation experiment, and for a model to analyze sustainable agricultural practices. At some point the effort required to gather validation data may no longer be feasible (De Wit, 1993).

The scientific literature is full of instances where the behaviour of complex systems is reduced to a set of basic emergent behaviours. The intrinsic complexity typically captured by many biophysical models may suggest a shift in emphasis from rigorous validation to a more exploratory approach characterizing the likelihood of distinct system behaviours rather than estimation of specific outputs (Bolte et al., 2004). The benefit of more flexible approaches to validation is clearly demonstrated in their ability to highlight model behaviour in extreme circumstances that might not appear in model validation datasets, but that certainly exist in the real world in which the model will be applied. This is the case, for instance, of high-magnitude events such as weather extremes that permit a form of model validation against past events, also called a "mental model" (Favis-Mortlock et al., 2001) based on the observer memory of landscape changes, i.e. massive soil degradation (with, possibly, the support of



- Increased ability to evaluate more components/estimates
- Reduced requirement for validation data.
- Decreased autocorrelation between parameters

- More complete entity representation
- Greater interconnection between processes
- More diverse range of sub-model outputs
- Finer scale of process representation

- Decreased statistical robustness.
- Greater probability of large difference between observed and estimated values.
- Generalised parameters.

- Decreased ability to validate inter-component or sub-process relationships and overall complete set of estimates.
- Increased requirement to test individual sub-processes by combined multiple numeric and statistical methods.
- Increased autocorrelation between parameters and probability of equifinality.
- Greater requirement for validation and meta data.
- Harder to calibrate between sub-model components

Fig. 2 Trade-off between model complexity and validation

photographic material and annotated texts). Sensibility tests (Huth and Holzworth, 2005) refer to as the comparison of model output against more subjective, local experts feeling for what the model should do. Model responses to various stimuli are evaluated against the regularly observed system responses that are often captured in the notion of "common sense". Examples from local agronomists include, for instance, statements like "under those conditions the model should have an average yield of x t ha^{-1} with a range of y to z". These tests are a way of making sure the model performs in situations where observed data for validation are not available, and their use in testing the correctness of the underlying model design should not be undervalued. The "hard" data available for model testing is indeed only a small snapshot of system behaviour, and some authors (Seibert and McDonnell, 2002 for a catchment's model; Pastres et al., 2004 for sea grass model) showed that a better process representation can be obtained by using "soft" data. This might protect against being the model "right for the wrong reasons" due to adapting a model to a small set of observations. One might become "less right" but for the right reasons.

2.6 Data Accuracy and Quality

The accuracy of a model is determined on one hand by the authenticity of the algorithms describing the processes of the real world, while on the other hand by the quality of both its input data and data used to evaluate its outputs. Inaccuracies are common in both inputs and measured outputs. Model validation must therefore be accompanied by critical examination of the source and nature of the data used. Random errors may occur when single, individual samples do not take into account temporal variability; when samples taken at different points do not represent the actual area of interest; or when the inputs have been modified by unnoticed factors (environmental or human). Random errors of sample collection and processing may also occur when the sample is contaminated by other environmental media, when the sample is modified during transit and storage, or when the sample data are misrecorded. Systematic errors might occur if instruments are miscalibrated, measurements are taken at inappropriate locations or seasons, no measurements or estimates are made of relevant factors, or the data are not representative of the same output as the modelled one.

Complex models must generally be tested against a broad range of data of varying types, quality, and coverage. In reality, modellers are often confronted with a poor database because data monitoring is normally limited to a few points where samples are collected and analysed at some intermittent frequency. This may also change with the variables analysed, e.g. only scattered field measurements for some variables, more complete time series data or even maps for other variables, only qualitative assessments for others again. In hydrology, for instance, consistent field data of extreme events are poor (Westrich, 2008). Due to their sparse resolution, the data are frequently subject to large errors and this is en ever-increasing problem in moving from plots or small catchments to watersheds and regions.

Weather and soil variables are key inputs for biophysical models. Common problems encountered with weather inputs are: records taken too far from the experimental site, errors under reporting values, poor maintenance and imperfect sensitivity of sensors. Soil properties are often essential input data, but soil name and classification are commonly reported in the literature for field sites based solely on location and defined soil series from published data, rather than from field measurements. The soil may only marginally meet the classification criteria and therefore have different characteristics (pH, particle size distribution, organic matter content, etc.) than a soil meeting the central trend of the stated soil, illustrating the need for an appropriate sampling strategy to capture the spatial variability (Wright et al., 2003). Inaccuracies in both weather and soil inputs may turn into combined effects, either able to strengthen or weaken each other's effect during model simulation (Fodor and Kovács, 2003) and being a source of errors in model estimates (Hoogenboom, 2000; Heinmann et al., 2002; Rivington et al., 2003, 2006). This makes it difficult to disentangle the impacts on outputs of the combined effects of error source manifesting themselves as compensating errors. Emphasis must then be to determine the uncertainties that imperfect data introduce to model estimates.

As regards model outputs, comparison of model results with observations is complicated by the fact that biophysical data are affected by errors. It is difficult to appropriately evaluate model performance if the uncertainty in the data is high. Fitting a set of data can therefore only establish legitimacy for a model under the specific circumstances for which it was evaluated (Oreskes et al., 1994). The data resource is often segregated into two groups: a sample is used to estimate model parameters (model calibration: the adjustment of model parameters to improve the fit); an independent sample is used to assess the fit of the model to the data. Validating the model against data not used to construct the model is probably the best and simplest way to assess uncertainty and derive the reliability of model estimates (e.g. Ljung, 1999; Gardner and Urban, 2003). However, biophysical data often exhibit a large degree of variability, and it is common that there are important discrepancies between model estimates and actual data despite significant calibration efforts (e.g. Gobas et al., 1998). Dedicated techniques do exist to generate reduced bodies of data (e.g. Stone, 1974; Efron, 1986; Breiman and Spector, 1992). Cross-validation is a random data split into a number (commonly in between 5 and 10) of roughly equal-sized parts; in turn, each part is used as a test set and the other parts for fitting the model. The prediction error is computed each time in the test set, and estimate of prediction error is the average of individual prediction errors. This technique is inappropriate for small sample size because the variance of the error is likely to increase considerably when splitting the data. The bootstrap technique is a valuable alternative (Wallach and Goffinet, 1989). A bootstrap sample is a sample created from random drawings with replacement from the original dataset (there can be repeats in a sample, and samples that do not contain an original point). It is also common in data-rich situations and for model comparison to generate three datasets: one to fit models, second part for estimating prediction error, third part for assessing prediction error of final selected model. As pointed out by Sinclair and Seligman (2000), a test of validity by separating data into sets may reflect only the success in splitting the data so that each group represents the same population of data. The same authors remarked: "a test of model performance is better served scientifically when based on all available data covering a wide range of conditions".

Extension of data series is also critical. Pastres et al. (2004) found that whilst traditional testing of their seagrass model gave an adequate description of the available model calibration data, the model failed to capture the known trends in sea grass evolution over a longer time frame.

Measurement uncertainty has important implications in modelling applications. Analysis of uncertainty in measured data which drive model calibration and validation improves model application and enhances decisions based on modelling results. However, the impact of uncertainty in model calibration and validation data is discussed but rarely included in the assessment of model accuracy. In order to change this omission, several goodness-of-fit indicators were modified to incorporate measurement uncertainty into model calibration and validation (Harmel et al., 2007, 2008).

2.7 Robustness of Model Results

Model robustness is its reliability under different sets of experimental conditions. Lack of robustness in model results may reflect an absence of explicit reference to physical processes in the construction of mathematical relationships. Concern is voiced about the fact that many model assessments are frequently limited to a small number of output variables and sets of conditions (Woodward, 2001). Well-known validation methods, whilst effective for some things, are not necessarily effective at ensuring that a model faithfully captures the underlying process driving the system in question and thus will be applicable in any new situations where a model may be employed. Simple comparisons can be misleading, for example, when only the final "end of growing season" results from a crop model are compared, because reasonable estimations of overall growth can be achieved by different pathways, not all of which are logically acceptable (Sinclair and Seligman, 2000). Performance of a model should be checked not only in terms of the major outcome of the model, but also in the estimations for critical constituent components throughout the simulation. Such checks give protection against spurious conclusions and indicate whether the model is conceptually consistent and related to reality, or specific areas require improvement. The validation of a sugarcane model by Keating et al. (1999) as cited by Sinclair and Seligman (2000), gives an example of a sound test of robustness for the following reasons: (1) a large number (i.e. 19) of data sets for sugarcane growth were used, (2) a broad range of conditions with differing locations, irrigation treatments, and nitrogen fertility treatments were explored, (3) the seasonal evolution of individual components of the model (leaf area index, green biomass, millable stalk biomass, stalk sucrose, and nitrogen accumulation) was compared with observed results. Qualified features were the discussion of the situations where relatively large deviations existed between simulated and observed results, and the presentation of hypotheses to stimulate further research to improve the understanding contained in the current model. Such model validation serves as the basis for building credibility with end users, and greatly improves the probability that the model will be accepted.

2.8 Time Histories

When simulating energy transfer or mass transformation in dynamic models, a time delay/anticipation frequently occurs if estimated versus measured values are compared. Peak synchronization between estimates and measurements most often will not occur. If synchronous comparison between estimates and measurements is applied, models which produce no response with respect to a specific process can yield better results, compared to models which show a time mismatch in the response (e.g. Vichi et al., 2004). In cases where poor parameterization produces a time shift of estimates, large residuals from few points may lead one to discount the model. Model re-parameterization can help correct time-related bias. If not, apparent misalignment of observed and modelled output may lead to a re-assessment of the daily time interval (common in many systems models) as the basis for comparing modelled and monitored event data (e.g. Yagow, 1997). Average values during multi-day periods (ten-day, month or year) can be used to calculate and compare occurrence of the event. This is particularly important where timing of events (i.e. crop phenology and management synchronization) estimated by a model are used to construct recommendations or optimized practises.

2.9 Summary

In this section, we summarized the vast international experience in the validation of biophysical models and ordered issues that we regard key to model validation into a list that may add value to the modelling work. Basically, we stressed that validation is purpose-dependent, based on equivalent definition of modelled and observed phenomena, to be substantiated over a variety of conditions (robustness), and possibly run at the level of individual processes in complex models. The discussion about data quality raises the need of a system for grading the relative quality of the input and the relative importance of the variables to be fit. Concerns were addressed regarding specific aspects such as predictions in the far future and synchronization of modelled and observed peak values. Moreover, model comparison was discussed as complementary to proper validation. Put into a logical structure, the ideas we have discussed are virtually applicable to validation of any model, and could equally be consistent with modelling in a variety of fields.

3 Validation of Models

A range of statistical measures and visual techniques can be used to assess goodness-of-fit of a given model and to compare the performance of a suite of models, as informed by the specific context of the problem (e.g., Berk et al., 2001). Recent review papers include: Mayer and Butler (1993), Janssen and Heuberger (1995), Smith et al. (1997), Martorana and Bellocchi (1999), Yang et al. (2000), Bellocchi (2004), Tedeschi (2006), Wallach (2006). Such papers provide and discuss a range of statistical measures and visual techniques that can be used to assess goodness-of-fit of a given model and to compare the performance of a suite of models. In this section we do not replicate a detailed examination of validation techniques, rather we consider validation approaches as developed by many authors and applied in numerous modelling studies in the domain of natural sciences. It is also beyond the scope of this paper to critically appraise in detail the usefulness of each method, as details are available in the review papers detailed above. The commonly used forms of numerical and statistical approaches are highlighted here with examples of criteria for determining model acceptability. The above cited papers (and the other papers in this section dealing with specific statistics) contain the equations of the statistics and methods used for model validation. So the equations are not reported here, but the rationale behind the choice of particular sets of statistics is given.

3.1 Validation Measures

There are two main categories of goodness-of-fit measures for testing of one-at-a-time output, which are: (a) residual-based; and (b) association-based. Residual-based measures such as the mean bias error (MBE), and root mean square error (RMSE) provide quantitative estimates of the deviation of modelled outcomes from measurements. On the other hand, measures of statistical association such as the Pearson's correlation coefficient (r) provide quantitative estimates of the statistical co-variation between observed and estimated values (Addiscott and Whitmore, 1987). Statistical measures aim to characterize the usefulness of a model for a specific application and may lead users to decide whether or not to use a model for that particular application. Moreover, visual comparison of modelled and measured data, and experience-based judgement on the part of the modeller have been deemed important by researchers for assessing model validity and applicability in decision making.

Mean residuals: Mean bias (MB), the mean difference between observed and model-estimated values, is likely to be the oldest statistic to assess model accuracy (Cochran and Cox, 1957). More common is the mean square error (MSE), or equivalently its square root, the root mean square error (RMSE, or derived statistics such as the relative root mean square error RRMSE). MSE is also the statistic whose value is usually minimized during the parameter calibration process (Soroshian et al., 1993; Makowski et al., 2006). Mean absolute error (MAE) measures the mean absolute difference between observed and estimated values (Mayer and Butler, 1993), and is also used as the mean absolute percent error. Fox (1981) proposed to use: (l) RMSE or MAE to quantify the average difference between estimates and measurements; (2) the mean bias error (MBE) to identify under- or over-estimates; and (3) the variance of the distribution of the differences to quantify error variability. Such proposals are reflected in the paper of Davies and McKay (1989) and Trnka et al. (2005) for validation of solar radiation estimates.

Modelling efficiency: Willmott (1981) developed an index of agreement (d) to be used in addition to the previous measures. The modelling efficiency statistic (EF, Nash and Sutcliffe, 1970), interpreted as the proportion of variation explained by the model, has been extensively used in plant and hydrology models (e.g. Greenwood et al., 1985; Loague and Green, 1991), and can certainly be used in biological and ecological models. Mayer and Butler (1993), likewise, indicated both RMSE and the MAE as stable statistics, and recognized modelling efficiency (EF) as an important overall measure of fit. Smith et al. (1997) pointed out that EF and a coefficient of determination (CD) should be used together for a better interpretation of RMSE when standard error of the measurements is unavailable. Alternative forms of the efficiency measures are given in Krause et al. (2005).

Correlation: The Pearson's correlation coefficient is largely used in validation. Fox (1981) and Willmott (1982) provided strong arguments against the use of this coefficient alone as a measure of performance. Its magnitude is indeed not consistently related to the accuracy of estimates, as correlation between dissimilar estimates and measurements can be high while, conversely, small differences between estimates and measurements may occur with low correlation values. Nonparametric correlation measures are also used for model validation such as concordance, Spearman and Kendall's coefficients (Press et al., 1992; Dhanoa et al., 1999; Agresti, 2002).

Linear regression: A linear regression between estimated and observed values is also commonly used. The hypothesis is that the regression passes through the origin and has a slope of unity (see Subsect. 3.3). The use of the r^2 regression statistic (least-squares coefficient of determination) for model performance is flawed, as it does not account for model bias (Mayer and Butler, 1993; Mitchell, 1997). Krause et al. (2005) proposed to use r^2 as a weighing factor of regression slope to quantify under- or over-estimates. An alternative (nonparametric) method to compute r^2 was proposed by Kvalseth (1985), resulting in a coefficient that is more resistant to outliers or extreme data points.

Combined graphical and statistical approaches: The factor of two (Fa_2) is currently used to evaluate air dispersion models (e.g. Kumar, 2000); combined with the values of different indices, MBE, fractional bias (FB), normalized mean square error (NMSE), correlation coefficient (r), geometric mean bias (MG), and geometric mean variance (VG). FB in the form of absolute differences is presented as fractional gross error (E_f) in Seigneur et al. (2000).

Loague and Green (1991) suggested the use of both statistical and graphical measures for validation. Model performance can be compared using either summary statistics (mean, range, standard deviation) or using individual measured versus estimated pairs of data, which can also be displayed in both statistical and graphical forms. Assessment of data pairs usually proceeds with an analysis of the residual errors in the forms of maximum error (MaxE), RMSE, EF, CD and coefficient of residual mass (CRM) (James and Burges, 1982; Green and Stephenson, 1986). Suggested graphical displays include: (1) comparison of measurements and estimates; (2) comparison of ranges, medians and means; (3) comparison of matched estimated and measured time-series values and/or residuals; (4) comparison of cumulative values; and (5) cumulative frequency distributions.

Zacharias et al. (1996) presented robust quantitative techniques, from median-based nonparametric statistical methods (MdAE: median absolute error; RMdAE: relative median absolute error; REF: relative modelling efficiency), that can be used when the distribution of the observed data is non-Gaussian or when the sample size is not large enough to determine the underlying data distribution. Zacharias and Coakley (1993) categorized validation techniques into three main categories: summary statistics, hypothesis testing, and measures of goodness-of-fit, i.e. MaxE, a relative measure of the root mean square error (RRMSE), CD, EF and CRM. They listed examples of summary statistics as the mean, standard deviation, and those statistics commonly used with box or whisker plots (range, inter quartile range, and median).

Yang et al. (2000) examined the correlation across different statistics, allowing one to choose from each correlated group without loosing accuracy. They argued that the same conclusion can be achieved by using together either RMSE, modified modelling efficiency (EF1), paired t-test and E, or MAE, EF and E.

Model assessment by Mankin et al. (1977) and improved by Scholten and van der Tol (1998) was based on a comparison between model estimates and observations by using Venn diagrams and measures such as model adequacy (number of agreements between model and experiments / number of experiments) and reliability (number of agreements between model and experiments / number of model responses). These are helpful in discriminating between a better and worse model, and to define cases of useless or good models. Gardner and Urban (2003) illustrated a general approach to test model performance across a spectrum of methods via receiver-operator characteristic curves (ROC). Such approaches are based on the classification of results into discrete categories and the concept of a "confusion matrix" (Campbell, 1996), and imply defining false and true positives in the estimation of binary variables. Some statistics based on the confusion matrix are presented and discussed in Beguería (2006). Pontius Jr. and Schneider (2001) described how to use the ROC as a quantitative measurement to validate a land-cover change model. In Barbottin et al. (2008), a ROC curve analysis was carried out to estimate the frequencies of correct and incorrect indicator-based and model-based decisions, using the area under the ROC curve as summary of the overall accuracy of a model.

Patterns: Change of patterns in the residuals can be assessed by testing the autocorrelation in the residuals (Vincent, 1998). Lin et al. (2002) developed model-checking techniques by taking the cumulative sums of residuals over certain coordinates to ascertain whether or not specific patterns exist in the residual plot. Donatelli et al. (2000, 2004a) proposed to quantify the presence of patterns of residuals versus independent variables (e.g., a model input or a variable not considered in the model), by computing pattern indices of two types: range-based (PI) and F-based (PI-F). Macro-patterns were revealed in model residuals by dividing the range of values of the external variable in two to five, fixed or varying sub-ranges. A pattern index in a percent relative-to-mean form was used as a validation measure by Bellocchi et al. (2003). In Trnka et al. (2006), estimated and observed herbage productions from permanent grassland were compared by using a vast array of pattern indices (against nitrogen fertilizer application rate, year, cut number, location, length of the growing season, date of the previous cut, number of snow days, two variants of accumulated air temperature, two variants of accumulated global solar radiation, and total precipitation during the period of sward growth) in conjunction with a set of performance statistics, i.e. MBE, RMSE, d, and Theil's inequality coefficient (U, Theil et al., 1970). The latter (ranging from 0 – perfect fit – to 1 – absence of any fit) penalizes large errors more than small ones and it also assesses a model's ability to duplicate a turning point or rapid changes in the data (Topp and Doyle, 2004). Pattern indices (versus month of year and minimum air temperature) in conjunction with error and correlation measures were also used by Diodato and Bellocchi (2007a) to assess the relative performance of three models of rainfall erosivity.

Correction factors: Correction factors were developed by Harmel and Smith (2007) for the error term in some goodness-of-fit indicators (modelling efficiency, index of agreement, root mean square error, and mean absolute error) to incorporate the uncertainty of measured data into model validation, later improved by Harmel et al. (2008) to consider the effect of model uncertainty.

In model predictions, one approach to estimating the prediction measures is to adjust the naïve measures to get less biased estimates of the prediction measures. Estimators that are based on this approach include the Mallows' statistic (C_p, Mallows, 1973), the Akaike's information criterion (AIC, Akaike, 1974), and the Bayesian information criterion (BIC, Schwartz, 1978). All of these estimators, functions of the naïve measures, size of the dataset, and number of parameters in the model, generally lack of robustness (Li et al., 2003).

3.2 Disaggregating Statistics

Once parameters of linear regression of model estimates versus actual data are estimated, the fitted line can be applied to generate new estimates of the variable under study. The difference between model-based and regression-based estimates defines the erratic portion of the error (and is the basis for computation of prediction error), while the systematic portion is described by the difference between regression-based estimates and actual data (the basis to assess the precision of the fitted linear regression, Tedeschi, 2006). Both model users and developers will focus on reducing the systematic error, the formers by model re-calibration, and the latters by better defining the basic equations. This is a basic concept by Aitken (1973) and Willmott (1981). More recently, Kobayashi and Salam (2000) developed the same concept to have residuals disaggregated into erratic and systematic components. They used the root mean square variation (RMSV) to indicate how much the model fails to estimate the variability of the measures around the mean, together with derived measures such as simulation bias (SB), square differences of the standard deviations (SDSD) and lack of positive correlation weighted by the standard deviations (LCS). These statistics are supportive in locating the causes of possible large deviations between estimates and measurements. The proportional contribution of systematic and erratic portions to the total error is helpful in determining areas in the model requiring further improvement. Further developing those findings, a different partitioning of mean square error into three additive components was given by Gauch and Fick (2003) and Gauch et al. (2003): they retained SB and derived the non-unity slope (NU), and the lack of correlation (LC). There is a unique quality to these approaches in the way the authors commented them in a letter exchange (Gauch et al., 2004; Kobayashi, 2004).

Trnka et al. (2006) called attention to disaggregating the Theil's coefficient (U). One of the main advantages of coefficient U is the possibility of calculating proportions of: estimated bias (U_B), indicating systematic errors; variance (U_S) that measures the ability of the model to replicate the degree of variability in the data; covariance (U_C), that is any remaining error, after accounting for the bias and variance effects. The ideal distribution of inequality over these three sources is for the bias and variance effects equal to zero, and the covariance equal to one.

3.3 Statistical Hypothesis Tests

Hypothesis testing is a formal approach to validation where either summary statistics or goodness-of-fit measures are tested against prescribed criteria (range of accuracy). In using statistical hypothesis testing to assess the validity of a model for its intended application, two hypotheses are formulated under the given set of experimental conditions: for the null hypothesis model is valid for an acceptable range of accuracy, and for the alternative hypothesis it is invalid under the same acceptable range of accuracy. Accepting the alternative hypothesis when the null hypothesis is true corresponds to the type-I statistical error, whose probability is also called model builder's risk. Accepting the null hypothesis when the alternative hypothesis is true matches the type-I statistical error, the second type of wrong decision with a probability called model user's risk (Balci and Sargent, 1982a).

Fundamental requirements: Statistical tests assume that the outcomes based on the model are statistically accurate representations of what they purport to estimate. However, both systematic and random errors in biophysical studies may influence the accuracy of the estimators (as seen in Subsect. 2.7). Pennell et al. (1990) stated that graphical analyses allow for identification of trends in the data, systematic errors, and other potential sources of error, such as outliers. Marcus and Elias (1998) elaborated on five major areas of concern when applying formal statistical tests: observational data may not have been collected for the purposes of model validation; the sample size may be too small, allowing inadequate power to detect model deficiencies or to discriminate among competing models; the sample size may be so large that even useful models may be rejected by a statistical test for deviations that have little practical importance; measurement errors may bias the test statistics in the direction of attenuating the apparent goodness of the model; the temporal rhythms of the output variable may be influenced by systematic and random errors occurring in environmental factors.

Typical forms of the goodness-of-fit test are the following: Does the observed value minus model-estimated value equal zero (showing that the estimates are unbiased)? Does the ratio of observed value to model-estimated value equal one (in which case the estimates are relatively unbiased)? Numerous approaches involving statistical analysis have been used to evaluate model adequacy and several forms of statistical hypotheses are structured to show the level of confidence that the hypothesis is not rejected.

Using regressions: The regression between observed and model-estimated values is commonly used because estimates of the intercept and the slope are good indicators of accuracy (the simultaneously closer to zero and unity, respectively, the higher the accuracy). Nonetheless, necessary assumptions have to be considered when performing a linear regression: the X-axis values are known without error; the Y-axis values have to be independent, random and with equal variance; residuals are independent and identically distributed. Some authors (e.g. Bland and Altman, 1995; Kleijnen et al., 1998) critically revisited the role of regression analysis in model validation, and suggested alternative approaches (difference between simulated and actual outputs against their sum, against their average, etc.) for achieving non-misleading regression testing. Fila et al. (2003a, b) suggested using the reduced major axis (RMA) method in place of the ordinary least squares method to estimate regression parameters. RMA has three desirable properties (Ricker, 1984): it is symmetric in X and Y (if the x and y axes are interchanged, the slope is replaced by its reciprocal and the line remains stationary about the data points); it is scale independent (the line does not change with a change of scale); it is robust to clusters of observations in the frequency distributions of data (the line usually describes the central trend even when the sample is not bivariate normal).

Thomann (1982) suggested using regression analysis and tests of slope (against one) and intercept (against zero) of a regression line in conjunction with other performance statistics. If the model is a good one, the regression will be a 45° line thorough the origin. Thus, the adequacy of the model can be determined by testing if intercept equals zero and slope equals one, separately using Student t-tests, or simultaneously using the F-test (Dent and Blackie, 1979; Mayer et al., 1994). These parametric tests for whether the regression line is significantly different from the 1:1 line assume that the data are normally distributed and independent, which is often not the case for data determined by non-linear biophysical processes.

Interpretation of statistics: The test statistics may also have ambiguous interpretations. A statistical test of observed versus estimated values which fails to achieve the desired level of confidence does not necessarily indicate a problem in the model (usually the specification of one or more key parameters), because the problem may (or may also) reside in the observed data (usually the result of measurement error). With the t-tests for the intercept and slope, the more scatter in the data points, the greater is the standard error of the regression parameters, the smaller is the computed value for the test statistic and therefore, the harder it is to reject the null hypotheses which states the parameters are equal to zero and one respectively. Therefore, the test can fail to reject the null hypothesis either because the regression parameters are really not different from the values desired or there is much scatter around the line (Harrison, 1990). The F-based calculation that tests if the intercept and the slope coefficients simultaneously are respectively not different from zero and unity is affected by the same ambiguity as the t-tests. As shown by Analla (1998), the greater the estimation error the more difficult it is to reject the null hypothesis. Alternatively, the confidence interval should be used to investigate the range of the slope (Mitchell, 1997).

Empirical confidence intervals were proposed by Parrish and Smith (1990) as practical test for model validity founded on an overlap between the ranges of values computed on both model outputs and observations. Upper and lower limits of the range were computed as division and multiplication of the nominal model estimate by a chosen factor. The factor of two (Fa_2) is commonly used in air dispersion modelling (Kumar, 2000). Summaries of confidence limits on normalized mean square error, geometric mean variance and geometric mean bias were used by Patel and Kumar (1998) to select the best out of three air dispersion models.

When replicated experiments are available, the lack-of-statistical-fit (LOFIT) can be calculated and tested against an F-distribution (Whitmore, 1991). Assuming random experimental errors, the LOFIT distinguishes the mean deviation as a source of error from the failure of the model. Smith et al. (1997) assessed the statistical significance of difference-based indices assuming a deviation corresponding to a given confidence interval of the measurements. Fila et al. (2003a, b) proposed statistical methods to compare experiments against estimates when both are replicated. The use of bootstrapping techniques for validation of simulation models when parametric assumptions are violated was proposed by Kleijnen et al. (2001).

Non-parametric tests: Contrary to parametric analyses, with nonparametric tests (i.e. variants of the Kolmogorov-Smirnov test as described in Stephens, 1974; the Wilcoxon-signed rank test, as described in Daniel, 1995) the assessment of adequacy of a model is related to its ability to yield the same ranking between observed and model-estimated values rather than model-estimates on observed values per se. Reckhow and Chapra (1983) listed measures of error, the t-test, the non-parametric Wilcoxon test, regression analysis, cross-correlation and box plots as appropriate statistical methods for deterministic models. Reckhow et al. (1990) also recommended various combinations of graphic (bivariate plots, histograms, and box plots) and statistical procedures based on the proposed analysis and intended use of modelling results. The χ^2 tests described by Agresti (2002) indicate whether the data points are homogenously distributed or if there is any tendency of over- or under-estimation.

The comparison of the distribution of the observed and model-estimated values has also been utilized to identify model adequacy for stochastic (Reynolds and Deaton, 1982) and deterministic models (Dent and Blackie, 1979). The common Kolmogorov-Smirnov's D test has been used to assess the probability that two data sets (observed and model-estimated values) have the same distribution. It consists to measure the overall difference of the area between two cumulative distribution functions (Press et al., 1992).

The use of multivariate statistics in model validation is not new (Balci and Sargent, 1982b). With multidimensional models, multivariate statistical analyses can be proficiently applied as in Mabille and Abecassis (2003), where a geometric model of wheat grain morphology was evaluated via principal component analysis and discriminating factorial analysis, and generating confidence limits in an elliptical plane.

3.4 Validation Criteria

One of the difficulties when evaluating assessment metrics is determining what values indicate "good" or "bad" models. This section outlines the development of criteria (not statistical) that have been applied within a range of published studies. Clouse and Heatwole (1996) stated that "primary usefulness is in assessing which modelling scenarios are better predicted than other scenarios". However, other authors have taken a different approach by setting definitive criteria for several statistics.

Criteria used in James and Burges (1982) included CD and EF > 0.97 for good hydrological model performance. Dillaha (1990) stated that good hydrologic model assessment should estimate observed values within a factor of two, where parameters are measured on site, or where the model is calibrated and within a factor of 10 otherwise. Kumar (2000) used a criterion that air dispersion model estimates be within a factor of two, by looking at the percentage of estimates meeting such a criterion ($\mathrm{Fa}_2 \geqslant 80\%$), combined with: NMSE $\leqslant 0.5$, $-0.5 \leqslant$ FB $\leqslant +0.5$, $0.75 \leqslant$ MG $\leqslant 1.25$, and $0.75 \leqslant$ VG $\leqslant 1.25$.

The general categorization for the range of values of Pearson's correlation coefficient (r) by Hinkle et al. (1994) may indicate a straightforward (non-statistically based) way of interpreting the calculated correlation between estimates and measurements: 0.0 to 0.3, little (very weak) if any correlation; 0.3 to 0.5, low (weak) correlation; 0.5 to 0.7, moderate correlation; 0.7 to 0.9, high (strong) correlation; 0.9 to 1.0 very high (very strong) correlation.

In the Erosion Productivity Impact Calculator validation performed by Chung et al. (1999), the following criteria were chosen to assess if the model results were satisfactory: RMSE and MdAE $< 50\%$, EF and REF$>$ 0.3, $-0.2 <$ CRM $+0.2$. Standards of $<$20% for the percentage error and $>$0.5 for r^2 were also set. In a following paper (Chung et al., 2000) the target criteria to judge if the model results were satisfactory were: EF $>$ 0.3, $r^2 > 0.5$, and P-value of the paired t-test between the observed and simulated values $>$0.025.

In Stöckle et al. (1999) RRMSE and Willmott's d index were taken together to evaluate the weather generator ClimGen. Upper and lower limits were suggested for a solid judgment on the model performance: good when RRMSE $\leqslant 10\%$ and d $\geqslant 0.95$, acceptable when $10\% \leqslant$ RRMSE $\leqslant 20\%$ and d $\geqslant 0.90$, poor with other values. In a following paper, Stöckle et al. (2004) adopted similar standards (but in a more restrictive fashion) for eva1uating evapotranspiration estimates: d $\geqslant 0.95$ and RRMSE $\leqslant 10\%$, very good; d $\geqslant 0.95$ and $15\% \geqslant$ RRMSE $> 10\%$, good; d $\geqslant 0.95$ and $20\% \geqslant$ RRMSE $> 15\%$, acceptable; d $\geqslant 0.95$ and $25\% \geqslant$ RRMSE $> 20\%$, marginal. Other combinations of d and RRMSE values indicated poor performance. In addition, all combinations with slope $>$1.1 or $<$0.9 and $r^2 < 0.85$ of the regression observed versus estimated values (forced through zero) were considered poor.

3.5 Combining Multiple Statistics

It emerges from the discussion above that each individual performance measure will only assess a single component of model behaviour and is not sufficient to judge model adequacy to a given purpose. It is possible that a model can be deemed unsuitable and rejected based on an assessment by one statistic assessing one form of model performance, whilst other attributes of the model may be desirable, as assessed by other measures. Similarly, a model may be seen as acceptable based on one performance statistic but still contains poor qualities not assessed by appropriate tests. The use of multiple metrics allows a greater range of model estimate behaviour to be tested, but still leaves the issue of how to achieve an overall model validation, i.e. weighing up the balance of positive and negative attributes.

The combination of multiple assessment metrics and the setting of criteria have evolved into formal structures, becoming attractive and being regarded as a positive step in achieving robust assessments. Bellocchi et al. (2002a) dealt with the need for integrated methods for model validation by introducing the concept of fuzzy multiple-metric assessment expert system to aggregate different metrics into one indicator for use in solar radiation model validation. The fuzzy system reflects the author's expert judgment about the quality of model performance. This approach enables the calculation of a single indicator made up of a number of individual metrics representing different indices or test statistics. Such an approach provides a comprehensive assessment, making it easier to identify best performing models.

The method exists as a flexible, open-ended structure (Fig. 3) in which a range of metrics can be aggregated into a single modular indicator, based on an expert weighting expression of the balance of importance of the individual indices and their aggregation into modules. The Bellocchi et al. (2002a) study used: relative root mean square error (RRMSE), modelling efficiency (EF), the probability of paired Student-*t* test (*P*(t)); the correlation coefficient of the estimates versus measurements (r) and the two pattern indices detailed in Donatelli et al. (2004a), one computed versus day of year (PI_{doy}), and the other versus minimum temperature (PI_{Tmin}). Values of each statistic are computed and then aggregated into modules: Accuracy (RRMSE, EF, *P*(t)); Pattern (PI_{doy}, PI_{Tmin}); and Correlation (r). A module is a validation measure calculated via a fuzzy-based procedure from one or more basic statistics. For each module, a dimensionless value between 0 (best model response) and 1 (worst model response) is calculated. The method adopts the Sugeno approach of fuzzy inference (Sugeno, 1985). Three membership classes can be defined for all indices, according to expert judgment, i.e. favourable, unfavourable and partial (or fuzzy) membership, using S-shaped curves as transition probabilities in the range favourable to unfavourable. A two-stage design of a fuzzy-based rules inferring system is applied, where firstly several metrics are aggregated into modules and then, using the same procedure, the modules are aggregated in a second level integrated index (again, ranging

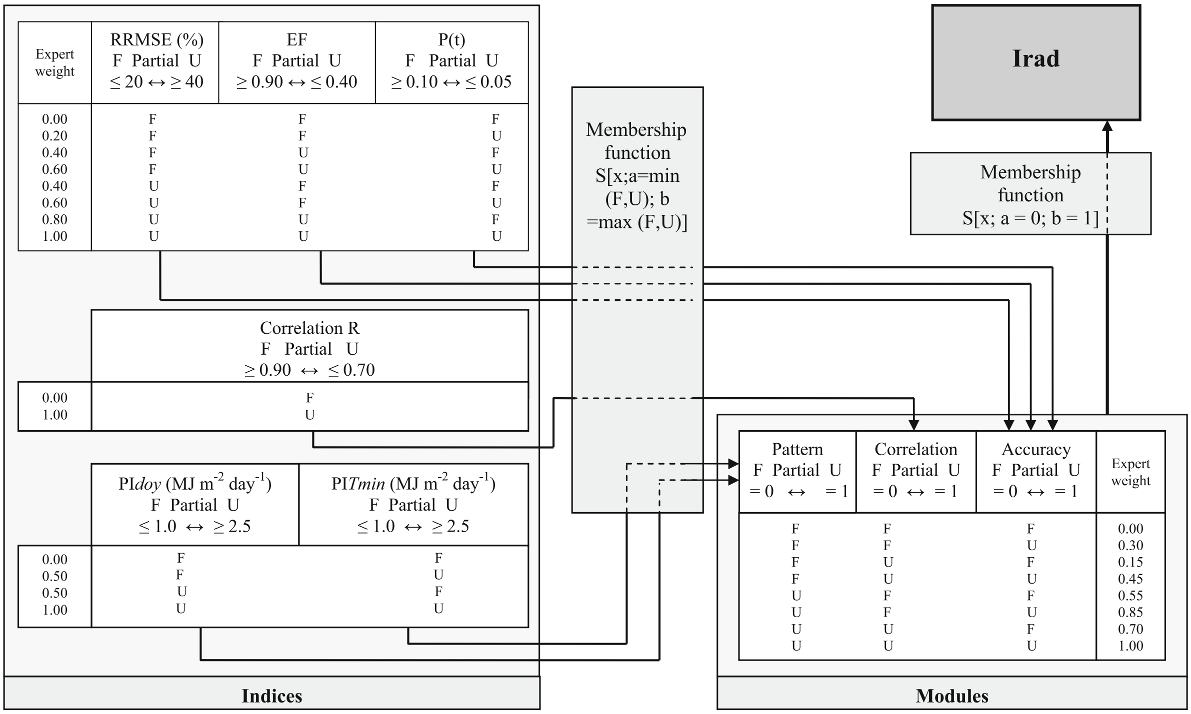


Fig. 3 Structure of the fuzzy-based integrated index for solar radiation model assessment (F = favourable; U = unfavourable; S = membership function; a = minimum value of F; b = maximum value of U; after Rivington et al., 2005)

from 0 to 1), called *indicator*. The expert reasoning runs as follows: if all input variables are favourable, the value of the module is 0 (good agreement between estimates and measurements); if all indices are unfavourable, the value of the module is 1 (poor agreement), while all the other combinations assume intermediate values. The weights can be chosen based on the users own experience in handling each statistic. In Bellocchi et al. (2002a) a decreasing importance was assigned to the modules: Accuracy, Pattern and Correlation. Rivington et al. (2005), Diodato and Bellocchi (2007b, c) and Abraha and Savage (2008) demonstrated the value of employing this method, in conjunction with using graphical illustrations, to gain a fine level of detail about model quality. Similarly to this, Donatelli et al. (2004b) developed integrated indices for evaluating the estimates from pedotransfer functions.

A fuzzy-based methodology was also proposed (Donatelli et al., 2002a) to identify mismatches between estimated and measured time series by using a fuzzy-based approach. The mismatch in time series comparison of LEACHM soil nitrogen estimates was identified by means of an integrated index derived aggregating RMSE and PI_{doy} (values $\leqslant 2$ as favourable and $\geqslant 12$ as unfavourable for both), 0.8 and 0.2 being the relative weights, and calculated reiterating the computation over a 100-day shift of model estimates. The same approach was integrated with a complementary set of statistics from the environmental modelling literature (e.g. Environmental Protection Agency, 1991) to evaluate SUNDIAL soil mineral nitrogen estimates (Bellocchi et al., 2004). Bellocchi et al. (2002b) extended the original fuzzy-based multiple-metric assessment system approach to aggregating the RRMSE values (values $\leqslant 20\%$ as favourable and $\geqslant 40\%$ as unfavourable) computed over different outputs in cropping systems modelling under different sets of conditions, thus allowing a comprehensive assessment of the model's performance by means of one integrated index. They attributed major weight to above ground biomass (i.e., 2), where a minor incidence of soil variables (water content: 1, nitrate content: 0.5) was recognized.

Aggregating measures of performance have in common that the information contained in the errors is aggregated into a single numerical value. Herbst and Casper (2008) argued that essentially different model results can be obtained with close to identical performance measure values. Because of their low discriminatory power, performance measures might not be well suitable to give evidence of the difference or equivalence between alternative model realizations. As a step towards improved extraction of information from existing data they introduced an approach, the Self-Organizing Map (SOM), which uses a number of performance statistics and represents, for each of them, the colour-coded spectrum of model realizations obtained from Monte-Carlo simulations. It was applied to a distributed conceptual watershed model. SOM is a type of artificial neural network and unsupervised learning algorithm that is used for clustering, visualization and abstraction of multi-dimensional data. Such an algorithmic approach mainly targets the optimization and identification of parameters that mostly affect the model output (sensitivity analysis), and is not of direct interest for this review.

3.6 Summary

Because of the vast collection and diversity of the approaches to assess models, we sorted through and straightened out validation statistics showing how they were introduced and applied to biophysical modelling. The review of the measures of performance that are commonly used in model validation reveals the positions assumed over time in the modelling literature mostly emphasising that a single statistic will only reveal one aspect of model performance. As each approach has its advantages and drawbacks, they are rather complementary and are generally used in combination in model validation. What values for assessment metrics indicate satisfactory models remains a subjective issue and no definitive guidance exists because of heterogeneity of approaches and application domains. While agreeing with many authors that model validation has to be performed using a set of dissimilar validation statistics, we in particular advocate the use of combined multiple statistics where several measures for validation can be considered both separately (each individual metric) and collectively (integrated indicator). Test statistics may be problematic because they rely on assumptions that are difficult to check in biophysical systems. Decomposition of statistics in basic terms may disclose the characteristic and the actual structure of the error, but the combination of multiple metrics into synthetic indicators where subjective choices (expert decisions) are converted into explicit and transparent rules reveals a more comprehensive picture. The lack of precise and undisputable criteria to consider a specific metric as more effective than others, and the multiplicity of aspects to be accounted for a multi-perspective evaluation of model performance, logically leads to some use of composite metrics for model validation. A composite metric is not the only output of composition: the modeller can "drill down" to module values, and finally to basic metrics to better understand the synthetic result provided by the composite indicator. In such respect, composition of metrics should be considered a shift of paradigm from merely selecting the best out of a set of evaluation metrics.

4 Conclusion

This paper discusses issues concerned with model validation and reviews the most commonly used forms of estimate testing. Exposition of material and explanation of concepts presented in Section 2 ("Issues on model validation") reflect the authors' perception of issues that are fundamental to understanding the factors that are related to model validation. The examples provided throughout the text demonstrate how previous instances of model use (and success or failure associated with that use) are the growing knowledge bases acquired from using different models for various applications. The publications cited show how the scope and capabilities of validation approaches have evolved and improved with time. Though finding solution of how best to evaluate numerical values produced by models will remain an issue, a range of approaches do exist for improving the testing of model estimates. Our hope is that these approaches will continue to evolve.

Our historical reconstruction of the approaches serving the validation purposes, as presented in Section 3 ("Validation of models"), points towards three main outcomes achieved: disaggregation of validation statistics into basic components, introduction of validation criteria, and combination of statistics into synthetic indicators. Baseline thresholds of validation measures (extracted from the international literature and recapped in sub-section "Validation criteria") provide users with the modellers' perception of good/bad performance statistics. Such criteria are presented and discussed not only to make available reference values of possible use in future validation studies, but also they call on the need for using expert rules to guide the validation process. This review of the methods available for numerical testing has shown that greater value can be gained through combined use and rule-based aggregation of multiple approaches to achieve a more complete form of validation.

Advancements in these numerical testing methodologies for validation need to be put into structured frameworks comprised of processes such as sensitivity and uncertainty analyses (parameter and input variable appraisal), parameter optimization, model structure assessment (expert review), software testing, etc. As such, validation must be seen as an integral part of the overall model development and application process, whilst also encompassing the requirement for better data quality control and meta data recording. This places a greater emphasis on the need to include validation plans within model project proposals and a higher level of support for validation work by funding organisations. As shown, models may come in a variety of time and space resolutions and scales. Matching these scales and ensuring consistency in the overall model is not a trivial process and may be difficult to fully automate. Techniques to validate models need to be developed at the same pace with which the models themselves are created, improved and applied. Also, validation steps must be clearly stated, accessible, transparent, and understandable to non-modellers. As discussed in the context of the current knowledge, this can be achieved by means of reliability statistics, history of previous use, or personal preferences. However, details about validation techniques development go beyond the aim of this review, and a second paper on this broad topic may be arranged later as a natural evolution of what has already been presented.

Acknowledgements This paper was developed with the support of the Italian Ministry of Agricultural Policies; project SIPEAA (http://www.sipeaa.it), paper No. 36. The authors also gratefully acknowledge the support of the Scottish Government Environment Rural Affairs Department. Thanks also to David Elston and David Walker of Biomathematics and Statistics Scotland (Macaulay Institute, Aberdeen, UK) for constructive criticism and suggestions.

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Cold Stress Tolerance Mechanisms in Plants

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Contents

Abstract The human population is increasing at an alarming rate, whereas at the same time agricultural productivity is decreasing due to the effect of various environmental problems. In particular, cold stress is a serious threat to the sustainability of crop yields. Indeed, cold stress can lead to major crop losses. Various phenotypic symptoms in response to cold stress include poor germination, stunted seedlings, yellowing of leaves (chlorosis), reduced leaf expansion and wilting, and may lead to death of tissue (necrosis). Cold stress also severely hampers the reproductive development of plants. The major negative effect of cold stress is that it induces severe membrane damage. This damage is largely due to the acute dehydration associated with freezing during cold stress. Cold stress is perceived by the receptor at the cell membrane. Then a signal is transduced to switch on the cold-responsive genes and transcription factors for mediating stress tolerance. Understanding the mechanism of cold stress tolerance and genes involved in the cold stress signaling network is important for crop improvement. Here, I review cold stress tolerance mechanisms in plants. The major points discussed are the following: (1) physiological effects of cold stress, (2) sensing of cold temperatures and signal transduction, and (3) the role of various cold-responsive genes and transcription factors in the mechanism of cold stress tolerance.

Keywords Cold stress • Signal transduction • Cold-responsive genes • Transcription factors

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_27,

from Yadav, Agron. Sustain. Dev. 30 (2010) 515–527. DOI: 10.1051/agro:2009050

1 Introduction

One-third of the total land area is considered as potentially suitable for arable agriculture. However, only some 10% of the world's 13 billion hectares are farmed. This is because abiotic stress in one form or another limits production on most of the world's 1.4 billion farmed hectares of land. This is a problem that is not going away. Among abiotic stresses, low and high temperature stress is very critical for determining the agricultural production. Plants exhibit a maximum rate of growth and development at an optimum temperature or over a diurnal range of temperatures (Fitter and Hay, 1981). When ambient temperature deviates from optimal, physiological, biochemical, metabolic and molecular changes occur within plants. This is an effort of plants to maximize growth and developmental processes and to maintain cellular homeostasis during such adverse conditions. Under increasingly stressful conditions, plants experience progressively more abnormal, impaired or dysfunctional cellular and whole-plant processes until the cardinal temperatures for survival are reached (Fitter and Hay, 1981). At the extremes of the natural temperature range of a plant, the degree of physiological, cellular, metabolic and molecular dysfunction becomes so severe that it leads to death.

Plants feel stress under high as well as low temperature exposure. Mesophilic temperate plants possess inducible temperature-stress tolerance. During high temperature exposure, stress tolerance can be induced by exposure to short-term elevated temperature and this is known as acquired thermotolerance (Kotak et al., 2007), while at lower temperatures, stress tolerance can be induced by exposure to reduced temperature and is known as chilling tolerance and/or cold acclimation. Chilling tolerance is the ability of a plant to tolerate low temperatures (0–15 °C) without injury or damage (Somerville, 1995), while cold acclimation is an enhanced tolerance to the physical and physiochemical vagaries of freezing stress (Guy, 1990; Thomashow, 1999). Both cold acclimation and chilling tolerance involve an array of biochemical, molecular and metabolic processes (Thomashow, 1999; Larkindale et al., 2005; Kotak et al., 2007; Zhu et al., 2007).

Exposure of plants to temperature stress leads to the modification of metabolism in two ways. First, plants try to adjust their cellular metabolism that altered due to rising or falling of temperatures. Temperature stress changes the structure, catalytic properties and function of enzymes (Kubien et al., 2003) and membrane metabolite transporters. Interestingly, regulatory mechanisms of plants become active and function to restore normal metabolite levels, and most importantly, metabolic fluxes (Schwender et al., 2004; Fernie et al., 2005). Secondly, the modifications of metabolism in response to temperature stress are mainly linked to enhanced tolerance mechanisms. Many metabolites thought to have important properties that could contribute to induced stress tolerance have long been linked to stress responses (Guy, 1990; Thomashow, 1999; Nayyar et al., 2005). Particular interest has been focused on metabolites that can function as osmolytes. Osmolytes are involved in the regulation of cellular water relations and reduce cellular dehydration. Their compatible solute behavior allows them to function to stabilize enzymes, membranes and other cellular components. Osmolytes are also involved in retailoring of membrane lipid composition to optimize the liquid/crystalline physical structure necessary for proper membrane function and energy sources. Such stress-responsive metabolites particularly include soluble sugars, amino acids, organic acids, polyamines and lipids (Levitt, 1972; Guy, 1990; Nayyar et al., 2005; Farooq et al., 2009).

Plants experience cold or chilling stress at temperatures from 0–15 °C. Under such situations, plants try to maintain homeostasis to acquire freezing tolerance and this involves extensive reprograming of gene expression and metabolism (Thomashow, 1999; Cook et al., 2004). In the recent past, great attention has been paid towards elucidation of the intricate signal transduction pathways responsible for low-temperature response (Lee et al., 2005). Fundamental responses of plants during cold stress exposure and acclimation mechanisms are presented in Fig. 1. Through work carried out in the last decade or so, several key components of signaling pathways and regulatory mechanisms contributing to freezing tolerance have been identified. This article summarizes the latest work that addresses the following questions: what are the physiological effects of cold stress in plants? How are cold temperatures sensed by plants? How is a cold stress signal transduced to the nucleus to regulate gene expression? What are different genes and transcription factors involved in the mechanism of low temperature tolerance?

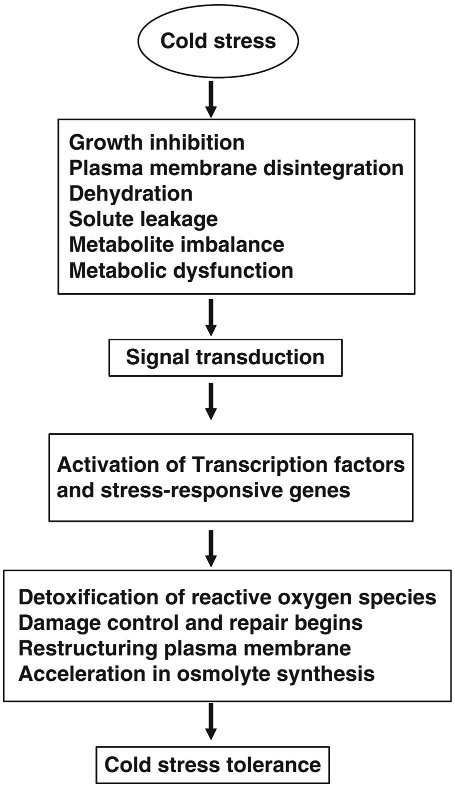

Fig. 1 Fundamental responses of plants during cold stress exposure. Cold stress exposure causes various physiochemical disturbances, leading to growth inhibition. Cold stress response is perceived by plants through a signal transduction that leads to the activation of transcription factors and cold-responsive genes. Such transcription factors and genes control the damage due to cold stress and help in providing tolerance to plants

2 Effect of Cold on Plant Physiology

Each plant has an optimum set of temperatures for its proper growth and development. A particular set of temperature conditions, which are optimum for one plant, may be stressful for another plant. It has generally been noticed that plants native to warm habitats exhibit symptoms of injury upon exposure to low non-freezing temperatures (Lynch, 1990). For example, plants such as maize (*Zea mays*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*), tomato (*Lycopersicon esculentum*) and banana (*Musa* sp.) show signs of injury upon exposure to temperatures below 10–15 °C (Lynch, 1990; Guy, 1990; Hopkins, 1999). However, the appearance of injury symptoms depends upon the sensitivity of a plant to cold stress and varies from plant to plant. Cold stress-induced injury in plants may appear after 48 to 72 h of stress exposure. Plants exposed to cold stress show various phenotypic symptoms that include reduced leaf expansion, wilting and chlorosis (yellowing of leaves) and may lead to necrosis (death of tissue). The phenotypic symptoms of rice seedlings upon exposure to cold stress are shown in Fig. 2. Cold stress also severely affects the reproductive development of plants and this has been seen in rice plants at the time of anthesis (floral opening), which leads to sterility in flowers (Jiang et al., 2002).

Cold stress generally results in poor germination, stunted seedlings, yellowing of leaves, withering and reduced tillering. The effects of cold stress at the reproductive stage of plants delay heading and result in pollen sterility, which is thought to be one of the key factors responsible for the reduction in grain yield of crops (Suzuki et al., 2008). The major adverse effect of cold stress in plants has been seen in terms of plasma membrane damage. This has been documented due to cold stress-induced dehydration (Steponkus, 1984; Steponkus et al., 1993). The plasma membrane is made up of lipids and proteins. Lipids in the plasma membrane are made up of two kinds of fatty acids: unsaturated and saturated fatty acids. Unsaturated fatty acids have one or more double bonds between two carbon atoms, whereas saturated fatty acids are fully saturated with hydrogen atoms. Lipids containing more saturated fatty acids solidify faster and at temperatures higher than those containing unsaturated fatty acids. Therefore, the relative proportion of these two types of fatty acids in the lipids of the plasma membrane determines the fluidity of the membrane (Steponkus et al., 1993). At the transition temperature, a membrane changes from a semi-fluid state into a semi-crystalline state. Cold-sensitive plants usually have a higher proportion of saturated fatty acids in their plasma membrane. Therefore, cold-sensitive plants have a higher transition temperature. On the contrary, cold-resistant plants have a higher proportion of unsaturated fatty acids and hence a lower transition temperature.

The agricultural crops which can withstand even during the freezing temperatures of late spring or early fall frost can be used more successfully for cultivation during cold stress. Therefore, selection of low temperature-tolerant crops is very important for the sustainability of agriculture. Additionally, understanding of how cold stress induces its injurious effects on plants is crucial for the development of frost-tolerant

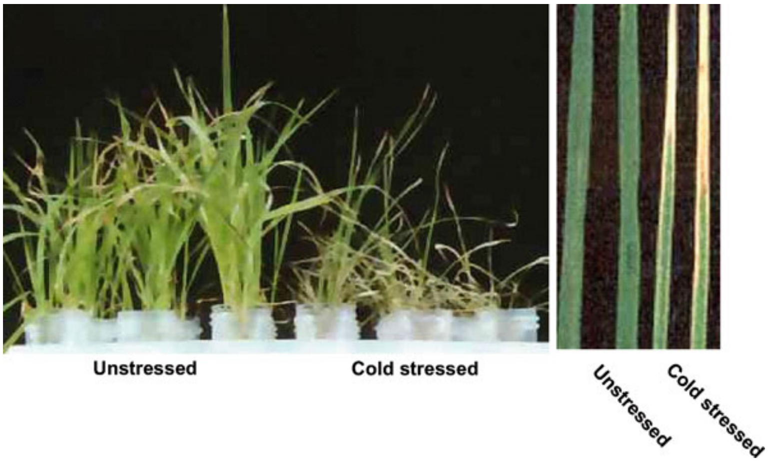

Fig. 2 Effect of cold stress on rice seedlings. Rice seedlings exposed to −2°C for 12 h and then allowed to recover for 1 week. Damage due to cold stress is seen as retardation in growth, reduced leaf expansion, wilting, chlorosis (yellowing of leaves) and necrosis (death of tissue). Picture on right hand side presents a closer view of the unstressed and cold stress-exposed leaves of rice

crops. It has been noticed that cold-induced ice formation is the real cause of plant damage. Ice formation in plant tissues during cold stress leads to dehydration. Ice is formed in the apoplastic space of a plant tissue because that has relatively lower solute concentration. It is known that the vapor pressure of ice is much lower than water at any given temperature. Therefore, ice formation in the apoplast establishes a vapor pressure gradient between the apoplast and surrounding cells. As a result of this gradient, the cytoplasmic water migrates down the gradient from the cell cytosol to the apoplastic space. This adds to existing ice crystals in the apoplastic space and causes a mechanical strain on the cell wall and plasma membrane, leading to cell rupture (McKersie and Bowley, 1997; Olien and Smith, 1997; Uemura and Steponkus, 1997).

In addition to the well-established harmful effect of cold stress alterations in lipid composition of the biomembranes, affecting their fluidity (Senser and Beck, 1982; Quinn, 1985; Williams, 1990; Welti et al., 2002), certain additional factors may also contribute to damage induced by cold stress. This includes synthesis and accumulation of compatible solutes, the synthesis of cold acclimation-induced proteins (Close, 1997; Shinozaki and Yamaguchi-Shinozaki, 2000), changes in the carbohydrate metabolism (Hansen and Beck, 1994; Hansen et al., 1997; Liu et al., 1998; Frankow-Lindberg, 2001) and the boosting of the radical scavenging potential of the cells (Tao et al., 1998; Hernández-Nistal et al., 2002; Baek and Skinner, 2003). Taken together, cold stress results in loss of membrane integrity, leading to solute leakage. Further, cold stress disrupts the integrity of intracellular organelles, leading to the loss of compartmentalization. Exposure of plants to cold stress also causes reduction and impairing of photosynthesis, protein assembly and general metabolic processes.

Recently, attempts have also been directed towards analyzing the effect of cold stress on the whole-plant metabolome. Metabolic profiling of *Arabidopsis* plants revealed that cold acclimation increases 75% of the 434 analyzed metabolites (Cook et al., 2004; Kaplan et al., 2004). However, metabolite profiles in *Arabidopsis* do not appear to correlate with cold acclimation capacity (Hannah et al., 2006). The role of such metabolites in plants has been known as osmoprotectants. In addition to their role as osmoprotectants and osmolytes, certain metabolites (individual metabolites or the redox state) induced during cold acclimation might act as signals for reconfiguring gene expression. For example, cold stress induces the accumulation of proline, a well-known osmoprotectant. Microarray and RNA gel blot analyses have shown that proline can induce the expression of many genes, which have the proline-responsive element sequence ACTCAT in their promoters (Satoh et al., 2002; Oono et al., 2003).

Cold stress affects virtually all aspects of cellular function in plants. One of the major influences of cold stress-induced dehydration is membrane disintegration. Such changes caused by cold stress adversely affect the growth and development of plants.

3 Sensing and Signal Transduction During Cold Stress

Plants are sessile in nature. Therefore, the only way for them to survive under adverse environmental conditions is to adapt to changing surroundings quickly and efficiently. The plastic nature of higher plants makes them able to react to different stresses with specific responses in growth, development and metabolism. In view of this, understanding of such plant responses could be of great importance for agriculture. This might be helpful in developing stress-tolerant crop varieties. Cold stress exposure reduces the fluidic nature of cellular membranes and increases their rigidity. This documents that the primary site of cold stress sense in plants could be associated with membrane fluidity, protein and nucleic acid conformation, metabolite concentration, and cellular redox status.

Cold stress is sensed by plant cells through its membrane rigidification effect. Rigidification of the plasma membrane during cold exposure has been shown to induce cold-responsive genes that help in cold acclimation in alfalfa and *Brassica napus* (Orvar et al., 2000; Sangwan et al., 2001). The *Arabidopsis* fad2 mutant defective in oleate desaturase exhibits membrane rigidification and cold stress sensitivity, suggesting the relation of cold stress sense to plasma membrane rigidification (Vaultier et al., 2006). Additionally, cold stress exposure in plants has been found to increase cytosolic calcium levels. This increase in calcium is mediated through membrane rigidification-activated mechano-sensitive or ligand-activated calcium channels. The higher level of calcium in the cytosol leads to signal amplification through phospholipids (Vergnolle et al., 2005; Williams et al., 2005; Chinnusamy et al., 2006; Komatsu et al., 2007).

Environmental stresses including cold stress are first perceived by the receptors present on the membrane of the plant cells. The specific receptor for cold stress is not yet known. The signal is then transduced downstream and many signaling pathways are activated. Studies have shown that such pathways are often activated in concert. The various components are calcium, reactive oxygen species, protein kinase, protein phosphatase and lipid signaling cascades. It is believed that specificity is achieved by the combination and timing of the activation of different signaling pathways. The change in cytosolic calcium level is sensed by calcium-binding proteins. These calcium-binding proteins do not possess enzymatic activity but undergo conformation changes in a calcium-dependent manner. The change in calcium-binding proteins makes them interact with other proteins and often initiates a phosphorylation cascade. Through this cascade, plant cells could target major stress-responsive genes or the transcription factors. Transcription factors also regulate the expression and function of genes, which ultimately leads to plant adaptation and survival during unfavorable conditions (Shinozaki and Yamaguchi-Shinozaki, 2000; Shinozaki et al., 2003; Mahajan and Tuteja, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006).

Individual plant cells respond to the environmental stress in the way described above and then the whole plant acts synergistically. The change in gene expression governed by the signal cascade mechanism also induces changes in genes participating in the formation of plant hormones such as abscisic acid, salicylic acid and ethylene. These hormones may amplify the same cascade or may initiate a new signaling pathway. Additionally, several other cellular components are also involved in the stress signal transduction mechanism. These accessory molecules may not directly participate in signaling but participate in the modification or assembly of major signaling components. Mainly such components are protein modifiers and act in post-translational modification of signaling proteins. Such modifiers are involved in myristoylation, glycosylation, methylation and ubiquitination of signaling proteins (Mahajan and Tuteja, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006).

Lipid molecules are also very important in signal transduction during cold stress. Though lipid signaling is relatively less studied, phosphatidic acid produced by both phospholipase D and the concerted action of phospholipase C and diacylglycerol kinase has been proposed as a membranous secondary messenger molecule. Phosphatidic acid is rapidly and transiently generated in response to various stresses and has been proposed to function as a second messenger (Meijer and Munnik, 2003). This phosphatidic acid constitutes a minor portion of membrane lipids under control conditions, but its levels significantly increased upon exposure to numerous stresses including cold stress (Munnik, 2001; Meijer and Munnik, 2003). Several modes of action for phosphatidic acid in signal transduction can be imagined. Its functions are mainly based on the recruitment and regulation of enzymatic activity of target proteins. The recruitment of soluble proteins to particular membranes can have numerous effects, such as localization of a protein to the site where it is active, localization of a protein to a site where it is modified and sequestration of a protein from the site where it is active. Alternatively, phosphatidic acid could influence the enzyme activity of proteins already residing in the membrane or of recruited proteins or protein complexes. A plethora of phosphatidic acid-binding proteins have been found in different organisms, and many have been proposed to function in signaling cascades (Testerink and Munnik, 2005).

Abscisic acid is an important phytohormone that plays a crucial role in several plant stress responses, including cold stress (Verslues and Zhu, 2005; Mahajan and Tuteja, 2005). Furthermore, phospholipase D has been linked to reactive oxygen species, which are known to be involved in abscisic acid and in cold stress responses (Laloi et al., 2004; Mahajan and Tuteja, 2005). In *Arabidopsis*, different phospholipases D such as *AtPLDa1* and *AtPLDd* have been implicated in both the production of and responses to reactive oxygen species (Zhang et al., 2003, 2005). The *AtPLDd* expression was shown to be induced by abscisic acid and cold in *Arabidopsis* plants (Katagiri et al., 2001). Cold-induced freezing tolerance is reportedly impaired in *Atpldd* T–DNA knock-out *Arabidopsis* mutant plants and enhanced in *AtPLDd*-overexpressing plants, which also display decreased and increased freezing-induced phosphatidic acid production, respectively (Li et al., 2004). As a whole, it seems that phospholipases D are involved in multiple aspects of both the overlapping and distinct signaling networks that are activated by cold stress. Cold stress-induced increase in phospholipases D is also involved in changes in membrane fluidity and osmotic balance.

Secondary signals, such as abscisic acid and reactive oxygen species, can also induce calcium signatures that impact cold signaling. *Arabidopsis* mutants defective in the activation of the molybdenum cofactor of abscisic aldehyde oxidase, namely aba3/freezing sensitive 1 (*frs1*) (Llorente et al., 2000), also known as *los5* (low expression of osmotically responsive genes 5) (Xiong et al., 2001), exhibit hypersensitivity to freezing stress. The *los5* mutant plants show a significant reduction in the expression of cold- and osmotic stress-induced genes (Xiong et al., 2001). Reactive oxygen species accumulate in plant cells during exposure to various abiotic stresses, and they appear to have a strong influence on cold regulation of gene expression. The *Arabidopsis fro1* (frostbite1) mutant, which constitutively accumulates high levels of reactive oxygen species, exhibits impaired expression of cold-responsive genes and hypersensitivity to chilling and freezing. The *FRO1* gene encodes the Fe–S subunit of complex I (NADH dehydrogenase) of the respiratory electron transfer chain in mitochondria, and its disruption leads to high levels of reactive oxygen species generation (Lee et al., 2002). Besides their effect on calcium signatures, reactive oxygen species signals can also exert their effects directly through the activation of redox-responsive proteins, such as transcription factors and protein kinases.

Ultimately, cold acclimation involves precise regulation of expression of transcription factors and effector genes, collectively known as cold-regulated genes (Thomashow, 1999; Viswanathan and Zhu, 2002). Significant progress has been made in identifying transcriptional, post-transcriptional and post-translational regulators of cold-induced expression of cold-regulated genes. Promoters of many of the cold-regulated genes contain cis-elements such as dehydration-responsive elements/C-repeat elements (A/GCCGAC), an abscisic acid-responsive element (PyACGTGGC), and myeloblastosis recognition sequences (C/TAACNA/G) and/or myelocytomatosis recognition sequences (CANNTG) (Yamaguchi-Shinozaki and Shinozaki, 2005). Additionally, abiotic stresses including cold stress regulate the expression and activity of various kinases of mitogen-activated protein kinase pathways. Under cold stress, reactive oxygen species activate the AtMEKK1/ANP1 (MAPKKK)-AtMKK2 (MAPKK)-AtMPK4/6 (MAPK) mitogen-activated protein kinase cascade that is necessary for cold acclimation in plants (Kovtun et al., 2000; Teige et al., 2004). This suggests that mitogen-activated protein kinase cascades act as a converging point in abiotic stress signaling.

Cold stress is sensed by a yet unknown receptor. The signal is then transduced through several components of signal transduction pathways. The components of signal transduction pathways discussed here are also involved in the transduction of other environmental stress signals, suggesting a common pathway for several responses in plants. The cold stress signal leads to changes in the expression of cold-regulated genes and the level of expression determines the fate of plants under such stress.

4 Role of Cold-Responsive Genes and Transcription Factors

Developing transgenic plants for cold tolerance is a fast and effective biotechnological tool to improve agricultural crops. Transgenic approaches have been used to improve cold tolerance in rice. Various studies on crop improvement have indicated that screening for genes involved in cold tolerance is a crucial initial step for crop improvement strategy using engineering (Hsieh et al., 2002; Dubouzet et al., 2003; Ohnishi et al., 2005; Ito et al., 2006). Among the genes identified for cold tolerance, genes encoding transcription factors are found to be better for improving stress tolerance in plants. Most of such transcription factors have been identified in *Arabidopsis* and rice. The functional and biochemical features of specific cell types are determined by their particular gene expression profiles. Such global gene expression patterns can be represented by a "transcriptome", which reveals the identity and the level of expression of each expressed gene in a defined population of cells (Velculescu et al., 1997; Tuteja and Tuteja, 2004). The transcriptome can be modulated by both external and internal factors, and thereby provide not only a wealth of basic biological insights but also a global view of biological responses to environmental stimuli.

Transcriptome expression profiles of a plant under normal and stressed conditions can be obtained and compared by various methods, such as ribonucleic acid (RNA)–deoxyribonucleic acid (DNA) hybridization measurements, subtractive hybridization, subtraction libraries and differential display (Donson et al., 2002). However, these methods have technical shortcomings and therefore, are unable to provide overall gene expression patterns. The recent DNA microarray technique allows larger-scale quantitative gene expression analysis. Microarray is a very useful technique for transcriptome analysis. This has been used in identifying many stress-inducible genes involved in stress response and tolerance (Shinozaki et al., 2003; Seki et al., 2004). A large number of genes responsive to various abiotic stresses have been identified using various microarrays (Seki et al., 2001,2002; Fowler and Thomashow, 2002; Kreps et al., 2002; Rabbani et al., 2003; Bray, 2004; Maruyama et al., 2004; Vogel et al., 2005). However, microarray has some innate limitations. It only allows the analysis of arbitrarily chosen genes (Duggan et al., 1999; Jones et al., 2001; Patankar et al., 2001; Lorenz and Dean, 2002; Gibbings et al., 2003). Another technology known as serial analysis of gene expression partially overcomes this limitation (Velculescu et al., 1995). It is an extremely promising, efficient and global approach for analyzing gene expression profiles under different physiological states. It has the advantage over various other techniques that it allows identifying novel genes along with their associated pathways and metabolic circuits. Also, the Affymetrix 22K GeneChip ATH1 has been used recently to identify more stress-inducible genes. The genes identified using the Affymetrix 22K GeneChip ATH1 can be seen at The Arabidopsis Information Resource Uniform Resource Locator (http://www.arabidopsis.org/).

Upon stress exposure, the expression of various genes alters. Such stress-responsive genes have been found to provide protection to plants in two ways. They protect from stress by producing important metabolic proteins. The products of stress-responsive genes are also involved in regulation of genes of signal transduction pathways. Based upon different functions of proteins encoded by stress-responsive genes, these are classified into two groups (Fowler and Thomashow, 2002; Kreps et al., 2002; Seki et al., 2002). The first group includes proteins that probably function in stress tolerance, such as chaperones, late embryogenesis abundant proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis such as proline, water channel proteins, sugar and proline transporters, detoxification enzymes, enzymes for fatty acid metabolism, proteinase inhibitors, ferritin, and lipid-transfer proteins. Some of these stress-inducible genes encoding key enzymatic proteins for osmolyte biosynthesis, late embryogenesis abundant proteins, and detoxification enzymes have been overexpressed in transgenic plants. Such transgenics have been found to be stress-tolerant (Cushman and Bohnert, 2000). This has evinced that the gene products of the stress-inducible genes really function in stress tolerance.

The second group contains protein factors involved in further regulation of signal transduction and gene expression that probably function in stress response. This group includes various transcription factors (Seki et al., 2003). These transcription factors regulate various stress-inducible genes cooperatively or separately, and may constitute gene networks. Functional analysis of such stress-inducible transcription factors should provide more information on the complex regulatory gene networks that are involved in responses to stresses including cold stress. There are a few more proteins involved in the complex network of stress regulatory mechanism of plants such as kinases, protein phosphatases, enzymes involved in phospholipid metabolism, calmodulin binding protein and 14-3-3 proteins. The functions of most of these genes are not fully understood yet. Some of these stress-inducible regulatory genes that encode transcription factors have been overexpressed in plants and generated stress-tolerant phenotypes in transgenic plants (Zhang et al., 2004; Tester and Bacic, 2005; Vinocur and Altman, 2005).

Cold stress-induced genes are known as cold-responsive genes. Most of such genes encoding polypeptides are homologs of late embryogenesis abundant proteins and the polypeptides that are synthesized during the late embryogenesis phase (Dure, 1993; Ingram and Bartels, 1996; Close, 1997). These late embryogenesis abundant-like proteins are mainly hydrophilic and have relatively simple amino-acid composition. They are composed largely of a few amino acids with repeated amino acid sequence motifs and are predicted to contain regions capable of forming amphipathic alpha-helices. The examples of cold-responsive genes include *COR15a* (Artus et al., 1996), alfalfa *Cas15* (Monroy et al., 1993) and wheat *WCS120* (Houde et al., 1992). The expression of cold-responsive genes has been shown to be critical for both chilling tolerance and cold acclimation in plants. The identified *Arabidopsis* cold-responsive genes are *COR78/RD29*, *COR47*, *COR15a* and *COR6.6* (Thomashow, 1999). These genes are induced by cold, dehydration or abscisic acid. A COR15A polypeptide encoded by the *COR15a* gene is targeted to the chloroplast. Formation of hexagonal II phase lipids is a major cause of membrane damage in non-acclimated plants during cold stress. However, *COR15a* expression decreases the propensity of the membranes to form hexagonal II phase lipids in response to freezing (Uemura and Steponkus, 1997). This observation suggests the role of COR15A polypeptide in providing freezing tolerance.

Every gene has promoter sequences upstream of their coding region. The expression of a gene is driven by its promoter. Promoters have regulatory elements in their sequences that control the expression of a gene. In view of this, analysis of the promoter elements of cold-responsive genes revealed that they contain dehydration-responsive elements or C-repeats. Some of the cold-responsive genes also contain an abscisic acid-responsive element in their promoter sequences (Yamaguchi-Shinozaki and Shinozaki, 1994; Stockinge et al., 1997). The expression of cold-responsive genes is induced during cold exposure. Exposure of plants to cold stress also induces the expression of a transcription factor, i.e. dehydration-responsive elements or C-repeat binding factors. This suggests that these binding factors regulate the expression of cold-responsive genes during cold stress. Overexpression of dehydration-responsive elements or C-repeat binding factors led to the induction of cold-responsive genes under unstressed conditions, documenting the regulatory role of binding factors in cold-responsive gene expression (Stockinge et al., 1997). Cold stress induces three dehydration-responsive elements or C-repeat binding factor genes in *Arabidopsis* such as AtCBF1 (DREB1B), AtCBF2 (DREB1C) and AtCBF3 (DREB1A). Dehydration-responsive elements or C-repeat binding factors bind to the promoter of cold-responsive genes and other cold-regulated genes. The overexpression of these regulatory elements not only resulted in increased freezing tolerance but also an increase in drought tolerance (Liu et al., 1998). This finding provides strong support that a fundamental role of cold-responsive genes is to protect the plant cells against cellular dehydration.

The role of these various factors has been substantiated by developing transgenics. Constitutive or stress-inducible overexpression of dehydration-responsive elements or C-repeat binding factor-1 or -3 in transgenic *Arabidopsis* plants resulted in constitutive or enhanced expression of cold-responsive genes. This increased abiotic-stress tolerance, including freezing tolerance, in *Arabidopsis* (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Kasuga et al., 1999). Overexpression of *Arabidopsis thaliana* dehydration-responsive elements or C-repeat binding factor-1 or -3 also enhanced chilling, freezing, drought and/or salt-stress tolerance in *Brassica* (Jaglo et al.,2001), tomato (Hsieh et al., 2002), tobacco (Kasuga et al., 2004), wheat (Pellegrineschi et al., 2004) and rice (Oh et al., 2005). Similarly, overexpression of rice (Dubouzet et al., 2003) and maize (Qin et al., 2004) dehydration-responsive element binding factor-1 in transgenic *Arabidopsis* induced constitutive expression of cold-responsive genes and conferred tolerance to freezing and drought stress. These studies have documented that dehydration-responsive elements or C-repeat binding factor-dependent gene expression is an important, evolutionarily conserved component of cold acclimation in diverse plant species. However, transcriptome analysis of dehydration-responsive elements or C-repeat binding factors overexpressing transgenic *Arabidopsis* revealed that only ∼12% of the cold-responsive genes are induced by dehydration-responsive elements or C-repeat binding factors (Fowler and Thomashow, 2002). This suggests the possibility of other transcriptional activators' or repressors' role in cold acclimation.

To find this, T-DNA insertion mutants of *Arabidopsis* were screened. Genetic analysis of mutants led to the identification of an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 as an activator of dehydration-responsive elements or C-repeat binding factor-3 (Chinnusamy et al., 2003). The inducer of dehydration-responsive elements or C-repeat binding factor expression-1 encoded a transcription factor that specifically recognized myelocytomatosis sequence on the promoter of dehydration-responsive elements or C-repeat binding factor-3. Interestingly, transgenic *Arabidopsis* overexpressing an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 did not express dehydration-responsive elements or C-repeat binding factor-3 at warm temperatures, but showed a higher level of expression for dehydration-responsive elements or C-repeat binding factor-3 as well as other cold-responsive genes such as *RD29* and *COR15a* at low temperatures. This suggests that cold stress induced an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 is a crucial activator of dehydration-responsive elements or C-repeat binding factor-3 in plants. In addition to the role of an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 in regulating dehydration-responsive elements or C-repeat binding factors, homeodomain leucine zipper has also been found to interact with dehydration-responsive elements or C-repeat binding factors. The expression of two dehydration-responsive elements or C-repeat binding factor-1 such as cDNAs CaCBFIA and CaCBFIB in hot pepper was induced in response to low temperature stress (4 °C) (Kim et al., 2004). The induction in expression of homeodomain leucine zipper and its interaction with CaCBFIB during cold stress suggested that homeodomain leucine zipper could also be an important regulon of dehydration-responsive elements or C-repeat binding factors. So far, studies have evinced the existence of one of the major and important molecular mechanisms of cold tolerance in plants, as presented in Fig. 3.

One of the largest families of transcription factors, myeloblasts also play a very significant role in regulating gene expression in response to environmental and developmental changes. In this category, myeloblasts are predominant transcription factors. Based on the number of tandem repeats of SANT 'SWI3, ADA2, N-CoR and TFIIIB' DNA-binding domains myeloblastosis transcription factors are divided into three subfamilies: myeloblastosis-like proteins (MYB1R factors), R2R3-type myeloblastosis factors and R1R2R3 myeloblastosis (MYB3R) factors with one, two or three repeats, respectively (Rosinski and Atchley, 1998).

In *Arabidopsis*, 198 genes encode myeloblastosis transcription factors and it is one of the largest families among all of the transcription factor families (Yanhui et al., 2006; Pasquali et al., 2008). The function of some of these myeloblastosis transcription factors has been elucidated through T-DNA insertional mutagenesis and transcript expression profiling analysis (Kranz et al., 2000; Stracke et al., 2001; Yanhui et al., 2006). This has revealed the roles of R2R3 myeloblastosis proteins in cell cycle control, secondary metabolism, cellular morphogenesis, meristem formation, hormonal signaling and stress responses (Salomoni et al., 1997; Meissner et al., 1999). Several R2R3 myeloblastosis genes have been found to be involved in the responses to environmental stimuli including cold stress (Yanhui et al., 2006). In this category, high expression of osmotically responsive genes such as *HOS10* encoding a R2R3-type myeloblastosis transcription factor is essential for cold acclimation (Zhu et al., 2005). A myeloblastosis 15 controls the expression of dehydration-responsive elements or C-repeat binding factors and other cold-responsive

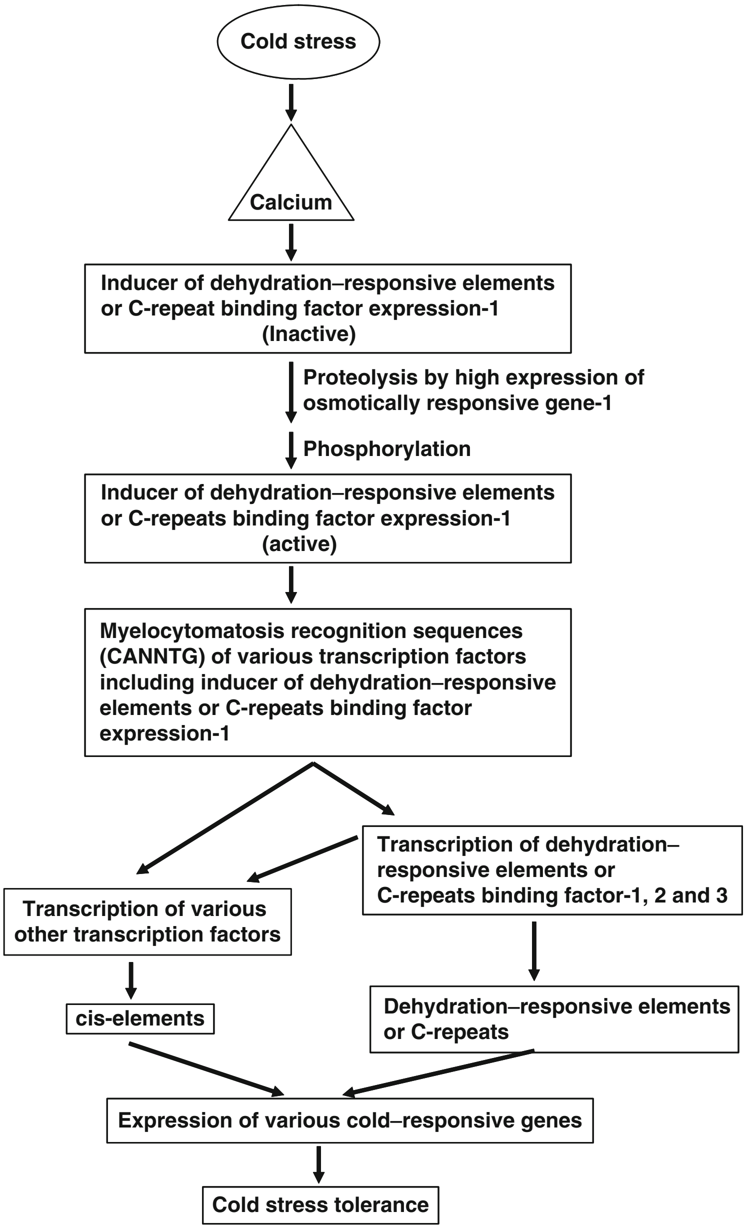

Fig. 3 Involvement of various transcription factors in the induction of cold-responsive genes during cold stress. Cold stress exposure in plants induces the calcium signature. This signature then induces an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 in its inactive form. The variant ring finger protein high expression of osmotically responsive gene-1 has been identified as a negative regulator of cold responses. The high expression of osmotically responsive gene-1 is an E3 ligase required for the ubiquitination of an inducer of dehydration-responsive elements or C-repeat binding factor expression-1. High expression of the osmotically responsive gene-1 physically interacts with an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 and mediates the proteolysis and phosphorylation of an inducer of dehydration-responsive elements or C-repeat binding factor expression-1, converting it from the inactive to active form. An active inducer of dehydration-responsive elements or C-repeat binding factor expression-1 regulates the expression of dehydration-responsive elements or C-repeat binding factors and other transcription factors by binding to myelocytomatosis recognition sequences in their promoter regions. The dehydration-responsive elements or C-repeat binding factors also regulate the expression of other transcription factors. These various transcription factors then regulate the expression of various cold-responsive genes by acting on cis-elements and C-repeats/dehydration-responsive elements in their promoter or upstream sequences. Induction in expression of cold-responsive genes determines tolerance during cold stress

genes (Agarwal et al., 2006). Overexpression of rice myeloblastosis transcription factors *OsMYB3R-2* and *OsMYB4* in *Arabidopsis* has been found to significantly increase the cold tolerance (Vannini et al., 2004; Dai et al., 2007; Pasquali et al., 2008).

In addition to the role of late embryogenesis abundant-like proteins and myeloblastosis transcription factors in cold acclimation, recent studies have found the essential role of the Zinc-finger group of proteins in cold stress tolerance. Overexpression of Zinc-finger genes such as *OsiSAP8*, *OsCOIN, OsISAP1 OsbHLH1*, *OsDREB1/CBF*, *ROs-bZIP*, *SNAC2* and *OsNAC6* confers cold stress tolerance at the seedling stage in rice (Wang et al., 2003; Mukhopadhyay et al., 2004; Ohnishi et al., 2005; Ito et al., 2006; Cheng et al., 2007; Liu et al., 2007; Nakashima et al., 2007; Hu et al., 2008; Kanneganti and Gupta, 2008).

The use of advanced biotechnological tools has enabled us to identify the role of several other signaling components and metabolic regulators in stress response. A *OsTPP1* gene encoding trehalose-6-phosphate phosphatase has been documented to confer cold stress tolerance in rice by inducing the expression of cold-responsive genes (Pramanik and Imai, 2005; Shima et al., 2007; Ge et al., 2008). Similarly, hydrophobic proteins encoded by *OsLti6* genes (*OsLti6a* and *OsLti6b*) also enhanced tolerance to cold stress in rice seedlings (Morsy et al., 2005). The *Arabidopsis FAD8* gene (Gibson et al., 1994) encodes a fatty acid desaturase that contributes to freezing tolerance by altering the lipid composition. The cold-responsive genes encoding molecular chaperones, including a spinach heat shock protein gene *hsp70* (Anderson et al., 1994), and a *Brassica napus* heat shock protein gene *hsp90* (Krishna et al., 1995), contribute to freezing tolerance by stabilizing proteins against freeze-induced denaturation.

Stress responses in plants are also mediated by the mitogen-activated protein kinase cascades as described under the signal transduction section. Overexpression of a rice mitogen-activated protein kinase *OsMAPK5* conferred tolerance to various environmental stresses including cold stress in rice seedlings (Xiong and Yang, 2003). Stress-responsive genes encoding calcineurin B-like protein-interacting protein kinases such as *OsCIPK03* and *OsCIPK12* also play important roles in conferring cold stress tolerance in rice (Xiang et al., 2007). The role of calcium-dependent protein kinases in signal transduction of plants has also been well documented. The calcium-dependent protein kinases encoded by *OsCDPK7* and *OsCDPK13* have been found to be positive regulators for enhancing cold stress tolerance (Saijo et al., 2000, 2001; Wan et al., 2007; Wang et al., 2008). Induction in expression of a rice calcium-dependent protein kinase *OsCDPK13* and accumulation of OsCDPK13 protein were upregulated in response to cold (Abbasi et al., 2004). A rice calcium-dependent protein kinase *OsCDPK13* overexpressing transgenic rice had higher recovery rates following cold stress in comparison with non-transgenic rice. Cold-tolerant rice varieties exhibited higher expression of a rice calcium-dependent protein kinase *OsCDPK13* than the cold-sensitive ones (Abbasi et al., 2004). These studies have documented the important role of calcium-dependent protein kinases in mediating tolerance in response to cold stress.

For acclimation to cold stress, plants involve precise signaling and regulation of the transcriptome. Among the various reported transcription factors, an inducer of dehydration-responsive elements or C-repeat binding factor expression-1 and dehydration-responsive elements or C-repeat binding factors play pivotal roles in the regulation of cold-responsive genes and providing cold tolerance to plants.

5 Conclusion

Cold stress is one of the major environmental stresses that limit agricultural crop productivity by affecting their quality and post-harvest life. Most temperate plants acquire chilling and freezing tolerance upon prior exposure to sublethal cold stress, a process called cold acclimation. Still, many agronomically important crops are incapable of cold acclimation. Cold stress affects virtually all aspects of cellular function in plants. Such changes caused by cold stress adversely affect the growth and development of plants. Cold stress is sensed by a yet unknown receptor. The cold stress signal is transduced through several components of signal transduction pathways. Major components are calcium, reactive oxygen species, protein kinase, protein phosphatase and lipid signaling cascades. Abscisic acid also mediates the response of cold stress. The cold stress signal leads to regulation of transcription factors and effector genes, collectively called cold-regulated genes. The effector genes encoding proteins under this category include chaperones, late embryogenesis abundant proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis such as proline, water channel proteins, sugar and proline transporters, detoxification enzymes, enzymes for fatty acid metabolism, proteinase inhibitors, ferritin, and lipid-transfer proteins. The transcription factors involved during cold stress response are inducers of C-repeat binding factor expression-1, C-repeat binding factors, myeloblasts and mitogen-activated protein kinase. Analyses of the expression of cold-regulated genes indicate the presence of multiple signal transduction pathways between the initial stress signals and gene expression. Use of these genes and transcription factors in genetic modification of agricultural crops can improve cold tolerance and productivity.

Acknowledgements I am grateful to Dr. P. S. Ahuja, Director, IHBT, for his useful suggestions. I would like to acknowledge the Council of Scientific and Industrial Research (CSIR) and Department of Science and Technology (DST), Govt. of India, for their financial help with conducting research in my laboratory.

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Part V
Alternative Pest Control

Defence Mechanisms of Brassicaceae: Implications for Plant-Insect Interactions and Potential for Integrated Pest Management

Ishita Ahuja, Jens Rohloff, and Atle Magnar Bones

Contents

Abstract *Brassica* crops are grown worldwide for oil, food and feed purposes, and constitute a significant economic value due to their nutritional, medicinal, bioindustrial, biocontrol and crop rotation properties. Insect pests cause enormous yield and economic losses in *Brassica* crop production every year, and are a threat to global agriculture. In order to overcome these insect pests, *Brassica* species themselves use multiple defence mechanisms, which can be constitutive, inducible, induced, direct or indirect depending upon the insect or the degree of insect attack. Firstly, we give an overview of different *Brassica* species with the main focus on cultivated brassicas. Secondly, we describe insect pests that attack brassicas. Thirdly, we address multiple defence mechanisms, with the main focus on phytoalexins, sulphur, glucosinolates, the glucosinolate-myrosinase system and their breakdown products. In order to develop pest control strategies, it is important to study the chemical ecology, and insect behaviour. We review studies on oviposition regulation, multitrophic interactions involving feeding and host selection behaviour of parasitoids and predators of herbivores on brassicas. Regarding oviposition and trophic interactions, we outline insect oviposition behaviour, the importance of chemical stimulation, oviposition-deterring pheromones, glucosinolates, isothiocyanates, nitriles, and phytoalexins and their importance towards pest management. Finally, we review brassicas as cover and trap crops, and as biocontrol, biofumigant and biocidal agents against insects and pathogens. Again, we emphasise glucosinolates, their breakdown products, and plant volatile compounds as key components in these processes, which have been considered beneficial in

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_28,

from Ahuja et al., Agron. Sustain. Dev. 30 (2010) 311–348. DOI: 10.1051/agro:2009025

the past and hold great prospects for the future with respect to an integrated pest management.

Keywords Brassicas • Insect pests • Chemical ecology • Trophic levels • Glucosinolates • Isothiocyanates • Defence mechanisms • Biocontrol • Trap crops • Integrated pest management

1 Introduction

1.1 The Origin and Excellence of "Brassicas"

"Brassicas: Oil-, food- and fodder-bearing crops with small seeds; that can grow as fast as Wisconsin rapid cycling brassicas; can grow as big as ornamental plants; can be seen as vast green fields of vegetable crops or as oilseed crops with fields of bright yellow flowers; lead to the production of economically important agricultural products; used as food for humans and animals; are important as valuable renewable bioenergy resources; are huge reservoirs of plant innate defences; show multiple defence responses in response to stresses; possess anticancer properties; hold ample potential for pest management" (*Ishita Ahuja*).

The Brassicaceae or Cruciferae, also known as crucifers, is a broad family of around 375 genera and 3200 species (LeCoz and Ducombs, 2006), which includes crops, ornamentals and many weeds. The genus *Brassica* belongs to the subtribe Brassicinae of the Brassicaceae family and comprises about 159 species, which, along with cultivated species, also includes wild brassicas (Zhou and Zhang, 2001; Zhang and Zhou, 2006).

The cultivated species of Brassicaceae include oilseed rape (Fig. 1), cabbage, cauliflower, broccoli, Brussels sprouts, turnip, kale, swede, various mustards and other leafy vegetables. They are grown worldwide with a wide spectrum of adaptation for cultivation under varied agro-climatic conditions (Suwabe et al., 2006; Hong et al., 2008). The archaeological evidence of importance of brassicas dates back to 5000 BC, and *Brassica* plants are considered among the oldest cultivated plants known to humans, with written records dating back to ca. 1500 BC (Raymer, 2002). The genetic relationship among different *Brassica* species was established in the classical work by U (U.N., 1935), which is now ascribed as U's triangle (Fig. 2).

The corners of the U-triangle include three diploid species: *B. rapa* L. (2n = 20; AA), *B. nigra* L. Koch (2n = 16; BB) and *B. oleracea* L. (2n = 18; CC), and the other three in the middle of the triangle are amphidiploid species: *B. napus* L. (2n = 38; AACC), *B. juncea* (L.) Czern. (2n = 36; AABB) and *B. carinata* Braun (2n = 34; BBCC) (Gomez-Campo, 1999). These six species of the U-triangle are also referred to as: *B. rapa* (*syn. B. campestris*) (Chinese cabbage and Turnip), *B. nigra* (Black mustard), *B. oleracea* (Cabbage, Brussels sprouts, Cauliflower and Broccoli),

Fig. 1 The classical view of yellow *Brassica napus* fields and a closer view of oilseed rape plants

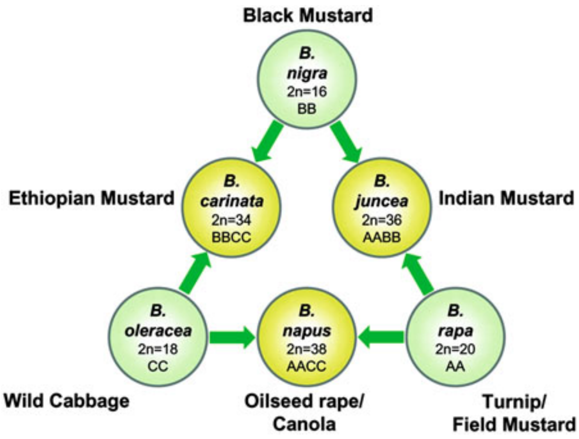

Fig. 2 U-triangle showing the affiliations among different *Brassica* species (U.N., 1935)

[*B. napus* var. *oleifera* (Oilseed rape, Rapeseed and Canola) var. *rapifera* (Swede, Rutabaga)], *B. juncea* (Indian mustard/Brown mustard) and *B. carinata* (Ethopian mustard) (Labana and Gupta, 1993). *B. rapa*, *B. nigra*, *B. oleracea*, *B. juncea*, *B. napus* and *B. carinata* are all designated as crop brassicas. Cauliflower, cabbage, Brussels sprouts, broccoli and turnip fall into the category of important vegetable crops. Among these vegetable crops, broccoli (var. *botrytis*) has been a valuable asset in the USA, both from a nutritional and economic point of view. Apart from being an excellent source of vitamin C, it provides dietary fibre, protein, iron, calcium and vitamin A and also contains anticancer components (Verhoeven et al., 1997; Cintas et al., 2002). *Brassica* crops carry properties of nutritional value, health benefits and biocontrol agents, and their use in crop rotations has been valued both by traditional and organic farmers (Guerena, 2006). The worldwide production of cabbages and kale-like brassicas in 2007 was 69 Mio t/3.1 Mio ha area, rapeseed 50 Mio t/30.2 Mio ha, cauliflowers and broccolis 19 Mio/1.0 Mio ha, and mustard seed 0.4 Mio t/0.7 Mio ha (FAOSTAT, 2009). Due to the increased oilseed production of *B. rapa*, *B. juncea*, *B. napus* and *B. carinata* in the past three decades, the oilseed brassicas have become an important source of oil and protein among cultivated brassicas (Font et al., 2003). Oilseed *Brassica* species are the major oilseed crops cultivated in India and around the world, and India produces about 11.3% of the world's rapeseed mustard (Damodaram and Hegde, 2002 as cited by Chattopadhyay et al., 2005). Oilseed *Brassica* species are also an important commodity in the world economy, as they are an important source of nutrition in developing countries (Rana, 2005). Amidst oilseed brassicas, oilseed rape has become a major crop in Europe, and one of the significant oil crops worldwide (Graner et al., 2003; Dubuis et al., 2005). The rapeseed and rapeseed oil production in 2007 from different countries that ranked in the first twelve worldwide are listed in Table 1.

B. napus stands as the third most important oilseed crop at the international level for both oil meal (after soybean and cotton) and vegetable oil (after soybean and oil palm) (Snowdon et al., 2007). The worldwide rapeseed oil production was 16.8 Mio t after palm and soybean (FAOSTAT, 2009). The significance of oilseed rape has increased during the present decade, not only because of its consumption as a nutritional food, but also due to its role as a renewable energy source as biodiesel in transport. Biodiesel is the methyl ester of oilseed rape oil (RME) (Souckova, 2006). *B. juncea*, an important source of edible oil, is cultivated in many countries across the globe. During the year 1999–2000, oilseed rape and mustard occupied, e.g., 6 Mio ha with an annual production of 5.8 Mio t in India (Dutta et al., 2005).

1.2 Important Insect Pests of Brassicas

"*An insect is considered a pest if it threatens a resource valued by human beings, including human health*" (Foster and Harris, 1997). Insect pests pose a great challenge to *Brassica* crop production worldwide. A huge number of insect pests attack brassicas, and several insect specialists have *Brassica* species as preferred host plants (Lamb, 1989; Sekhon and Åhman, 1993; Sibanda et al., 2000). These insect pests are the major un-equalisers of growth and crop yield of brassicas and their importance varies by geographical location (Hokkanen and Wearing, 1996; Kanrar et al., 2002). Some of the major insect pests that attack Brassicacea crops worldwide are listed in Table 2 and presented in Fig. 3.

Sources: AgroAtlas, 2009; Bromand, 1990; Bartlet et al., 1996; Hokkanen and Wearing, 1996; Ruther and Thiemann, 1997; Girard et al., 1998; Kift et al., 2000; Ulmer, 2002; Ester et al., 2003; Du Toit, 2007; Kazana et al., 2007; Khattab, 2007; Lehrman, 2007; Valantin-Morison et al., 2007; Smallegange et al., 2007; Knodel and Ganehiarachchi, 2008; Cartea et al., 2009.

Table 1 Rapeseed and rapeseed oil production in the first 12 highest producing countries worldwide in 2007 (FAOSTAT, 2009)

Country	Rapeseed (Mio t)	Rapeseed oil (Mio t)
China	10.38	4.35
Canada	8.86	1.30
India	7.10	2.34
Germany	5.32	2.20
France	4.55	1.05
Poland	2.11	0.68
United Kingdom	2.11	0.72
Australia	1.07	0.17
Ukraine	1.06	0.06
Czech Republic	1.04	0.31
USA	0.66	0.50
Russian Federation	0.60	0.07

1.2.1 The Diamondback Moth (*Plutella xylostella*)

The diamondback moth (*P. xylostella*) (Fig. 3) is a highly mobile insect, and is considered as the most damaging insect pest of *Brassica* crops worldwide. With an estimated control cost of nearly US$ 1 billion annually (Talekar and Shelton, 1993; Pivnick et al., 1994; Sarfraz et al., 2006; Golizadeh et al., 2007; Shelton et al., 2008), it has been the greatest threat to *Brassica* production in many regions of the world, with crop loss of 90% in some cases (Verkerk and Wright, 1996; Charleston and Kfir, 2000). The diamondback moth was first noticed as a pest in South Africa in the early 1900s (Charleston and Kfir, 2000). In Canada, the short generation time and high fecundity of the diamondback moth allows it to become a significant pest of oilseed crops in this region (Ulmer et al., 2002). Diamondback moth can attack plants at all stages of growth and female moths attach their eggs singly or in groups of two or three to the underside of leaves. Larval chewing make small holes in leaves, with larger larvae making larger holes. Their chewing may make leaves appear “windowpaned”, with a clear cuticle left after feeding. Diamondback moth larvae feed on most of the *Brassica* plants such as *B. campestris*, *B. napus*, *B. juncea* and *B. oleracea*.

1.2.2 The Cabbage Looper (*Trichoplusia ni*)

The cabbage looper feeds on a diverse range of plants and is an important pest on Brassicaceae plants (Chow et al., 2005). The cabbage loopers (Fig. 3), which often attack broccoli, cauliflower, cabbage, kale, collards and mustard, are pale green larvae of a grey-brown moth, feed on foliage and tender above-ground parts and generally lead to plant decline (Du Toit, 2007; Capinera, 2008). Young larvae initially are dusky white, but become pale green as they begin to feed on foliage. The cabbage looper is found throughout Canada, Mexico and the United States, wherever crucifers are cultivated, and on other continents (Capinera, 2008). The cabbage looper females can produce 300 to 600 eggs in their 2 week life span, laying eggs singly and mostly on the lower surface of leaves. Larvae emerge from eggs in 3 to 4 days and feed on leaves (Chow et al., 2005; Mossler, 2005).

1.2.3 The Cabbage Moth (*Mamestra brassicae*) and Bertha Armyworm (*Mamestra configrata*)

The cabbage moth, *M. brassicae* (Fig. 3), is a polyphagous insect, and the observed food plants of the cabbage moth include more than 70 species of 22 families, of which Brassicaceae and Chenopodiaceae are among the most preferred (Popova, 1993 as cited by Rojas et al., 2001; Ulland et al., 2008). The cabbage moth is widely distributed throughout most of Europe and Asia, from 30°N to about 70°N (Klingen et al., 2002b and references therein). The cabbage moth is native to Norway and is an important pest on various cabbage crops in Southern Norway up to about 62°N (Johansen, 1997). The Bertha armyworm, also referred to as the ‘Miller Moth’ or ‘climbing cutworm’, is native to North America and is a pest of oilseed and canola production in the northern Great Plains (Ulmer, 2002; Knodel and Ganehiarachchi, 2008).

Table 2 List of some important insect pests that attack Brassicaceae plants worldwide

Common names		Scientific names	Host plants
Lepidoptera			
1	Diamondback moth/Cabbage moth (Specialist)	*Plutella xylostella* L.	Broccoli, Brussels sprouts, cabbage, cauliflower, kale, mustard, turnip
2	Cabbage looper (Generalist)	*Trichoplusia ni* Hübner	Broccoli, cabbage, cauliflower, kale, collards, mustard, rutabaga, turnip
3	Cabbage moth (Generalist)	*Mamestra brassicae* L.	Cabbage, mustard, turnip,
4	Bertha armyworm (Generalist)	*Mamestra configrata* Walker	Canola, rapeseed, mustard
5	Small white butterfly/Imported cabbage worm/Cabbage butterfly (Specialist)	*Pieris rapae* L.	Broccoli, Brussels sprouts, cabbage, cauliflower, kale, kohlrabi
6	Large white butterfly (Specialist)	*Pieris brassicae* L.	Kale, cabbage, turnip, black mustard, Ethiopian mustard, swede
7	Green-veined white (Specialist)	*Pieris napi* L.	Cabbage, Brussels sprouts, turnip, swede, mustard, oilseed rape
Hymenoptera			
8	Cabbage sawfly/Turnip sawfly (Specialist)	*Athalia rosae* L.	Mustard, turnip, oilseed rape, cabbage
Diptera			
9	Cabbage maggot/Cabbage root fly/Cabbage fly (Specialist)	*Delia radicum* syn. *brassicae* L.	Broccoli, Brussels sprouts, cabbage, kale, swede
10	Turnip root fly (Specialist)	*Delia floralis* Fallen	Turnip
11	Brassica/Pod midge (Specialist)	*Dasineura brassicae* Winnertz.	Oilseed rape
Coleoptera			
12	Flea beetles/pollen beetles	*Phyllotreta*, *Psylliodes* and *Meligethes* spp.	
	Crucifer flea beetle (Specialist)	*Phyllotreta cruciferae* Goeze	Broccoli, Brussels sprouts, cabbage, cauliflower, kale, collards, turnip, mustard, oilseed rape
	Striped flea beetle (Specialist)	*Phyllotreta striolata* F.	Broccoli, Brussels sprouts, cabbage, cauliflower, kale, collards, canola, turnip, mustard, oilseed rape
	Cabbage stem flea beetle (Specialist)	*Psylliodes chrysophela* L.	Cabbage, mustard, turnip, oilseed rape
	Pollen beetles	*Meligethes* spp.	Turnip, swede, mustard, oilseed rape
13	Weevils	*Ceutorhynchus* spp.	
	Rape stem weevil (Specialist)	*C. napi* Gyll.	Oilseed rape, cabbage
	Cabbage seed weevil (Specialist)	*C. assimilis* Paykull	Cabbage, turnip, oilseed rape
	Cabbage seedpod weevil (Specialist)	*C. obstrictus* Marsham	Turnip, mustard, cabbage, brown mustard, canola, cabbage, broccoli
	Cabbage stem weevil (Specialist)	*C. pallidactylus* Marsham	Oilseed rape, cabbage, cauliflower, turnip
Homoptera			
14	Aphids		
	Cabbage aphid/Mealy cabbage aphid (Specialist)	*Brevicoryne brassicae* L.	Cabbage, mustard, turnip, oilseed rape, broccoli, Brussels sprouts
	Mustard aphid (Specialist)	*Lipaphis erysimi* Kaltenbach	Mustard
	Turnip aphid (Specialist)	*Hyadaphis erysimi* Kaltenbach	Turnip
	Green peach aphid (Generalist)	*Myzus persicae* Sulzer	Broccoli, Brussels sprouts, cabbage, cauliflower, turnip

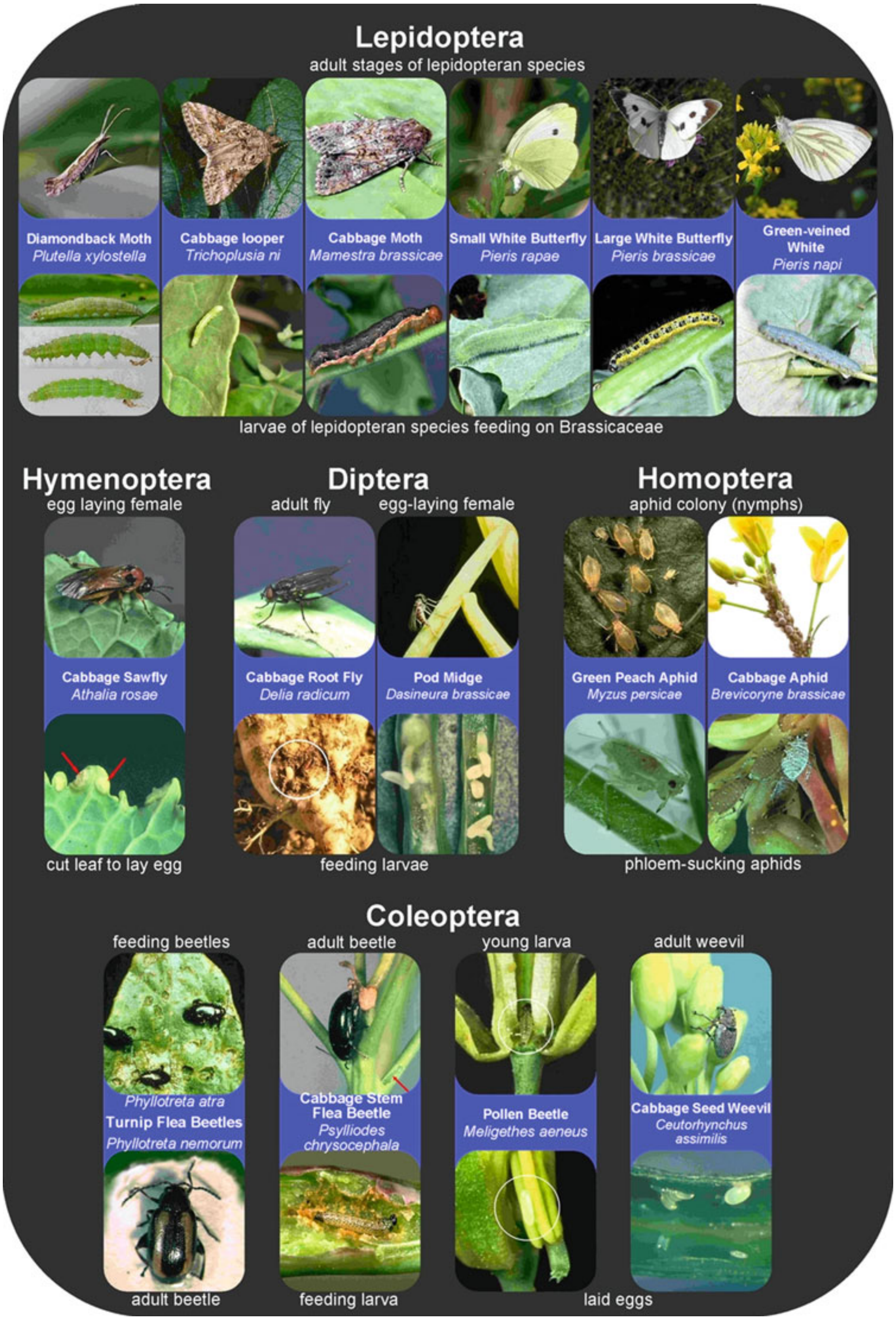

Fig. 3 Important insect pests that attack Brassicaceae plants worldwide

Young larvae feed on the underside of leaves and chewing makes irregular-shaped holes. The economic damage occurs due to significant larval feeding on foliage as well as on developing seedpods of canola. In years with outbreaks, larval feeding has resulted in economic crop losses and increased production costs from spraying insecticides (Knodel and Ganehiarachchi, 2008).

1.2.4 The Cabbage White Butterflies (*Pieris brassicae, Pieris rapae* and *Pieris napi*)

The cabbage white butterflies *P. brassicae, P. rapae* and *P. napi* (Fig. 3), are specialised on the Brassicaceae family and they have been used as a model species in the field of insect pest biology (Smallegange et al., 2007 and references therein). *P. brassicae* and *P. rapae* are cabbage herbivores that are closely related, yet show drastic contrasts in the amount of eggs they lay on plants (Bruinsma et al., 2007). *P. rapae* occurs in temperate regions around the world, and is generally confused with other common cabbage white butterflies (Capinera, 2004). In North America, it is known as "imported cabbageworm" and in Europe it is known as the small white cabbage butterfly. *P. rapae* is a cosmopolitan species, which is widespread throughout Europe, Asia and North America. The damage to foliage caused by *P. rapae* is slight, although it can be severe in seasons with a high infestation of caterpillars (Hern et al., 1996). *P. napi* is spread throughout the northern hemisphere ranging from North America, Europe and Asia to North Africa. Although larvae mainly feed on wild Brassicaceae species, infestation of *Brassica* vegetable crops such as cabbage, turnip and swede occurs and potentially leads to significant crop losses.

1.2.5 The Cabbage Sawfly/Turnip Sawfly (*Athalia rosae*)

The turnip sawfly, *A. rosae* (Fig. 3), is oligophagous, i.e. it feeds on a few types of plants in nature. The pest eats leaf mass, buds, flowers and young pods (AgroAtlas, 2009). Among cruciferous plants its preferred hosts are white mustard (*S. alba*) and turnip (*B. rapa*), but the pest can also rear on young oilseed rape (*B. napus*) crops (Barker et al., 2006).

1.2.6 The Cabbage Root Fly (*Delia radicum* syn., *brassicae*) and the Turnip Root Fly (*Delia florales*)

The cabbage root fly (*D. radicum* syn. *brassicae*) (Fig. 3) and the turnip root fly (*D. florales*) are considered to be economically important pests on *Brassica* crops such as broccoli, Brussels sprouts, cabbages and kales, and are members of a large family of root flies (Klingen et al., 2002b and references therein; Ester et al., 2003). The larvae of *Delia* flies cause damage to plants by feeding on plant roots and through eggs and neonates (Klingen et al., 2002b). The plant mortality rate is unusually high, and the recovered plants are of reduced marketable quality (De Jong and Städler, 1999). Cabbage root fly and turnip root fly damaging *Brassica* spp. roots lead to significant reductions in yield, flowering and seed production as well as leaf, stem and root biomass (Blossey and Hunt-Joshi, 2003). Both cabbage root fly and turnip root fly are oligophagous in nature. Gravid females of the cabbage root fly arrive at a host-plant odour source by a series of short upwind flights, landing and reorientating into the wind between flights (Hopkins et al., 1999). The adult female oviposits in the soil close to the stem of *Brassica* plants (Nottingham and Coaker, 1985; De Jong and Städler, 1999), while damage to the plants is caused by hatched larvae feeding on the roots.

1.2.7 The Brassica Pod Midge (*Dasineura brassicae*)

The brassica pod midge is a common pest on Brassicaceae plants, particularly on oilseed rape, throughout north-western Europe and is attacked by over 20 species of hymenopteran parasitoids, among them *Omphale clypealis* and members of the genus *Platygaster subuliformis* (Murchie et al., 1997 and references therein; Murchie and Hume, 2003). Females deposit their eggs inside *Brassica* pods, where larvae feed and develop. Larvae spend their feeding period within the same pod (Åhman, 1985).

1.2.8 The Flea and Pollen Beetles (*Phyllotreta, Psylliodes* and *Meligethes* spp.)

Phyllotreta nemorum and *Phyllotreta undulata* are the main flea beetle species infesting *Brassica* crops, such as Brussels sprouts, broccoli and cauliflower (Ester et al., 2003). The crucifer flea beetle, *Phyllotreta cruciferae*, and the striped flea beetle, *Phyllotreta striolata*, are the most serious insect pests of canola in North America and both were introduced from Eurasia. *P. cruciferae* has become the dominant flea beetle pest of canola. Adult flea beetles emerge in the spring and feed on the cotyledons and true leaves. When emerging in huge numbers, they can quickly devastate a seedling canola field. Therefore, a timely detection and management of this pest is important. The damage to oilseed *Brassica* crops through flea beetles exceeds US $ 300 million annually in North America (Knodel and Olsen, 2002). *Psylliodes chrysocephala* (cabbage stem flea beetle) is the major stem-mining pest of oilseed rape (Barari et al., 2005) and an important pest of other *Brassica* spp. in Europe (Bromand, 1990; Bartlet and Williams, 1991; Bartlet et al., 1996; Vig, 2002; Valantin-Morison et al., 2007). The genus *Meligethes*, generally known as pollen beetles (Fig. 3), occurs worldwide and includes more than 400 species (Kirk-Spriggs, 1996 as cited by Blight and Smart, 1999). Of the 10 *Meligethes* species reported from brassicaceous plants in Europe, *M. aeneus* is by far the most common on cultivated brassicas (Blight and Smart, 1999). Pollen beetles are considered important pests on oilseed brassicas and cause serious yield losses to oilseed rape crops, with yield reductions of more than 80% reported (Lamb, 1989; Ekbom, 1995; Ekbom and Borg, 1996; Ruther and Thiemann, 1997; Ekbom and Ferdinand, 2003; Bartlet et al., 2004; Hansen, 2004; Williams, 2006; Kazachkova, 2007; Lehrman, 2007). Although pollen beetles are important pests, particularly for oilseed rape in Northern Europe (Jönsson and Anderson, 2007), and turnip rape, they do not oviposit on all Brassicacea plants (Bartlet et al., 2004).

1.2.9 The Weevils (*Ceutorhynchus* spp.)

The cabbage seed weevil (*C. assimilis*) (Fig. 3) is an important pest of oilseed rape in Europe and North America (Lamb, 1989; Bartlet et al., 1997; Smart and Blight, 1997; Girard et al., 1998; Ferguson et al., 1999b), causing reduction in crop yield in heavily infested fields (Smart et al., 1997; Girard et al., 1998). The cabbage seed weevil is an oligophagous insect that feeds and develops on *Brassica* species. The cabbage seed weevil invades oilseed rape crops, the adults colonise flowering host plants and feed on pollen, and the larvae feed on the developing seeds before leaving the pods and pupating in the soil. The cabbage seedpod weevil (*C. obstrictus*) is native to Europe, and is a serious pest of brassicaceous oilseed crops in Europe and North America (Bartlet et al., 1993; Ferguson et al., 1999b; Ulmer and Dosdall, 2006; Valantin-Morison et al., 2007). The cabbage seedpod weevil is a small, dark grey “snout beetle” normally occurring after peak flowering and lays eggs in the pods of cruciferous plants. The larvae feed on seeds in the pods, which results in seed loss (Bartlet et al., 1993; Du Toit, 2007). When mature, the larvae leave the pods, and fall on the ground to pupate. Adults emerge in late July and feed before diapausing until the following spring. The cabbage seedpod weevil therefore needs to find a host plant at two stages of its life cycle: upon emergence from pupation in the summer (pre-diapause weevils), and upon emergence from hibernation the following spring (post-diapause weevils) (Bartlet et al., 1993). The cabbage stem weevil (*C. pallidactylus*) is the major stem-mining pest of oilseed rape (Barari et al., 2005).

1.2.10 The Aphids (*Brevicoryne brassicae, Myzus persicae* and *Lipaphis erysimi*)

Aphids have been considered as the main insect pests in India and northern European agriculture, and are also important pests in horticulture both in field and greenhouse production, causing damage to crops either directly by feeding or by transmitting plant viral diseases (Dawson et al., 1990; Sekhon, 1999). Aphids feed by piercing plant tissue with their needle-like mouthparts (stylets), sucking water and nutrients from the phloem vascular system of the plant. Feeding damage and toxins in the saliva cause thickening, crumpling and downward curling of leaves (Mossler, 2005). The nymphs and adults suck sap from leaves, stem, flowers and pods, hence resulting in poor pod formation and reduced oil content in grains. A severe aphid attack can result in up to 75% loss to *Brassica* crops (Sekhon, 1999). *B. brassicae* (Fig. 3) is a global problem with a strong negative impact on agriculture and horticulture. *B. brassicae* is controlled by multiple insecticide treatments (Kift et al., 2000; Pontoppidan et al., 2003) and is a severe pest on brassicas (Cole, 1994; Kift et al., 2000). *B. brassicae* is highly host-specific, feeding almost exclusively on the phloem sap of *Brassica* or other closely related plant species (Cole, 1997). *B. brassicae* produces parthenogenic, viviparous females throughout the year, which overwinter on horticultural brassicas and forage crops of rape and swede (Schroeder and Dumbleton, 2001). During spring, these females change into winged forms, fly to seedlings of brassicas and produce offspring (nymphs). The peach aphid (*M. persicae*) is a generalist (Fig. 3) reported to have more than 400 species as host plants (Quaglia et al., 1993 as cited by Francis et al., 2001). The mustard aphid (*L. erysimi*) is the most important pest of cruciferous crops worldwide, causing damage of 10–90% depending upon the severity of the infestation and crop stage. Apart from causing damage as a sapsucker, it is also a vector of several viral diseases (Rana, 2005). A 2-year study on the preference and performance of *L. erysimi* on different *Brassica* species in the field and under greenhouse conditions revealed that rapeseed (*B. campestris* varieties BSH-1 and YSPB-9) and mustard (*B. juncea* RH-30) were better hosts for this aphid than other *Brassica* species (*B. napus*, *B. nigra*, *B. carinata*) (Rana, 2005). Moreover, *L. erysimi* is a harmful insect on *Brassica* oilseeds, especially on *B. juncea* in India and in other tropical regions of the world, causing up to 83% yield loss (Sekhon and Åhman, 1993; Mandal et al., 1994 as cited by Chattopadhyay et al., 2005; Agarwala and Datta, 1999; Aslam and Ahmad, 2001; Dutta et al., 2005; Hossain et al., 2006 and references therein). Aphids reproduce at a higher rate during the early vegetative stage of mustard plants when the developmental period is shortest and the production of winged morphs is lowest (Agarwala and Datta, 1999). The nymphs and adults cause damage by sucking away the plant sap, often covering the entire surface of the shoots, floral buds and pods. The pest breeds parthenogenetically, and an individual female gives birth to nymphs, which grow very fast and are completely bred in 7–10 days. About 45 generations are completed in a year. The high propagation rate of the pest affects the crop vitality, because the flowers fail to bear healthy pods, subsequently producing seeds of poor quality (Hossain et al., 2006 and references therein).

1.3 Plant Defence Mechanisms – General Information

Typical defence mechanisms that exist or are expressed in plants include constitutive, inducible, induced, direct and indirect defences. The existence, expression and functioning mechanism of these defences show both parallelism and contrasts to each other. These defence mechanisms are defined and addressed in this section, and are referred to and documented in other sections of the article with regard to the chemistry of Brassicaceae plants towards insect interactions.

Plants and animals both possess the potential to differentiate between self and non-self (hostile organisms), which may vary according to the heredity and environment. A major difference between plants and animals is that plants are sessile and animals mobile and the latter therefore can spread infections more easily (Jones and Takemoto, 2004). In order to overcome infections, plants are not only equipped with diverse constitutive/innate/preformed but also adapted defence mechanisms, to defend themselves. Likewise, striking similarities and obvious differences have evolved in animals which also form the basis of inducible or induced defence mechanisms (Nürnberger and Brunner, 2002; Montesano et al., 2003). Constitutive defence mechanisms, which are also regarded as ancient defence systems, are weapons that involve various receptors that recognise classes of microbial cell-surface molecules, the related signal transduction pathways that activate transcription of genes related to host defence, and the ubiquitous cationic peptides and proteins that act as antimicrobial effectors (Boman, 1995; Borregaard et al., 2000; Thomma et al., 2002).

An ecosystem comprises plants circumvented by herbivorous insects that continuously affect plant fitness. In order to overcome these herbivorous insects or to protect themselves from damage by herbivoral arthropods, plants have developed physical and chemical defences, which can either be innate or inducible in response to a certain attack (Takabayashi and Dicke, 1996; Karban and Baldwin, 1997; Paré and Tumlinson, 1999; Dicke and Hilker, 2003). The occurrence of both defence responses establishes an intricate network of defences for plants against insect herbivores. Inducible defences may provide an adaptive defensive strategy in which non-lethal cues from predators, herbivores or parasites provide a reliable indicator about the future risk of attack (Agrawal et al., 1999). In order to reduce the impact of insect attacks, plants have developed different defence strategies that include chemical and physical barriers such as induction of defensive proteins, volatiles that attract predators of the insect herbivores, toxic secondary metabolites, and trichome morphology and density (Mello and Silva-Filho, 2002). These defence components produced by plants act both as constitutive substances to repel herbivores through direct toxication, or by lowering the digestibility of plant tissues, and as inducible substances produced in response to tissue damage by herbivores.

Induced defences are activated in the presence of an enemy, and then emerge or develop to their full strength. This particular kind of defence is important with regard to innate resistance, in case the defence is metabolically expensive, and when the attack is unpredictable but frequent (Haukioja, 1999). Plant responses that affect herbivore arthropods directly through systemic production of toxic metabolites are named direct defences, whereas responses that result in the attraction of natural enemies of the herbivores are designated as indirect defences (Dicke, 1999; Mattiacci et al., 2001). The term indirect defence, that is generally used when plants attract, nourish or house other organisms to reduce pressure from their enemies, has been referred to in the literature only in the last 20 years (Dicke and Sabelis, 1989; see review by Heil, 2008). Indirect defence mechanisms contribute towards the efficiency of the natural enemies of herbivores, e.g. through the emission of blends of volatile compounds and other secondary metabolites (Vet and Dicke, 1992; Hilker and Meiners, 2002; Kessler and Baldwin, 2002; Dicke et al., 2003; Rohloff and Bones, 2005). Such release of volatile compounds from plants has been considered as the cry or call for help by the plant from the carnivorous enemies (which are predators of herbivores) that might assist in reducing damage to plants. The release of volatile compounds from damaged plants derives from at least three biosynthetic pathways: first, the fatty acid (or octadecanoid) pathway that produces green leaf volatiles and (Z)-jasmone; second, the shikimic acid pathway that produces indole and methyl salicylate; and third, the isoprenoid pathway which produces terpenes (Hilker and Meiners, 2002). Indirect defences are mostly referred to as an environmentally-friendly crop protection strategy, but their plant fitness effects require more information in order to understand their ecological and evolutionary relevance before trophic interactions can be used as a reliable tool in agriculture (Heil, 2008).

2 Defence Components of Brassicaceae: Glucosinolates, the Glucosinolate-Myrosinase System, Plant Volatiles, Phytoalexins, Phytoanticipins and Sulphur

2.1 Glucosinolates

The characteristic feature of the Brassicaceae family is their production of specific secondary metabolites, the so-called glucosinolates (anionic thioglucosides) (Fahey et al., 2001; Bones and Rossiter, 2006). The glucosinolates constitute a large group of non-volatile and sulphur-containing secondary plant metabolites, which occur in all economically important *Brassica* crops (Tripathi and Mishra, 2007). Their known number totals almost 140 structures to date, 30 of which are present in *Brassica* species (Bellostas et al., 2007). Glucosinolates are β-thioglucoside N-hydroxysulphates with at least two sulphur atoms, one originating from cysteine, the other from phosphoadenosine phosphosulphate, possessing a β-D-glucopyranose moiety and a side chain derived from the amino acids methionine, tryptophan or phenylalanine (Fahey et al., 2001; Wittstock and Halkier, 2002; Rausch and Wachter, 2005). The content and composition of glucosinolates varies depending on *Brassica* species, the cultivar plant parts within the same plant, agronomic practices and climatic conditions (Sang et al., 1984; Clossais-Bernard and Larher, 1991; Rangkadilok et al., 2002; Font et al., 2005; Tripathi and Mishra, 2007). Glucosinolates are known to mediate interactions between Brassicaceae and their associated insect herbivores. They have been recognised as a class of natural pesticides since they exhibit toxic or repellent effects, by which they establish a significant defence mechanism to protect *Brassica* plants against pests and diseases (Mithen, 1992; Zukalová and Vašák, 2002). Glucosinolate levels and proportions of individual glucosinolate compounds have been demonstrated to be altered due to the damage caused by several insect pests (Koritsas et al., 1991; Bodnaryk, 1992; Hopkins et al., 1998). The glucosinolate concentration can increase in response to herbivore feeding, and this high level of glucosinolates can affect both generalist and specialist herbivores, and glucosinolates can be equally effective as stimulants as well as deterrents (Bodnaryk, 1992; Bartlet et al., 1999; Li et al., 2000; Rask et al., 2000; Agrawal and Kurashige, 2003; Kuśnierczyk et al., 2007, 2008; Gols et al., 2008a).

2.2 The Glucosinolate-Myrosinase Defence System

The term *myrosin cell*, initially used by (Guignard, 1890), was discovered by (Heinricher, 1884). The myrosin cells can be easily distinguished from their neighbouring cells by light, electron and confocal microscopic observations (Bones and Iversen, 1985; Thangstad et al., 1990, 1991; Bones et al., 1991; Thangstad et al., 2004; Kissen et al., 2009). The myrosin cells contain less lipids, a high content of endoplasmic reticulum and harbour smooth-looking protein bodies referred to as myrosin grains (Bones and Iversen, 1985; Thangstad et al., 1991). Myrosin cells exist as scattered cells in stems, leaves, seeds, seedlings, petioles and roots. *Brassica* plants contain the enzyme myrosinase (β-thioglucoside glucohydrolase, thioglucosidase, EC 3.2.3.147 (formerly EC 3.2.3.1) (Bones and Slupphaug, 1989; Bones, 1990; Bones and Rossiter, 1996, 2006), which is thought to be exclusively present in myrosin cells (Thangstad et al., 1990, 1991; Bones et al., 1991; Höglund et al., 1991; Husebye et al., 2002; Thangstad et al., 2004; Kissen et al., 2009). In brassicas, myrosinases can be divided into three different gene families; the MA, MB and MC families (Xue et al., 1992; Chadchawan et al., 1993; Lenman et al., 1993; Thangstad et al., 1993; Falk et al., 1995). Furthermore, myrosinases are glycosylated dimeric proteins with subunit molecular weights from 62 to 75 kDa (Bones and Slupphaug, 1989; Bones and Rossiter, 1996). Myrosinases that belong to the MA family occur as free dimers (140 kDa), while members of the MB and MC families are found in high molecular complexes (200–1000 kDa), with myrosinase binding proteins (MBP) and the myrosinase associated proteins (MyAP) (Rask et al., 2000).

Glucosinolates and myrosinases are spatially segregated (Kelly et al., 1998; Koroleva et al., 2000; Husebye et al., 2002), but insect herbivory or tissue damage bring them together, which facilitates glucosinolate hydrolysis into thiocyanates, isothiocyanates, nitriles, oxazolidine-2-thiones and epithionitriles, depending upon pH and other conditions (Fig. 4) (Pivnick et al., 1992; Bones and Rossiter, 1996, 2006; Wittstock and Halkier, 2002).

The biosynthesis of glucosinolates still poses questions with, e.g., respect to transport and tissue, cell and subcellular spatial separation and organisation (Svanem et al., 1997; Thangstad et al., 2001; Halkier and Gershenzon, 2006). Glucosinolates such as progoitrin, sinigrin, gluconapin and glucobrassicanapin can give rise to cyanoepithioalkanes (epithionitriles). The exact mechanism behind the formation of epithionitriles from these glucosinolates is not known, but depends on the presence of a protein known as epithiospecifierprotein (ESP) (MacLeod and Rossiter, 1985; Foo et al., 2000; Zabala et al., 2005; Bones and Rossiter, 2006). The breakdown products resulting from glucosinolate hydrolysis represent the 'defence-active' components and the dual functioning of glucosinolates and myrosinases coming into contact upon tissue disruption is designated as the glucosinolate-myrosinase defence system. This system has been shown to have multiple roles in plant-insect interactions and insect pest management (Bones and Rossiter, 1996, 2006; Rask et al., 2000).

It should be noticed that some insects such as *B. brassicae* and *L. erysimi* actively take advantage of the defence compounds produced by their host plants by sequestering toxic compounds from the plant and using these compounds to protect themselves from predators. *B. brassicae* and *L. erysimi* sequester glucosinolates which can be degraded by a thioglucosidase endogenously produced by the insect, when the latter is crushed (Jones et al., 2001, 2002; Bridges et al., 2002; Rossiter et al., 2003; Husebye et al., 2005). These crushed insects likely both smell/taste badly and release volatiles, alarming other aphids in the colony.

2.3 Plant Volatiles

Throughout evolution, higher plants have evolved complex mechanisms to be able to communicate with their environment. Based on their capability for gas exchange, plants can release mixtures of plant volatiles having distinct biological functions related to plant-insect, plant-pathogen and plant-plant communication, and adaption to stresses (Kishimoto et al., 2005;

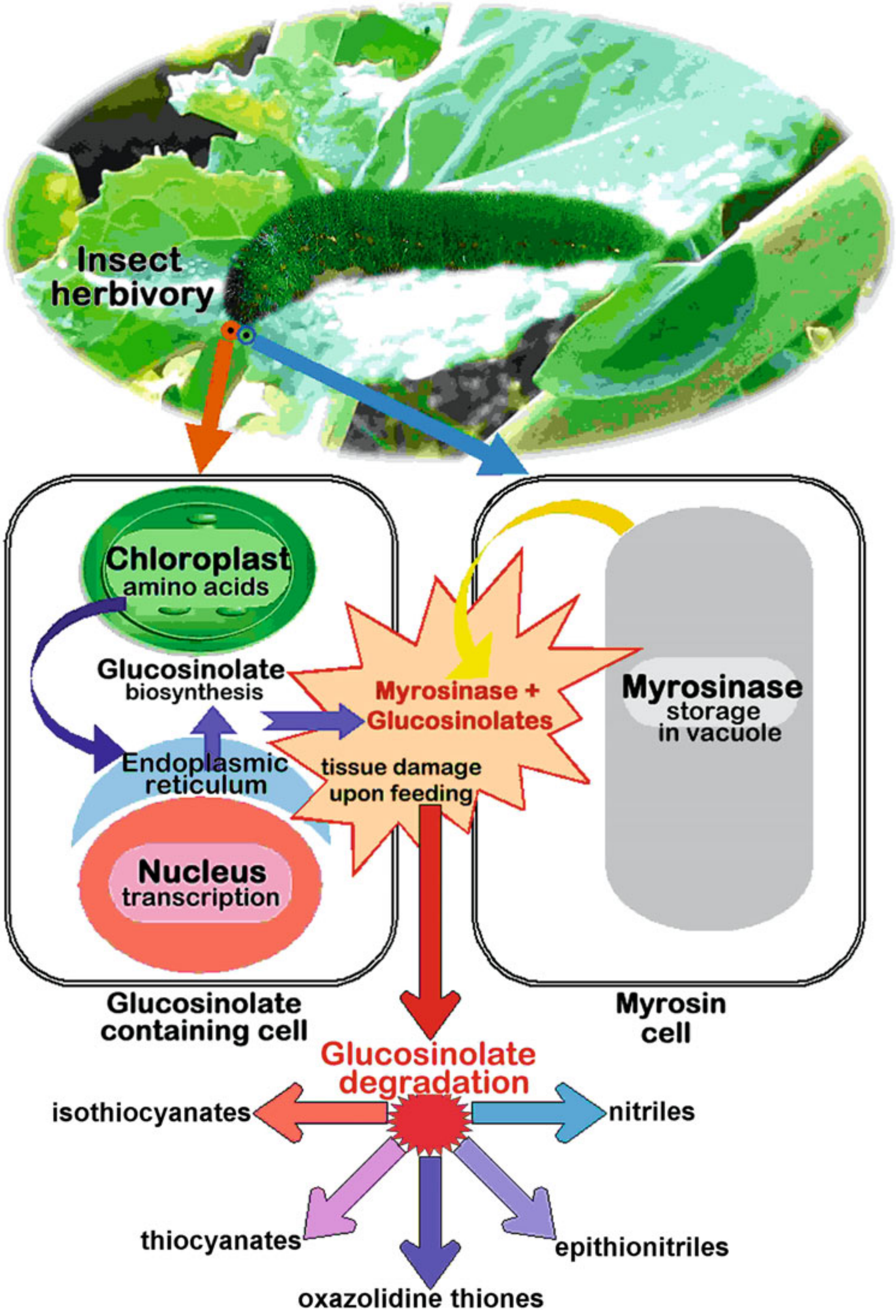

Fig. 4 Insect herbivory brings glucosinolates and myrosinase together and facilitates the hydrolysis of glucosinolates

Baldwin et al., 2002). Brassicaceae produce volatile and semi-volatile toxic compounds based on glucosinolate degradation upon tissue damage, thus directly functioning in plant defence. However, plant volatile communication is a much more sophisticated process (described in Sect. 3.2) of trophic interactions where, e.g., herbivore-attacked plants release volatile signals to attract predators of the feeding insects. In order to

Table 3 The diversity of plant volatiles and signalling compounds in Brassicaceae plants (Rohloff and Bones, 2005)

Compound group	Plant volatiles	Plant organ	Function
Green leaf volatiles	C_6-alcohols, aldehydes and acetates	Green plant parts	Plant-plant signalling, predator attraction, antimicrobial activity
Plant hormones	Jasmonic acid and salicylic acid derivatives, ethylene	Whole plant	Plant-plant signalling, induction of plant defences
Terpenes	Mono- and sesquiterpenes	Flowers, leaves, roots	Flower pollinator attraction, attraction of predators, antimicrobial activity
Aromatics	Benzyl and phenylethyl-derivatives	Mainly flowers	Flower pollinator attraction, antimicrobial activity
Glucosinolate-derived volatiles	Isothiocyanates, thiocyanates, oxazolidine thiones, nitriles, epithionitriles	All plant parts containing myrosinase and glucosinolates	Plant defence, herbivore attraction
Sulphur-containing compounds	Sulphides, elemental sulphur	Probably whole plant	Plant defence

distinguish between the multiple roles and functions of plant volatiles in Brassicaceae, Table 3 briefly summarises the known chemical structure groups found in different plant parts and tissues.

2.4 Phytoalexins and Phytoanticipins

Phytoalexins are low-molecular-weight antimicrobial compounds or secondary metabolites that are synthesised de novo, while phytoanticipins are pre-formed inhibitors of infection (Dixon, 2001; Rouxel et al., 1991). However, the distinction between phytoalexin and phytoanticipin is not always clear as some compounds may be phytoalexins in one species, and phytoanticipins in others (Dixon, 2001). Phytoalexins and phytoanticipins are also referred to as two significant classes of natural pesticides exerting different methods of action (Zukalová and Vašák, 2002). Phytoanticipins emerge from already created precursors, and phytoalexins commence as the result of an external affect due to distinct metabolic activity. Glucosinolates and the glucosinolate-myrosinase system represent an example of such a type of phytoanticipin since myrosinase and glucosinolates are already biosynthesised as precursors before insect attack (Zukalová and Vašák, 2002). The glucosinolate-myrosinase system, which has been throuoghly investigated in the past decades, is to a large extent considered as a constitutive, as well as inducible type of defence system. It is highly dynamic, interactable with insect pests and a well-established mechanism towards integrated pest management (Bones and Rossiter, 1996, 2006; Rask et al., 2000; Wittstock et al., 2004; Müller and Sieling, 2006). Isothiocyanates produced after glucosinolate hydrolysis by myrosinases play crucial ecological roles in protecting plants against various pests, including insects and microbial systems. Therefore, isothiocyanates are part of a group of basic plant chemical defences known as phytoanticipins (Pedras et al., 2007a). Moreover, phytoalexins from the Brassicaceae family are the only sulphur-containing and nitrogen-containing phytoalexins including an unexpected range of functional groups and indolyl structures (Pedras et al., 2007b). Brassinin, 1-methoxy brassinin, brassilexin and cyclobrassinin are sulphur-containing indole phytoalexins, which have been isolated from different *Brassica* species (Rouxel et al., 1991). Brassinin and 1-methoxybrassinin, which contain a dithiocarbamate group, were the first phytoalexins to be reported. Dithiocarbamates have been recognised as important pesticides and herbicides and until now crucifers are the only plants known to produce such compounds (Pedras et al., 2000).

2.5 Sulphur

The plants of the family Brassicaceae are known to be rich in sulphur (Williams and Cooper, 2004). Sulphur is necessary for plant development and sulphur-containing compounds such as sulphur-rich antifungal proteins, phytoalexins and glucosinolates play an important role in plant defence against pathogens (Dubuis, 2004). *Brassica* plants use sulphur (S) to synthesise glucosinolates and phytoalexins. Cysteine, the primary product of sulphur assimilation, is incorporated into sulphur-rich proteins (SRPs; including thionins) and glutathione. Furthermore, cysteine is the donor of reduced sulphur for glucosinolate biosynthesis and for the synthesis of phytoalexins (including camalexin) (Rausch and Wachter, 2005). Low sulphate availability has also been shown to induce the expression of myrosinase proteins in *Sinapis alba* plants (Bones et al., 1994; Visvalingam et al., 1998). Pathogen attack and abiotic elicitors lead to the synthesis of sulphur-containing phytoalexins such as brassinin and concentration at the site of pathogen attack. Moreover, leaves of some *Brassica* varieties possess the constitutive elemental sulphur (S^0) that may be related to an alternative process of S^0 biosynthesis, such as from the degradation of certain glucosinolates (see reviews Bones and Rossiter, 1996; Williams and Cooper, 2004). Elemental sulphur as cyclooctasulphur S^8 has been reported to have antimicrobial activity in *Theobroma cacao* (Cooper et al., 1996) and similar inorganic sulphur compounds are also present in Brassicaceae (Rohloff and Bones, 2005).

3 Chemical Ecology and Insect Behavioural Aspects

The chemical ecology of plant-insect interactions deals with chemical signals mediating all aspects of insects' lives, their ecological interactions through identification, and defining the chemicals involved in these interactions (Cardé and Millar, 2004). These chemical signals and ecological interactions include: chemical mediators modifying insect behaviour, plant chemicals to protect from insect herbivores (below- and above-ground), multitrophic interactions among plants-herbivores-parasitoids-hyperparasitoids, oviposition, semiochemical-mediated interactions, and the chemical cues that parasitoids use to find their herbivore hosts. Plant chemicals that elicit immediate behavioural responses in insects are generally categorised as attractants (eliciting oriented movements towards the source), arrestants (causing aggregation), stimulants (eliciting feeding, phagostimulation, oviposition, etc.), repellents (causing oriented movements away from the source) and deterrents (inhibiting feeding or oviposition) (Ryan, 2002).

In an article entitled "Developing sustainable pest control from chemical ecology" (Pickett et al., 1997), the significance of chemical ecological relations is emphasised in order to understand insect/insect and insect/plant interactions, and insect behaviour influenced by pheromones and other semiochemicals. This type of knowledge opens up promising pest control methods as alternative strategies to the exclusive use of broad-spectrum pesticides. In general, insect behaviour results from the integration by its central nervous system of a variety of inputs that derive from stimuli acting on exteroceptors (that sense events external to the insect), enteroceptors (that sense the internal physiological state of the insect), and proprioceptors (that sense the relative positions of parts of the body) (Foster and Harris, 1997).

On the background of chemical ecological and insect behavioural aspects, we have mainly focused on and reviewed regulation of oviposition during insect attack and behaviour of the different herbivore insects that attack brassicas, and their trophic interactions. The oviposition and trophic interaction studies mainly revolve around the response of plant cues and chemicals from *Brassica* plants towards these insects, which have been discussed with special emphasis on glucosinolates, their breakdown products, and blends of plant volatiles that are released during oviposition and multitrophic (second, third or fourth level) interactions. Figures 5 and 6 show the diagrammatic presentation of larvae feeding, oviposition process by adult insects, the trophic interactions of *Brassica* plants as hosts, their herbivore insects and the predators or parasitoids of herbivores.

3.1 Oviposition

The search for an oviposition site by flying insects comprises two phases of behaviour, i.e. pre-alighting and post-alighting (Hopkins et al., 1999). The pre-alighting behaviour of an insect, captivated by a range of visual and odour stimuli, ends up in contact with a potential host plant, and the post-alighting behaviour of the female on the host plant depends upon the balance of the internal physiological condition and

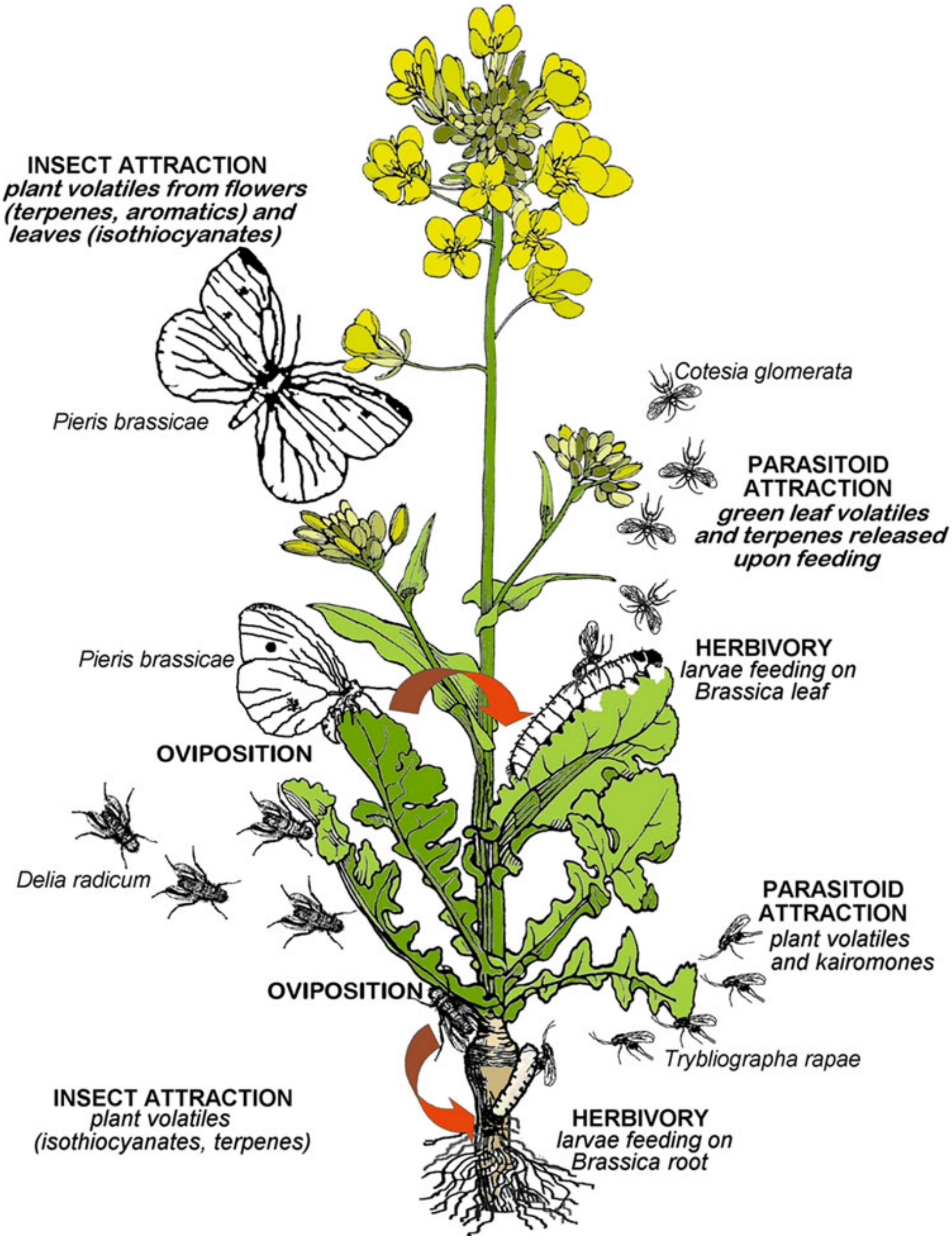

Fig. 5 *Brassica* plant as host showing leaf feeding, oviposition and trophic interactions by larvae and adults of *Pieris brassicae* and *Delia radicum* and their parasitoids (modified after Ross-Craig, 1949)

external stimuli that she perceives (Hopkins et al., 1999). Olfaction (the sense of smell) has an important role after an insect has landed on a plant, and before it moves to leaves or down to the soil to oviposit. Physical factors such as size, colour and leaf structure characteristics have been shown to influence oviposition behaviour, since bright green surrogate leaves (sprayed with a leaf surface extract of the host plant, with a stem, possessing vertical folds to mimic veins, and with a paraffin cover to mimic the wax layer) received the most eggs (Roessingh and Städler, 1990). Most herbivorous insects firstly attack plants by oviposition. Most lepidopterans do not feed on leaves as adults, but females deposit their eggs on those plants or plant parts

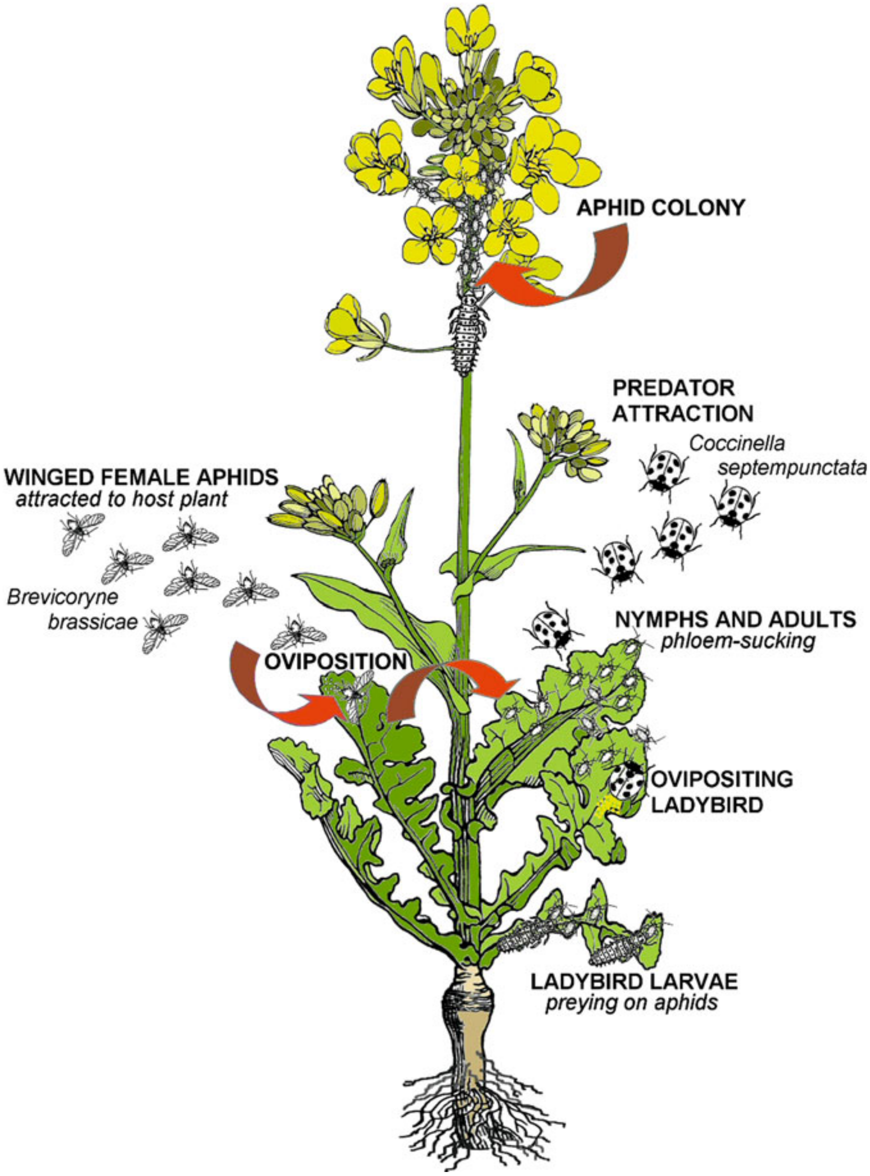

Fig. 6 *Brassica* plant as host showing aphid oviposition, colony and trophic interactions between cabbage aphid (*Brevicoryne brassicae*) and its ladybird predators (*Coccinella septempunctata*) (modified after Ross-Craig, 1949)

where hatching larvae will find suitable food (Hilker and Meiners, 2006). In order to protect themselves against feeding damage, the oviposition-induced plant responses are targeted against eggs laid on the plant, the hatching larvae, or the egg-laying gravid female (Hilker and Meiners, 2002, Hilker and Meiners, 2006).

Chemical stimulation of oviposition is a complex process, and specific chemicals are involved in the acceptance of hosts and rejection of non-host plants (Hamilton et al., 2005). Ovipositing females seem to employ plant volatiles as cues for orientation to host plants and the following contact evaluation of plants by means of less- or non-volatile secondary metabolites has a great significance in host recognition (Keiichi, 1995). It was further highlighted that the acceptance or rejection of a particular plant by females is regulated not only by the presence or absence of oviposition stimulants, but by negative stimuli evoked by co-occurring deterrents. Moreover, oviposition-deterring activity has also been observed

Table 4 Definitions of significant terms mentioned in the article

Term		Definition	Reference
1	Allelochemical	An infochemical that mediates an interaction between two individuals that belong to different species.	(Dicke and Sabelis, 1992)
2	Antixenosis	A term that is derived from the Greek word 'xeno' (guest) that describes the inability of a plant to serve as host to an arthropod, e.g. insect, represents plant traits conferring non-preference of herbivores, i.e. reduced acceptance for oviposition or feeding.	(Smith, 2005)
3	Deterrent	A chemical that inhibits behaviour, such as feeding or oviposition, when applied to a site where such behaviour normally occurs.	(Blossey and Hunt-Joshi, 2003)
4	Infochemical	A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response.	(Dicke and Sabelis, 1992)
5	Semiochemical	A term that is derived from the Greek word 'semion' (a mark or a signal). Chemicals, which function in communication between and among species, as well as those that serve as messengers between members of the same species.	(Law and Regnier, 1971 as cited by Paré and Tumlinson, 1999)

in leaves without eggs, but adjacent to those carrying eggs, hence indicating a systemic effect (Hilker and Meiners, 2002). Plant secondary compounds, influencing antixenosis (Table 4), are mentioned as a link with oviposition specificity or feeding stimulation of more than 20 insect species (Hopkins et al., 1997). Moreover, plant secondary compounds (or allelochemicals) (Table 4) from a particular plant may be oviposition stimulants or deterrents for insects, which feed on that plant (Renwick and Radke, 1981, 1985) and oviposition deterrence by these compounds possibly has significant consequences for crop pest management (Tabashnik, 1987). These chemical deterrents/stimulants evidently play a significant role in the acceptance or rejection of plants as hosts by ovipositing female butterflies.

It has been stated that the induced defence responses both in above-ground and below-ground plant parts are common (Van Dam and Raaijmakers, 2006). Feeding damage by below-ground herbivores may cause a systemic increase in defensive compounds in above-ground parts, hence leading to interactions between above-ground and below-ground herbivores feeding on the same plant. These kinds of above- and below-ground interactions stimulated by induced responses may alter damage patterns and, finally, affect fitness or plant survival (Bezemer and Van Dam, 2005; Van Dam and Raaijmakers, 2006). Furthermore, the root herbivores of oilseed rape, kale, swede and canola have been shown to affect plant performance, commercial yield and plant chemical defence.

Based on oviposition behaviour of different insects and plant defence, we have reviewed essential works that have been conducted on insects that oviposit on *Brassica* plants. In recent years, most of the investigations pertaining to insect oviposition behaviour and related chemical aspects of *Brassica* plants have been focused on white butterflies (*Pieris* spp.), cabbage seed and seedpod weevils (*Ceutorhynchus* spp.), and cabbage root and turnip root flies (*Delia* spp.), with few studies on diamondback moth (*P. xylostella*), cabbage looper (*T. ni*), cabbage moth (*M. brassicae*) and brassica pod midge (*D. brassicae*).

3.1.1 White Butterflies (*Pieris brassicae* and *P. rapae*)

Almost thirty years ago, it was observed that gravid female *P. brassicae* butterflies show discrimination behaviour in their choice of a plant to oviposit on (Behan and Schoonhoven, 1978). In another study from the 1980s regarding oviposition preferences of field-collected *P. rapae* butterflies towards *B. nigra* plants, leaf water content was considered as a phenotypic characteristic associated with oviposition preference (Wolfson, 1980). Renwick and Radke (1985) further showed that oviposition by *P. rapae* on cabbage was deterred by homogenised cabbage tissue sprayed onto intact plants. Polar as well as non-polar extracts of non-host plants inhibited oviposition. In another investigation, oviposition by

P. rapae butterflies was deterred by spraying the plant secondary compounds coumarin and rutin on cabbage plants in greenhouse tests. Both coumarin and rutin deterred oviposition primarily by affecting prealighting rather than postalighting behaviour, indicating that deterrence was mediated by non-contact cues (Tabashnik, 1987). In a comparative study of oviposition responses of *P. rapae* and *P. napi* to nine crucifers, the results showed that the two *Pieris* spp. have apparently evolved differential sensitivities to the chemical stimuli that trigger or deter oviposition. The balance of positively and negatively interpreted sensory signals generated by plant chemicals obviously plays an important role in acceptance or rejection of a plant by both species. Moreover, in an investigation of leaf volatiles, while the large white butterfly (*P. brassicae*) was laying eggs, it was found that the young cabbage leaves on which *P. brassicae* had laid eggs emitted larger amounts of the monoterpene α-thujene than young clean leaves (Bergström et al., 1994). Furthermore, the oviposition-deterring pheromones (ODPs) of *P. brassicae* and *P. rapae* have been considered to be produced in the female accessory glands, and contain volatile and non-volatile components. Oviposition-deterring pheromones are natural and species-specific compounds that inhibit oviposition, and seem suitable chemicals to reduce crop infestation by insects utilising them, since they produce very low environmental risks (Schoonhoven, 1990).

The potential of glucosinolates in stimulating feeding by larvae and oviposition by adults has been confirmed for *P. brassicae* and *P. rapae* through studies showing that ovipositing *P. rapae* adults respond more strongly to indole glucosinolates, such as glucobrassicin, and less strongly to aliphatic glucosinolates, such as glucocheirolin (Rodman and Chew, 1980; Renwick et al., 1992; Van Loon et al., 1992; Huang and Renwick, 1993; Renwick, 2001). Some of these studies showing response of these insects towards glucosinolates and isothiocyanates are described in more detail below.

Already in 1980, Rodman and Chew showed that the oviposition and larval feeding by *P. napi* is associated with glucosinolate profiles of plant species. Later, Klijnstra and Roessingh (1986) suggested that foretarsal taste hairs of females, apart from the glucosinolate cells, also possess sense cells specifically sensitive to the oviposition-deterring pheromones. These different sense cells are responsible for the sensitivity of tarsal "B-type hairs" to eggwash and glucosinolates. The so-called "B-type hairs" comprise one type of trichoid sensilla which is recognised on the tarsi of *P. brassiace* (Ma and Schoonhoven, 1973). The oviposition by *P. rapae* on cabbage was shown to be stimulated by the glucosinolate glucobrassicin. Other studied glucosinolates were sinigrin, which was slightly active, and glucoiberin, which was completely inactive as a stimulant (Renwick et al., 1992). Another investigation involving oviposition bioassays between cabbage (*B. oleracea*) leaves and *P. brassicae* also identified glucobrassicin as the oviposition stimulant (Van Loon et al., 1992). Moreover, Traynier and Truscott Traynier and Truscott (1991) showed that solutions of glucobrassicin purified from foliage and sinigrin elicited oviposition by the cabbage butterfly, *P. rapae*, at threshold concentrations as low as 10^{-6} M while at higher concentrations, glucobrassicin elicited a faster oviposition rate and a stronger visual response to the substrate through associative learning. In another comparative study on the relative activities of 10 glucosinolates in stimulating oviposition by *P. rapae* and *P. napi,* it was observed that in most cases, *P. rapae* was more sensitive to aromatic and indole glucosinolates than to aliphatic ones (Huang and Renwick, 1994); however, *P. napi* responded strongly to aliphatic glucosinolates.

(Bruinsma et al., 2007) investigated the effect of jasmonic acid (a key hormone involved in plant defence responses) on Brussels sprouts (*B. oleracea* var. *gemmifera*) and their acceptability for oviposition by the butterflies *P. rapae* and *P. brassicae*. The investigation showed that both butterfly species laid fewer eggs on leaves of jasmonic acid-treated plants than on leaves of control plants. It was further shown that application of jasmonic acid doubled the concentration of the glucosinolate glucobrassicin and lowered the concentrations of the glucosinolates glucoiberin and 4-hydroxyglucobrassicin.

3.1.2 Cabbage Seed and Cabbage Seedpod Weevils (*Ceutorhynchus assimilis* and *C. obstrictus*)

In order to develop resistant crop germplasm, it is important to understand how host-plant characteristics affect behavioural and physiological responses of insect herbivores. With this hypothesis, Ulmer and

Dosdall (2006) investigated feeding, oviposition preference, larval development and oviposition behaviour of the cabbage seedpod weevil (*C. obstrictus*) on eight Brassicaceae species that differ in their glucosinolate profiles. Among these eight *Brassica* species, the preferred host plant for feeding and oviposition was *B. carinata*, larval development occurred most rapidly on *B. rapa*, and the larval weight was highest on *B. napus*. Total glucosinolate levels did not influence *C. obstrictus* larval growth or development; however, high levels of specific glucosinolates such as sinalbin and gluconapin were associated with increased developmental time or reduced weight.

In a linear track olfactometry test to observe responses of the cabbage seed weevil (*C. assimilis*) to volatiles from oilseed rape (*B. napus*), weevils showed attraction towards the odour of rape during a short period before diapause and for most of their postdiapause life (Bartlet et al., 1993). Apart from that, attraction was also observed for 3-butenyl and 4-pentenyl isothiocyanate, but not for 2-phenylethyl isothiocyanate. A mixture of the three isothiocyanates was more attractive than the individual isothiocyanates. Furthermore, the effects of extracted and artificial oilseed rape odours on the behavioural response of male and female cabbage seed weevils were investigated in a wind tunnel experiment (Evans and Allen-Williams, 1998). Omission of two isothiocyanates from the artificial extract significantly reduced the upwind movement of females.

After oviposition into a pod of oilseed rape, the female cabbage seed weevil marks the pod with oviposition-deterring pheromones by brushing it with her eighth abdominal tergite (Ferguson et al., 1999b). This oviposition-deterring secretion of the cabbage seed weevil was demonstrated to contain iso- and n-alkanes, dimethylalkanes, alkenes, fatty acids, 15-nonacosanone, 15-nonacosanol, and cholesterol (Mudd et al., 1997). Extracts of volatiles entrained from ovipositing weevils failed to inhibit oviposition. The authors evidenced that the oviposition-deterring pheromones of the cabbage seed weevil are sensed primarily by contact chemoreception at the sensilla chaetica of the antennae, and the electrophysiological responses recorded from these gustatory sensilla retain significance as a bioassay to assist identification of the active constituent(s) of the pheromone (Ferguson et al., 1999a). In their previous study, it was reported that the decision of a seed weevil to accept or reject a pod for oviposition to some extent depends upon cues perceived via antennal sensilla. In order to select an oviposition site, a female weevil walks back and forth along the pod, antennating the substrate much more intensively than when walking on the stem or petiole (Ferguson and Williams, 1991).

3.1.3 Cabbage and Turnip Root Flies (*Delia radicum* and *D. floralis*)

The very first detailed work published on the behavioural responses of cabbage root fly (*D. radicum*) (Traynier, 1967a, b) to host plants specified that the odour stimulates activity and that chemical contact stimulates oviposition (Hawkes and Coaker, 1979). In a visual host finding, and shape recognition study on the cabbage root fly, using four shapes of yellow sticky traps as plant models, Tuttle et al. (1988) indicated that yellow discs or crosses at ground level baited with allyl isothiocyanate effectively monitored female cabbage root fly. The main factors affecting female landing were suggested to be the colour of substrate, height above the ground, presence of host volatiles in the vicinity, visual prominence, and size of the total area of ‘attractive’ colour. The initial landing phase of turnip root fly (*D. floralis*) (period between landing on the leaf and the first movement across the leaf) was shown to be crucial for host-plant expedition prior to oviposition site selection (Hopkins et al., 1997). The behavioural sequence analysis of individual gravid female turnip root flies showed that during the postalighting behaviour of turnip root fly, the decision to reject a highly resistant plant was predominantly based on plant cues received during a stationary period immediately after landing on the leaf (the leaf contact phase) (Hopkins et al., 1999). The host-plant acceptance by the cabbage root fly seems to result from a synergistic response to simultaneously perceived olfactory and contact chemostimulation (De Jong and Städler, 1999; De Jong et al., 2000). It was elucidated that female flies, after landing on a potential host, explore the plant by walking on the leaf surface and stem. Due to this exploration, the taste receptors located on the tarsi detect the presence of host-specific cues which stimulate the flies to descend to the soil and oviposit.

Glucosinolates, glucosinolate hydrolysis products such as isothiocyanates, and other volatile compounds are important cues for the cabbage root fly in locating and recognising a suitable host, and are involved in the feeding behaviour. Only mated gravid females respond to them (Hawkes and Coaker, 1979; Nottingham and Coaker, 1985; Renwick et al., 1992; Roessingh et al., 1992; Simmonds et al., 1994; De Jong and Städler, 1999; Hurter et al., 1999). In a study examining the role of glucosinolates towards oviposition behaviour of the cabbage root fly using egg counts and electrophysiological recordings from tarsal chemoreceptors, Roessingh et al. (1992) showed that the D sensilla on segments 3 and 4 of the tarsus of cabbage root fly females contain a sensitive receptor cell for glucosinolates. The flies clearly distinguished between model leaves with and without glucosinolates, but a clear dose response curve was only obtained for the indole glucosinolate glucobrassicin, which indicates that glucobrassicin on the cabbage leaf surface stimulates oviposition. Another study involving behavioural and chemosensory responses of the turnip root fly to glucosinolates demonstrated that slight modifications in chemical composition of glucosinolates resulted in alterations in neural activity (Simmonds et al., 1994). Furthermore, of the eleven glucosinolates tested in these studies, the flies reacted most to glucobrassicanapin, gluconapin and glucobrassicin. Investigation of *B. nigra* and *B. oleracea* plants against cabbage root fly displayed a local increase in indole glucosinolates in the main roots, with *B. oleracea* plants showing a stronger increase in indole glucosinolate levels than *B. nigra*, which was the preferred feeding site of cabbage root fly larvae (Van Dam and Raaijmakers, 2006). Moreover, the increase in indole glucosinolates in *B. nigra* main roots was counterbalanced by a significant decline in aromatic glucosinolates.

Apart from glucosinolates, *Brassica* phytoalexins have been documented as playing a significant role in oviposition (Roessingh et al., 1997; Baur et al., 1996, Baur et al., 1998; De Jong et al., 2000; Hurter et al., 1999; Marazzi et al., 2004a, b). Two compounds, the so-called "Cabbage Identification Factors" (CIFs) are isolated from the surface of *B. oleracea* cv. *botrytis* leaves and identified. These compounds, perceived by a specific receptor neuron in the tarsal sensillum C5 of the female fly (Roessingh et al., 1997), are strong stimulators of oviposition in the cabbage root fly. Spectroscopic data indicated that the main CIF compound (1,2-dihydro-3-thia-4,10,10b-triaza-cyclopenta[.a.]fluorene-1-carboxylic acid) is a novel compound related to *Brassica* phytoalexins such as brassicanal C and is accompanied by its glycine conjugate. Cabbage (*B. oleracea*) leaves contain these compounds in extremely low concentrations but higher levels were detected in the roots of *B. napus* var. *napobrassica* (rutabaga) (De Jong et al., 2000). Furthermore, surrogate leaves treated with methanolic leaf surface extracts of *B. napus* plants that received three different sulphur fertilisation treatments showed even more marked differences by the oviposition choice of cabbage root fly than the potted plants. The oviposition data was shown to be positively correlated either with CIF or glucosinolates (Marazzi et al., 2004a). Investigation on application of eleven crucifer-specific phytoalexins and related synthetic compounds on surrogate paper leaves, being offered to cabbage root flies in oviposition assays, showed three of them (methoxybrassinin, cyclobrassinin and brassicin) to be significantly stimulatory, whereas the remaining metabolites had no effect, suggesting that the reaction of the fly appears to be structure-specific (Baur et al., 1998). In their previous observations, the authors suggested that the cabbage root fly tends to choose plants infected by suitable bacteria, and thus phytoalexins produced by the infected plant might be providing the specific guiding signal (Baur et al., 1998). The cabbage root fly seems to present another example of a herbivore preferring already attacked plants, which is consistent with the possibility that some phytoalexins act either as a signal for host-plant detection or as a marker for optimally preconditioned plants.

3.1.4 Diamondback Moth (*Plutella xylostella*)

The oviposition preference of *P. xylostella* for cabbage, broccoli and cauliflower was observed in the field (Hamilton et al., 2005). No difference was observed in the number of eggs found on the broccoli or cauliflower cultivars. The eggs that were laid on cultivar *Savoy King* were higher than any of the cultivar tested. Larvae development was more rapid and longer on the cabbage cultivar *Green Coronet* than the cultivar *Savoy King*. It was concluded that in the field, *Savoy King* (cabbage cultivar) is more attractive to oviposition.

Renwick et al. (2006) demonstrated isothiocyanates to be oviposition stimulants for the diamondback moth. Hughes et al. (1997) showed that potent oviposition stimulants for the diamondback moth are extracted from cabbage foliage by soaking the intact leaves in chloroform. Analysis of these extracts revealed the presence of two isothiocyanates, iberin and sulphoraphane. Other isothiocyanates with sulphur in the side chain were also reported to be active.

In 1960, Gupta and Thorsteinson studied the effect of sulphur mineral nutrition on two Brassicaceae species (*S. alba* and *B. nigra*) and demonstrated that the constituents of host plants affected larval feeding and oviposition of *P. xylostella*. In order to observe sulphur perception by the diamondback moth, Marazzi et al. (2004a) raised *B. napus* plants under three different sulphur regimes: sulphur-free, normal field concentration and sulphur-rich (twice the normal field concentration). In addition, they performed dual oviposition assays with the diamondback moth, using either *Brassica* plants or artificial leaves sprayed with methanolic leaf-surface extracts. It was shown that chemical compounds on the leaf surface mediate the oviposition preference and that the female insect can perceive the quality of the host plants in terms of their fertilisation status. Since the leaf content of the volatile isothiocyanates is influenced by sulphur nutrition, the authors analysed the extracts for the presence of these compounds. Eleven glucosinolates were identified, with progoitrin and gluconapoleiferin being the most abundant ones. Sulphur nutrition has also been reported to differentially affect the expression of the glucosinolate-hydrolysing myrosinases (Bones et al., 1994).

3.1.5 The Cabbage Looper (*Trichoplusia ni*)

The cabbage looper exhibits a chemical spacing mechanism. This chemical spacing mechanism was noticed as feeding larvae deterred oviposition by gravid females and larval frass, since debris or excrement produced by insects were found to contain the biologically active material (Renwick and Radke, 1980 as cited by Renwick and Radke, 1981). The concept that host-plant chemicals play a role in the spacing of phytophagous insects was introduced by Cirio in 1971 (as cited by Renwick and Radke, 1981). Based on this concept, Renwick and Radke (1981) also demonstrated that the cabbage looper depends on the host plants to avoid overcrowding. Furthermore, the results by Landolt (1993) suggested an important role for damage-induced plant volatiles in host location as well as host acceptance by *T. ni.*

Chow et al. (2005) observed the effects of larval experience with complex plant latex (*Hoodia gordonii*) on subsequent feeding and oviposition by the cabbage looper moth. The study showed that naïve groups of *T. ni* moths are deterred from feeding and ovipositing on cabbage leaves treated with the *H. gordonii* latex, and that larval feeding experience can lessen or reverse this deterrence. It was further suggested that moths may be acquiring oviposition preferences from larval feeding experience as described by Hopkins' host selection principle (HHSP). This principle, also called theory of larval memory, postulates that the adult females of phytophagous insects will prefer to feed or oviposit on the same plant species upon which they themselves developed as larvae (Hopkins, 1917 as cited by Barron, 2001 and stated by Chow et al., 2005).

3.1.6 The Cabbage Moth (*Mamestra brassicae*) and Bertha Armyworm (*Mamestra configrata*)

The cabbage moth (*M. brassicae*) often chooses *Brassica* plants as hosts for oviposition (Ulland et al., 2008). The mortality of eggs, larvae, pupae and larval dispersal of the cabbage moth on white cabbage (*B. oleracea* var. *capitata*) was investigated in a series of small-scale field experiments and in the laboratory (Johansen, 1997). The highest mortality was found in young larvae and in hibernating pupae. The main mortality factor was found to be unfavourable weather conditions, and cold stress.

Furthermore, in other oviposition studies, Rojas and Wyatt (1999) analysed the influence of pre-imaginal (larval conditioning) and post-imaginal experience (adult conditioning) on the orientation, landing and oviposition of the female cabbage moth in a wind tunnel. The females were initially attracted to and landed on chrysanthemum whether or not they fed on this plant species. In addition, the oviposition preference for cabbage plants was not changed by the larval feeding regimen (a regulated system of a diet). Overnight exposure of females to chrysanthemum or cabbage plants decreased the subsequent orientation/landing on the same species offered in non-choice tests. However, it had no effect on subsequent oviposition as females from both treatments oviposited more on cabbage. In another study, Rojas et al. (2000) investigated the orientation and oviposition behaviour of *M. brassicae* on the most preferred host (cabbage) and two other host plants, tomato and chrysanthemum. It was observed that after landing on the plant, the insects were most likely to lay eggs on cabbage and tomato, but the behavioural sequence on these plants was shown to be different. Half of the females laid eggs on tomato only after dragging the ovipositor on the leaf, whereas almost all females laid eggs on cabbage after touching the surface with the ovipositor only briefly. The authors proposed that an understanding of these behaviours could help establish appropriate conditions for future studies on chemical identification of plant semiochemicals mediating host finding of the cabbage moth. The same group also investigated the age at which females begin to lay, the daily pattern of oviposition and the influence of host-plant material on egg-laying, as well as the host preference of *M. brassicae* under laboratory conditions (Rojas et al., 2001). It was observed that the females started ovipositing during the third, fourth and fifth scotophases after emergence. Maximum oviposition occurred during the second hour of scotophase. The females without host-plant material laid fewer eggs than females with host-plant material (*B. oleracea* var. *capitata*). In two-choice tests, the females preferred to oviposit on cabbage rather than chrysanthemum, but there was no difference in the mean number of eggs laid on cabbage and tomato.

Ulland et al. (2008) identified methyl salicylate as primary odorant of a specific receptor neuron type, and showed that it inhibits oviposition by *M. brassicae*. The behavioural effect of methyl salicylate was studied in outdoor test arenas with *B. napus* and artificial plants (Ulland et al., 2008). The experiments indicated that mated *M. brassiace* females avoid plants with dispensers emitting methyl salicylate. It was further pointed out that as methyl salicylate is induced by caterpillar feeding, it may mediate a message to mated *M. brassicae* females that the plant is already occupied.

The oviposition biology of the Bertha armyworm was studied in relation to the effect of conspecific eggs on oviposition site selection (Ulmer et al., 2003). Females strongly preferred to oviposit on leaves with eggs of a different female than on leaves without eggs. Gravid females preferred leaves that were treated with methanol, highlighting that the source of oviposition stimulation is possibly chemical-based.

3.1.7 The Brassica Pod Midge (*Dasineura brassicae*)

The oviposition behaviour of the brassica pod midge was compared on a preferred host (*B. napus*) with that of a non-preferred, less suitable host (*B. juncea*) for larval growth (Åhman, 1985). The number of landing females was significantly higher on *B. napus* than on *B. juncea*, indicating host differences in olfactory and/or visual stimuli. After landing, the females showed different behaviour on the two species by staying longer and laying more egg batches on *B. napus* than on *B. juncea* plants (Åhman, 1985). In another study by the same author looking at the toxicities of *Brassica* secondary compounds to the eggs of *D. brassicae*, the nitrile compound 1-cyano-2-phenylethane was shown to be more toxic than a "green leaf alcohol", (*Z*)-3-hexen-1-ol (Åhman, 1986). The author further suggested that a crucifer specialist may be restricted in its use of particular hosts due to the compositions and concentrations of glucosinolate compounds.

3.2 Trophic Interactions Among Brassica Crops-Herbivores-Parasitoids-Hyperparasitoids

"Nearly 75% of the world's macroscopic biodiversity is tied up in the look between plants, herbivores, predators and decomposers. In this context, the study of trophic interactions, involving plants, herbivores, and their predators or parasitoids represents a frontier in ecology, and this knowledge can be integrated in environmentally sound agricultural pest management" (Sergio Rasman, Cornell University, USA).

Multitrophic interactions among host plants (first trophic level), herbivores (second trophic level), natural enemies of herbivores/carnivorous arthropods/parasitoids/predators (third trophic level), and hyperparasitoids (fourth trophic level) have been well documented (Agrawal, 2000; Shiojiri et al., 2002; Ode, 2006; Heil, 2008). Parasitoids and predators of herbivores have evolved and generally perform their activities within a multitrophic framework. Plants emit diverse blends of volatile compounds from their leaves, flowers and fruits, which affect a range of organisms in the environment including pollinators, herbivores, neighbouring plants and carnivores (Takabayashi and Dicke, 1996; Neveu et al., 2002; Shiojiri et al., 2002). The emission of these volatiles has mutualistic effects being clearly beneficial for the plant such as in the attraction of pollinators to flowers. Several investigations and documentations from tritrophic level interactions among plants, herbivores, and parasitoids or predators have illustrated that these components are tightly interwoven. Interactions between plants and natural enemies can be antagonistic, additive or synergistic, which illustrates the importance of multitrophic perspectives for effective and sustainable pest management strategies (Wright and Verkerk, 1995; Gange and Brown, 1997; Lewis et al., 1997; Tscharntke and Hawkins, 2002; Gripenberg and Roslin, 2007; De Boer et al., 2008). Apart from bi- or tritrophic, the infochemicals released by an infested plant and/or herbivores are available to other trophic levels. The same information used by the parasitoid to locate the herbivore may be utilised by hyperparasitoids (the fourth trophic level) in order to locate the parasitoids.

Plants under herbivore attack produce chemical cues due to the mechanical damage. These chemical cues are important signals for orientation of both carnivorous enemies and herbivores, including distant host location by arthropods (Karban and Baldwin, 1997; Dicke, 1999; Dicke and Van Loon, 2000; Arab and Bento, 2006). Plants' responses upon damage caused by herbivores occurs through the regulation of several biochemical pathways that lead to the release of chemical compounds, which cause either repellence to herbivore insects or attraction to natural enemies (predators or parasitoids) of herbivore insects (Karban and Baldwin, 1997; Dicke and Van Loon, 2000; Pickett et al., 2003; Arab and Bento, 2006; Gols and Harvey, 2009). Attracted by herbivory-induced plant volatiles, parasitoids of herbivores perform host searching to lay their eggs in or on them as shown in recent studies (Mattiacci et al., 1994; Takabayashi and Dicke, 1996; Dicke, 1999; Hilker and Meiners, 2002; Fatouros et al., 2005b; Heil, 2008). Apart from being chemical cues for parasites and predators, these so-called semiochemical volatiles may induce defence responses in neighbouring plants (Paré and Tumlinson, 1999). Semiochemicals emitted from a diverse group of plants and insects mediate key processes in the behaviour of specific insects.

In addition, several ecological approaches have emphasised the significance of plant traits in plant-insect interactions (Agrawal, 2000), which may vary due to genetic variations among plants and/or induced responses in individual plants upon herbivore attack. These effects are determined mainly by nutritional quality, physical structure, defence-related volatiles and other secondary metabolites. Furthermore, non-lethal exposure of an animal to carnivores, and a plant to herbivores, not only induces a defence, but causes the attacked organisms to produce offspring that are better defended than offspring from untreated parents. This is generally referred to as the transgenerational effect (Agrawal et al., 1999) and likely involves epigenetic modifications. The transgenerational induction of defences has been described as a new level of phenotypic plasticity across generations which might be an important component of predator-prey interactions (Agrawal et al., 1999).

Moreover, the tritrophic role of plant chemistry is a key to various aspects of trophic phenomena (Ode, 2006). This includes top-down effects (controlled by predators) versus bottom-up effects (controlled by resources) and enemy-free space and host choice. The tritrophic effects of plant chemistry are valuable to assess the degree of compatibility between biological control and plant resistance approaches to manage pests (Ode, 2006). Therefore, the study of trophic interactions and their manipulation has the potential to lead to effective ways of biological pest control, and thus reduce pesticide use (Agrawal, 2000; Dicke et al., 1990). Some of the multitrophic (second, third or up to fourth level) studies on *Brassica* species will be reviewed below. Most of these studies revolve around one single herbivore species such as *Pieris* spp., *P. xylostella*, *Delia* spp. and aphids, but studies investigating different herbivore insects simultaneously are also described.

3.2.1 Parasitoids or Predators

Cotesia rubecula Marshall is a solitary endoparasitoid of the small white butterfly (*P. rapae*) and *Cotesia glomerata* L., a gregarious parasitoid of both *P. rapae* and *P. brassicae*. *P. rapae* and *P. brassicae* are also the hosts for the egg parasitoid *Trichogramma evanescens* Westwood in cabbage (Noldus and Van Lenteren, 1985a, b). *Cotesia plutellae* Kurdjumov is a dominant solitary koinobiont, larval endoparasitoid of *P. xylostella*, and generally regarded as being highly specific to *P. xylostella* (Talekar and Shelton, 1993; Agelopoulos and Keller, 1994a–c; Geervliet et al., 1994, 1998; Mattiacci et al., 1994, Mattiacci et al., 2001; Harvey et al., 2003; Fatouros et al., 2005b). *Diadegma semiclausum* Hellén is a specialised parasitoid of *P. xylostella* (Bruinsma et al., 2009). *Platygaster subuliformis* Kieffer and *Omphale clypealis* Thompson are among the more than 20 species of hymenopteran parasitoids that attack *D. brassicae* (Murchie et al., 1997). *Lysibia nana* Gravenhorst is a solitary hyperparasitoid of newly cocooned prepupae and pupae of several microgastrine braconids, including *C. glomerata* (Harvey et al., 2003). *Adalia bipunctata* L. (predator) is known for its polyphagy against many aphid species (Hodek, 1959, as referred to by Francis et al., 2001). The seven-spot ladybird *Coccinella septempunctata* L. is recorded as the only parasitoid of *B. brassicae* (Acheampong and Stark, 2004). *Diaeretiella rapae* M'Intosh, a predominant parasitoid of *Brassica*-feeding aphids, attacks the mustard aphid *L. erysimi* at a greater rate than the generalist feeding aphid *M. persicae* (Blande et al., 2007). The parasitoid *Trybliographa rapae* Westwood is a specialist larval endoparasitoid of *D. radicum* (Neveu et al., 2002). *Phradis interstitialis* Thomson, *Phradis morionellus* Holmgr. and *Tersilochus heterocerus* Thomson are among the most frequent pollen beetle parasitoids (Jönsson et al., 2005). Among these three parasitoids, the parasitoid *P. morionellus* attacks larvae inside oilseed rape buds and flowers and also feeds on the flowers (Jönsson and Anderson, 2007 and references therein). *Microplitis mediator* Haliday is an important parasitoid of early instar larvae of the cabbage moth *M. brassicae* (Lauro et al., 2005). The root-lesion nematode (*Pratylenchus penetrans* Cobb) is a migratory endoparasite with a broad host range (Baldridge et al., 1998 and references therein).

3.2.2 Host Plants (*Brassica* spp.)-Herbivores (*Pieris* spp.)-Parasitoids-Hyperparasitoids

Among the four different crucifer-specific compounds tested towards perception of *P. rapae*, phenylacetonitrile elicited a higher response than ally1 isothiocyanate, benzyl isothiocyanate and 2-phenylethyl isothiocyanate (Hern et al., 1996). Furthermore, *P. rapae* is considered to be an important pollinator for many plant species, since the butterfly occurs three to four times a year and tends to visit a diversity of flowers (Ômura et al., 1999). As an innate preference, butterflies are attracted by specific aromatic volatiles from rape flowers such as benzaldehyde, phenylacetaldehyde, benzyl alcohol, 2-phenylethanol, phenylacetonitrile and indole, in decreasing order of quantity (Ômura et al., 1999). Moreover, experience-based food consumption studies with larvae of *P. rapae* have shown that plant host preference is dependent on the abundance of glucosinolates in brassicas, independent of the chemical structure of these compounds (aliphatic or aromatic) (Renwick and Lopez, 1999). The role of glucosinolates acting as feeding stimulants for larvae of the large white butterfly *P. brassicae* was recognised long ago (Verschaffelt, 1910) as mentioned by David and Gardiner (1966). David and Gardiner (1966) in their *P. brassicae* and glucosinolate-related study demonstrated that *P. brassicae* reared on fresh cabbage leaf to the end of the fourth instar would not accept diets containing sinigrin (and sucrose) in the fifth instar. Another interesting point is that although glucosinolates act as feeding stimulants for *P. brassicae* larvae, about 50% of unfed, newly hatched larvae will accept a diet which contains no glucosinolates. It was noted that out of the nine effective glucosinolates, four (glucoiberin, glucoerucin, sinigrin and progoitrin) were from cabbage. Agrawal and Kurashige (2003) analysed the classical interaction between *P. rapae* and isothiocyanates. Using whole plants, root extracts and a microencapsulated formulation of allyl isothiocyanate, it was shown that isothiocyanates reduce herbivore survival and growth, and increase development time, each in a dose-dependent manner. Neither the substrate allyl glucosinolate, nor myrosinase negatively affected *P. rapae*, hence presenting strong evidence for a role for isothiocyanates in plant resistance against the specialist herbivore *P. rapae.*

Karowe and Schoonhoven (1992) determined the relative suitability of *Brassica* as host plants both for unparasitised *P. brassicae* caterpillars and for *C. glomerata* developing in *P. brassicae*. Of all these *Brassica* plants (Brussels sprouts, Swedish turnip and rape varieties of *B. napus*), the host-parasitoid complex attained a lower final weight than unparasitised

P. brassicae, probably due to reduced consumption by the parasitised *P. brassicae*. In contrast, Sato and Ohsaki (2004) elucidated that *C. glomerata*, although a potential parasitoid of *P. brassicae*, did not effectively lower the population density of *P. brassicae* immediately after the first invasion due to its reluctance to parasitise.

The introduction of a new species can alter the attributes of other species within a community, which may affect discontiguous trophic levels via adjacent trophic levels (Tanaka et al., 2007). The exotic large white butterfly *P. brassicae* invaded Hokkaido Island, Japan, and quickly spread throughout the island. Prior to the invasion, the small white butterfly *P. rapae* was the host of the primary parasitoid *C. glomerata*, on which both the larval hyperparasitoid *Baryscapus galactopus* and the pupal hyperparasitoid *Trichomalopsis apanteroctena* depended. At the time of the invasion, *C. glomerata* generally laid eggs exclusively in *P. rapae*. During the five years following the invasion, however, the clutch size (number of eggs laid in a single nesting) of *C. glomerata* in *P. rapae* gradually decreased, whereas the clutch size in *P. brassicae* increased. As a consequence, the invasion of *P. brassicae* changed the host use of the primary parasitoid *C. glomerata* and the pupal hyperparasitoid *T. apanteroctena* within a very short time (Tanaka et al., 2007).

In a study assessing the attractive role of infochemicals originating from either the host, *P. brassicae*, or its food plant, cabbage, it was shown that *C. glomerata* responds to chemical signals emitted from herbivore-damaged plants rather than infochemicals from *P. brassicae* (Steinberg et al., 1993). Geervliet et al. (1997) demonstrated that the parasitoids of *Pieris* species, *C. glomerata* and *C. rubecula*, showed differential responses towards various herbivore-infested food plants. Herbivore-infested plants emit a huge number of compounds to their maximum level, and the parasitoids that search for hosts have to deal with variability in the availability of chemical cues emitted by the food plants and their host. In a tritrophic study with *P. brassicae* and *C. glomerata*, Mattiacci et al. (1994) investigated the significance of herbivory-induced plant volatiles in Brussels sprouts leaves (*B. oleracea*) versus infochemicals released by the larvae. Chemical analysis of the headspace of undamaged, artificially damaged, caterpillar-infested and caterpillar regurgitant-treated leaves showed that the plant responds to damage with an increased release of volatiles. Another tritrophic study revealed that the solitary parasitoid *C. rubecula* discriminated among volatile blends from Brussels sprouts plants treated with regurgitants of unparasitised *P. rapae* or *P. brassicae* caterpillars over blends emitted by plants treated with regurgitant of parasitised caterpillars (Fatouros et al., 2005b). The parasitoid *C. glomerata* discriminated between volatiles induced by regurgitant from parasitised and unparasitised caterpillars of its major host species, *P. brassicae*. Another study of a system comprising *Brassica-Pieris-Trichogramma* suggested that egg deposition induces alteration in plant surface chemicals, thus causing the arrest of egg parasitoids by contact cues around the eggs (Fatouros et al., 2005a).

Studies by Blaakmeer (1994) regarding infochemicals in a tritrophic system of *Brassica*, *Pieris* and *Cotesia* identified the glucosinolate glucobrassicin as an oviposition stimulant. In another tritrophic study, Blaakmeer et al. (1994) analysed headspace composition, collected either from intact cabbage plants or cabbage plants infested with either *P. brassicae* or *P. rapae* first instar larvae. They identified twenty-one volatiles in the headspace of intact plants. The major differences between intact and caterpillar-damaged plants regarding the headspace profile were revealed for hexyl acetate, (*Z*)-3-hexenyl acetate, myrcene, sabinene and 1,8-cineole. The larval endoparasitoid *C. glomerata* was attracted by the volatiles released from *B. oleracea* damaged by *P. brassicae* first instar larvae. *C. rubecula*, a specialised larval endoparasitoid of *P. rapae*, was attracted by the volatiles released from the *B. oleracea-P. rapae* plant-host complex. This shows that cabbage plants kept under the conditions of headspace collection produce attractive volatiles for both parasitoids. Furthermore, Harvey et al. (2003) examined the interactions over four trophic levels. The study involved *B. oleracea* and a naturally occurring population of *B. nigra* because of the difference in their glucosinolate content. The concentrations of glucosinolates were more than 3.5 times higher in young shoots of *B. nigra* than in corresponding shoots of *B. oleracea*. The study observed that the cocoon mass of *C. glomerata* was not affected by the host-plant species on which *P. brassicae* fed; however, *L. nana* survival was greater and the body size was larger when *P. brassicae* fed on *B. oleracea*. It was demonstrated that the qualitative differences in herbivore diet can differentially affect the performance of interacting organisms across several trophic levels with a proposition that the bottom-up forces may also play a role in mediating interactions involving plants-herbivores-parasitoids and hyperparasitoids (Harvey et al., 2003).

In a perspective paper, Dicke et al. (2004) presented a tritrophic system comprising host cabbage plants, herbivorous larvae of *P. brassicae* and the parasitoid *C. glomerata*. The damage caused by caterpillars feeding on cabbage plants differentially regulates the expression of various genes in the plants, up-regulates biosynthesis of certain types of glucosinolates, and emits bouquets of volatile organic compounds (VOCs). These VOCs are shown to act as an indirect defence by attracting parasitoids that laid eggs in the caterpillars. Among these volatile organic compounds, the green-leaf volatile (*Z*)-3-hexen-1-ol and the terpenoid 1,8-cineole were shown to be the main volatiles emitted by the cabbage plants. Mumm et al. (2008) investigated the significance of terpenoids in a 'cabbage' system consisting of Brussels sprouts plants, large and small cabbage white butterflies (*P. brassicae* and *P. rapae*), and the larval parasitoid *C. glomerata.* The terpenoid emission was manipulated by treating the plants with fosmidomycin, which inhibits one of the terpenoid biosynthetic pathways, and consequently terpenoid emission. The study demonstrated that inhibitors such as fosmidomycin can be used to investigate the role of terpenoid infochemicals in plant defence mechanisms against herbivores.

3.2.3 Host Plants (*Brassica* spp.)-Herbivore (*P. xylostella*)-Parasitoids

Olfactory attraction of female diamondback moths (*P. xylostella*) to the odours of intact and homogenised host plants was investigated using behavioural and electrophysiological methods (Pivnick et al., 1994). Allyl isothiocyanate from *B. juncea* and *B. napus* plants was the most attractive component, being absent in odours from intact plants. It was further suggested that certain elements of this fraction, possibly in combination, are important olfactory cues for host-plant finding by the diamondback moth, with isothiocyanates playing an important and synergistic role, particularly when plants are damaged. Van Loon et al. (2002) showed that the diamondback moth larvae employ a combination of biosynthetically distinct categories of feeding stimulants which allows for a higher degree of discriminatory ability than glucosinolates alone.

Karimzadeh and Wright (2008) used a tritrophic crucifer-*P. xylostella*-*C. plutellae* experimental system in order to test the hypothesis of host-plant effects challenging the innate immune system of an insect host. Using measures of the two principal immune effectors against parasitoids, encapsulation and phenoloxidase activity, it was shown that despite having strong plant effects on parasitism, parasitoidal effects on immune effectors of the host were transitory. These varied levels of parasitism of *P. xylostella* mediated by plant quality are stated to be an outcome of behavioural and fitness factors rather than a reduced immune challenge.

With a consideration that the parasitoids *Trichogramma chilonis* and *C. plutellae*, and the predator *Chrysoperla carnea* are the potential biocontrol agents, Reddy et al. (2002) conducted olfactory response studies with *P. xylostella* on cabbage plants. Among the four larval frass that were tested, only allyl isothiocyanate elicited significant responses in the parasitoids and predator, but *C. plutellae* and *C. carnea* responded well to all four volatiles (dipropyl disulphide, dimethyl disulphide, allyl isothiocyanate and dimethyl trisulphide). The results indicated that the sex pheromone and larval frass volatiles from the diamondback moth and volatile compounds from cabbage may be used as natural enemies to locate diamondback moth (Reddy et al., 2002). Furthermore, the same group performed another study on the host plant-mediated orientation and oviposition by the diamondback moth and its predator *Chrysoperla carnea* in response to four different *Brassica* host plants: cabbage, cauliflower, kohlrabi and broccoli (Reddy et al., 2004). The results indicated that the orientation of female diamondback moths and *C. carnea* females towards cabbage and cauliflower was significantly greater than toward broccoli or kohlrabi plants. Furthermore, in free-choice tests, oviposition by the diamondback moth was significantly greater on cabbage, followed by cauliflower, broccoli and kohlrabi, while *C. carnea* preferred to oviposit on cabbage and cauliflower, followed by broccoli and kohlrabi.

3.2.4 Host Plants (*Brassica* spp.)-Herbivores (*M. brassicae* and *P. brassicae*)-Parasitoids

Lauro et al. (2005) examined the attack responses of female *M. mediator* to the first three larval instars of *M. brassicae*. Their results suggested that first and second instar larvae of *M. brassicae* are suitable hosts for *M. mediator*. Third instar larvae are suboptimal because of unsuccessful oviposition attempts and immature parasitoids failed to complete development. However, naïve attacking parasitoids exhibited minimal discrimination among instars, although experienced parasitoids most frequently attacked first instar larvae.

In a four-armed airflow olfactometry study with the egg parasiotoid *T. evanescens*, Noldus and Van Lenteren (1985a) observed that the females were attracted by a volatile substance (or substances) released by virgin females of cabbage white butterfly (*P. brassicae*). *T. evanescens* was also observed to be attracted by the volatiles released by calling virgin cabbage moths, *M. brassicae*. The results of the experiments with *M. brassicae* showed that *T. evanescens* is attracted by volatiles which are probably the sex pheromone released by calling virgin females, but not by the main component (Z)-11-hexadecenylacetate, nor by a crude extract of the sex pheromone gland (at the concentrations tested). The study suggested the significance of volatile kairomones for the egg parasite *T. evanescens* in the host-habitat location. In another follow-up study, Noldus and Van Lenteren (1985b) showed that contact kairomones are involved in host location by *T. evanescens*. Indeed, *T. evanescens* females searched significantly longer on cabbage leaves treated with the wing scales of two hosts, *P. brassicae* and *P. rapae.* Furthermore, egg washes of *P. brassicae* containing an oviposition deterrent pheromone for the butterflies were found to have a contact-kairomonal effect on the parasite.

Gardner et al. (2007) performed an oviposition experience study to observe the response of egg parasitoid (*T. vanescens*) towards contact kairomones of two different host species *M. brassicae* and *P. brassicae*. The response of *T. evanescens* was influenced by the number of eggs it had laid, but oviposition did not result in a significant change in behaviour. Parasitoids readily accepted an egg of a second species, and the time spent searching in a particular kairomone area appeared to depend on the reproductive state and expected survival of a parasitoid rather than the development of any host preference. It was further proposed that *T. vanescens* seems to select patches on the basis of reward probability rather than maximising reward size.

3.2.5 Host Plants (*Brassica* spp.)-Herbivore (*A. rosae*)-Parasitoids

Müller and Arand (2007) tested whether adults of *A. rosae* innately prefer the plant species for oviposition that allows the best larval performance with regard to both developmental conditions and (plant-derived) defence efficiency against predators. It was observed that in *A. rosae* innate preferences of ovipositing, females seem to be mainly influenced by the host-plant effects on larval developmental times rather than potential defence efficiency of larvae against predators. The study demonstrated that for preference and performance studies, not only the plant-insect interactions should be considered, but also the biotic determinants (Müller and Arand, 2007). In a previous study by the same group, Müller and Brakefield (2003) tested the role of the *A. rosae* sawflies' haemolymph and of the glucosinolate sinalbin in the defence against wasps by using manipulation assays. The haemolymph released by easy bleeding and its chemical components proved to be in part responsible for the efficient defence of the sawfly against wasps (Müller and Brakefield, 2003).

3.2.6 Host Plants (*Brassica* spp.)-Herbivores (*Delia* spp.)-Parasitoids

Birch et al. (1992) showed that different *Brassica* genotypes such as kale, rape and swede after inoculation with turnip root fly (*D. floralis*) eggs showed a similar response in glucosinolate metabolism. Total glucosinolate content in roots increased due to a two- to fourfold increase in indole-based compounds. The largest increase for an individual glucosinolate after attack was found for glucobrassicin. Root damage did not significantly affect stem glucosinolate composition but resulted in an increase in aliphatic glucosinolates with a corresponding decrease in indole-based compounds in leaves. Furthermore, Griffiths et al. (1994) investigated induced changes in the indole glucosinolate content of oilseed and forage rape (*B. napus*) plants in response to either turnip root fly (*D. floralis*) larval feeding or artificial root damage. Larval damage increased the total glucosinolate content of the roots. In contrast, artificial damage reduced the total glucosinolate content of the roots. It was also interesting to note that the concentration of glucobrassicin present in the oilseed rape cultivar *Ariana* was consistently higher in both the larval and artificially root-damaged plants compared with undamaged plants of the same cultivar.

The long-range plant involvement of volatiles in host location of the herbivore *D. radicum* on the parasitoid *T. rapae* was investigated by Neveu et al. (2002). It was shown that the infested turnips systemically emit herbivore-induced plant volatiles that attract the parasitoid *T. rapae,* implying that the production of parasitoid-attracting volatiles appeared to be systemic in this particular tritrophic system.

3.2.7 Host Plants (*Brassica* spp.)-Herbivore (*D. brassicae*)-Parasitoids

The responses of *D. brassicae* and its parasitoids *P. subuliformis* and *O. clypealis* to allyl- and 2-phenylethyl isothiocyanates were investigated using traps in winter oilseed rape (Murchie et al., 1997). In this study it was observed that the traps baited with allyl isothiocyanate caught more male and female *D. brassicae* and more female *O. clypealis* than traps baited with 2-phenylethyl isothiocyanate or unbaited traps. Contrarily, traps baited with 2-phenylethyl isothiocyanate caught more male and female *P. subuliformis* than traps baited with allyl isothiocyanate or unbaited traps.

3.2.8 Plants (*Brassica* spp.)-Herbivores (*Phyllotreta* spp. and *Meligethes aeneus*)-Parasitoids

Already in 1956, Görnitz showed that flea beetles are attracted to allyl isothiocyanate, which is a volatile hydrolysis product of the glucosinolate sinigrin. Based on these results, Pivnick et al. (1992) performed trapping experiments to test attraction of crucifer-feeding flea beetles to volatile glucosinolate hydrolysis products released from glass vials. The pattern of attraction was the same for the flea beetle species *P. cruciferae* and *P. striolata.* When captures of the two species were pooled, 3-methylthiopropyl isothiocyanate, methyl isothiocyanate and n-butylisothiocyanate were found to be significantly attractive, while nitriles were the least attractive compounds. Flower and leaf extracts of rape were found to be attractive in the field from at least 20 m, and the pollen beetles were proposed to use odour-mediated upwind anemotaxis to locate oilseed rape plants (Evans and Allen-Williams, 1994). The results from Y-tube olfactometer bioassays indicated that *M. aeneus* is able to locate its host plant by olfactory stimuli in the early bud stage, i.e. the stage at which the infestation begins in the field and when the typical yellow colour and floral scent of oilseed rape are absent (Ruther and Thiemann, 1997). In a field study on the effect of trap design, trap colour and isothiocyanate lures on the capture of the pollen beetle, *M. aeneus* was shown to be attracted to four alkanyl, three alkenyl and 2-phenylethyl isothiocyanate lures (Blight and Smart, 1999). The response of *M. aeneus* to yellow water traps baited with individual lures of 25 floral volatile compounds was also investigated in 17 field experiments with *B. napus* (Smart and Blight, 2000). These compounds comprised seven nitrogenous amino acid derivatives, five non-nitrogenous amino acid derivatives, nine fatty acid derivatives and four isoprenoids. The results showed most compounds to be attractive, but four fatty acid derivatives were repellent. 1-Hexanol was either attractive or repellent, depending on the release rate. It has been further suggested that because of its polyphagous nature, *M. aeneus* responds to a large number of chemically diverse compounds. The attraction to the most effective unbaited yellow traps was enhanced 1.7–3.3 times with the addition of a lure comprising a mixture of allyl, 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanate.

Jönsson et al. (2005) analysed behavioural responses in three ichneumonid pollen beetle (*M. aeneus*) parasitoids (*P. interstitialis*, *P. morionellus* and *T. heterocerus*) to volatiles emitted from different phenological stages of oilseed rape. All three parasitoid species were attracted to odours from the bud stage of oilseed rape. *T. heterocerus* was attracted to odours of flowering rape, but the two *Phradis* species avoided the flower odours. However, when the odours of flowering rape were in combination with yellow-coloured flowers, and odours of the bud stage were in combination with green-coloured buds, *P. interstitialis* was equally attracted to both stimuli, and *T. heterocerus* showed an increased preference for flower odours, while no effect of colours could be found in *P. morionellus*. The terpenes sabinene, myrcene, limonene and (E, E)-α-farnesene were the dominant volatiles in the bud and flower headspace. A group of aromatic compounds including benzaldehyde, methyl benzoate and phenyl acetaldehyde were mainly released from the flowering rape. Furthermore, Jönsson and Anderson (2007) investigated emission of oilseed rape volatiles after pollen beetle infestation by analysing behavioural and electrophysiological responses in the parasitoid *P. morionellus*. It was observed that both starved and fed parasitoids preferred infested rape, but the proportion of responding female *P. morionellus* was significantly lower for the group that was starved. Six of the 20 volatiles identified were released at higher rates from infested than from non-infested rape. The volatiles released at a significantly higher rate from infested rape and detected by *P. morionellus* antennae were (Z)-3-hexenylacetate, (Z)-3-hexenol, 3-butenyl isothiocyanate and (E, E)-α-farnesene.

3.2.9 Plants (*Brassica* spp.)-Herbivores (*Ceutorhynchus assimilis*)

In a study analysing the peripheral olfactory perception of isothiocyanates, cabbage seed weevil (*C. assimilis*) antennal receptors were able to locate a broad range of compounds associated with the odour of its host plant (Evans and Allen-Williams, 1992). Green leaf volatiles and specific host-related compounds were demonstrated to play a significant role in overall perception and recognition of host odour. In a response study with cabbage seed weevil antenna to volatiles in air entrainment-derived extracts of oilseed rape, using electroantennograms coupled with GC-single cell recording, it was shown that isothiocyanate perception is mediated by three types of olfactory cells, which show differential response to 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanate (Blight et al., 1995). The responses of cabbage seed weevil to other electrophysiologically-active volatiles from rape were tested in a linear track olfactometer (Bartlet et al., 1997). Attraction was shown to nitriles (phenylacetonitrile, 4-pentenenitrile and 5-hexenenitrile) and to volatiles emitted by a wider spectrum of plant families, (Z)-3-hexen-1-ol and methyl salicylate. The combination of an isothiocyanate mixture with phenylacetonitrile increased attraction, but there was no such increase when the isothiocyanate mixture was combined with methyl salicylate. Furthermore, the response of the cabbage seed weevil to yellow water traps baited with some components of oilseed rape odour was studied in a series of field experiments (Smart and Blight, 1997). Four isothiocyanates, five other amino acid derivatives (aromatic compounds) and two fatty acid derivatives were detected by peripheral olfactory receptors of *C. assimilis*. The results suggested that yellow traps baited with 2-phenylethyl isothiocyanate can be used to monitor immigration into crops in the spring and that phenylacetonitrile may be useful for assessing numbers of weevils colonising the crop throughout the summer. After several field experiment studies on trap design, trap colour, and a mixture of isothiocyanates on the capture of *C. assimilis*, Smart et al. (1997) showed that the sticky card trap, mounted at 45° to the vertical and baited with the isothiocyanate mixture, may be useful for monitoring movement of *C. assimilis* during migratory periods.

3.2.10 Host Plants (*Brassica* spp.)-Aphids-Parasitoids/Predators

Chemicals that influence aphid behaviour have been studied for many decades, but a major breakthrough occurred in the early 1970s, due to chemical identification of an alarm pheromone for a number of species by several groups (as reviewed by Dawson et al., 1990). This pheromone was shown to comprise the sesquiterpene hydrocarbon (E, E)-α-farnesene. The chemical potential of glucosinolates and the glucosinolate-myrosinase system has been shown for cabbage and mustard aphids. The cabbage aphid, designated as the walking mustard oil bomb (Bridges et al., 2002; Jones et al., 2001, 2002; Kazana et al., 2007), is not only capable of sequestering harmful glucosinolates but also catalyses the hydrolysis of accumulated glucosinolates upon predator feeding in order to generate biologically active and toxic isothiocyanates. Both *B. brassicae* and *L. erysimi* produce an endogenous insect myrosinase, thus mimicking the plant glucosinolate-myrosinase system and its spatial organisation (Rossiter et al., 2003; Jones et al., 2001, 2002; Bridges et al., 2002; Husebye et al., 2002; Kazana et al., 2007). Recent studies with the model plant *A. thaliana* have shown the induction of aliphatic glucosinolates (Mewis et al., 2006), the contribution of indole glucosinolates towards aphid deterrence of *M. persicae* (Kim and Jander, 2007), and alterations in metabolism and chemical defence by *B. brassicae* (Kuśnierczyk et al., 2007, 2008). It is likely that similar defence responses exist in important *Brassica* crops.

For the mustard aphid (*L. erysimi*), isothiocyanates together with (E, E)-α-farnesene have been reported to work as alarm signals (Dawson et al., 1987). The sequestration of glucosinolates by the specialist aphid, *B. brassicae*, may provide protective compounds against attack by predators or parasitoids. It was suggested that the functioning of glucosinolate utilisation may be important in understanding the exploitation of biological control agents such as predators and parasitoids to control specialist *B. brassicae* and generalist *M. persicae* aphid species (Cole, 1997). Electrophysiological recordings together with high-resolution gas chromatography identified metabolites of glucosinolate precursors in the cruciferous host plants as being synergists for the alarm pheromone of *L. erysimi*. The most active, allyl isothiocyanate, significantly improved the activity of an aqueous formulation of $(E,E)-\alpha$-farnesene (Dawson et al., 1990). The volatile (Z)-jasmone was shown to repel aphids while being an attractant to parasitoids (Birkett et al., 2000). Feeding behaviour of the specialist *B. brassicae* and the generalist *M. persicae* on the range of *Brassica* species tested indicated that generalist and specialist aphids are influenced differently by the host plant (Cole, 1997). *M. persicae* did not generally accept or reject *Brassica* species due to the presence of phagostimulants, such as glucosinolates at the leaf surface or along the stylet pathway, unless the concentration was very high.

Francis et al. (2001) performed a tritrophic interaction study on different *Brassica* crops with variable glucosinolate profiles, with *M. persicae* and *B. brassicae* and the predator *A. bipunctata*. It was suggested that the pest management must include both herbivore and plant trophic levels to determine the plant allelochemical impact on the third trophic level, the beneficial entomophagous insects. It was further added that the semiochemicals from plants, directly or through herbivore prey, must be considered as a potential toxin or reliable infochemical in relation to the efficacy of pest control by natural enemies. In another study either *B. nigra* plants (characterised by high levels of sinigrin), or an artificial aphid diet to which sinigrin was selectively added, were used to rear the specialist *B. brassicae* and aphids were provided as a food source to two species of polyphagous ladybirds, *A. bipunctata* and *C. septempunctata* (Pratt et al., 2008). The results indicated that the presence of sinigrin in the diet of *B. brassicae* makes this aphid unsuitable as a food source for *A. bipunctata*, but not for *C. septempunctata*. However, there appear to be costs associated with *C. septempunctata* feeding on aphids that contain this secondary metabolite. Blande et al. (2007) investigated the orientation behaviour of the parasitoid *D. rapae* to the semiochemicals produced when the two aphid species *L. erysimi* (specialist) and *M. persicae* (generalist) were fed on turnip. Isothiocyanates were among the compounds emitted by Brassicaceae plants in response to insect feeding damage, including damage caused by aphids. The results suggested that similar cues may be utilised by *L. erysimi* and *M. persicae* for host location, whereas the acceptance of hosts and their suitability may involve aspects of non-volatile aphid chemistry. In an olfactory perception and orientation behaviour study of the aphid *B. brassicae* and the parasitoid *D. rapae* to alkenyl glucosinolate hydrolysis products, electroantennogram responses indicated peripheral odour perception in *D. rapae* females to all 3-butenylglucosinolate hydrolysis products (Pope et al., 2008). Nevertheless, rearing *D. rapae* either on *B. nigra*, which accumulates 2-propenylglucosinolate, or *B. rapa*, which accumulates 3-butenylglucosinolate, changed the parasitoids' response to 3-isothiocyanatoprop-1-ene and 4-isothiocyanatobut-1-ene.

3.2.11 Host Plants (*Brassica* spp.)-Herbivores [(*P. xylostella/Pieris*) (*P. xylostella/M. persicae*)]-Parasitoids and [*P. rapae* and Two Different Root Feeders]

Some of the tritrophic studies involving two herbivores documented below are based on the hypothesis suggesting that plants in both natural and cultivated ecosystems are subjected to insect infestation by more than one species and that induced responses occur both locally and systemically throughout the plant (Agelopoulos and Keller, 1994a–c; Shiojiri et al., 2000; Agbogba and Powell, 2007).

The role of volatiles in attracting *C. rubecula* to cabbage infested by the host *P. rapae* was elucidated (Agelopoulos and Keller, 1994b). The study showed that *C. rubecula* was attracted to cabbage previously infested by *P. rapae*. Additionally, females were also attracted to mechanically damaged cabbage and cabbage previously infested by *P. xylostella* (a non-host lepidopteran herbivore). Furthermore, another tritrophic study by the same group focused on the identification of the volatile compounds that could be involved in the searching behaviour of the parasitoid *C. rubecula* (Agelopoulos and Keller, 1994c). The volatiles emitted by the intact cabbage were α-pinene, β-pinene, myrcene, 1,8-cineole, n-hexyl acetate, (Z)-3-hexen-1-yl acetate and dimethyl trisulphide. Mechanical damage on an intact plant induced the release of two more compounds, $(E)-2-$hexenal and l-methoxy-3-methylene-2-pentanone. Feeding by larvae of *P. rapae* induced the plant to release all the compounds upon mechanical damage and additionally 4-methyl-3-pentenal and allyl isothiocyanate. On the other hand, feeding by larvae of *P. xylostella* induced the plant to release all the compounds present after mechanical damage with addition of allyl isothiocyanate. Shiojiri et al. (2000) performed a comparative study of host-searching behaviour with two parasitoid species (*C. plutellae* and *C. glomerata*) on a cabbage plant, infested by the host larvae *P. xylostella.* It was found that the parasitoids showed their antennal-searching behaviour only on the host-infested site. The searching time of *C. plutellae* on a piece of cabbage leaf infested by host larvae was significantly longer than a piece infested by non-host (*P. rapae*) larvae. Likewise, the searching time of *C. glomerata* on the host (*P. rapae*)-infested piece of cabbage was significantly longer than that on a leaf infested by non-host (*P. xylostella*) larvae. Furthrmore, Shiojiri et al. (2002) conducted another study by using the same system as previously (Shiojiri et al., 2000) confirming that the fitness effects of induced chemical production by plants should be studied in tritrophic interaction webs, because chemicals induced by one herbivore species may indirectly affect oviposition preferences of heterospecific herbivores by altering the effectiveness of their respective parasitoids. Such indirect interactions between host and non-host herbivores are referred to as infochemical-mediated indirect interactions. In a recent study, Bruinsma et al. (2009) showed that feeding by *P. rapae* and *P. xylostella* resulted in increased endogenous levels of jasmonic acid in *B. oleracea* (Brussels sprouts) plants. However, the levels of the intermediate 12-oxophyto-dienoic acid (OPDA) were induced only after *P. rapae* feeding. Moreover, jasmonic acid-induced volatiles of *B. oleracea* attracted parasitoids (*C. glomerata*, *C. rubecula* and *D. semiclausum*) in a time- and dose-dependent application (Bruinsma et al., 2009). In a study using two insects, the peach aphid (*M. persicae*) and the caterpillar (*P. xylostella)* on cabbage plants, Agbogba and Powell (2007) looked at the responses of the aphid parasitoid *D. rapae* by Y-tube olfactometry. Their results indicated that the aphid and the caterpillar induce different changes in the volatile profile of cabbage plants and that *D. rapae* females readily distinguish between the two. In a study, involving *B. nigra* plants, *P. rapae* and two different root feeders (the endoparasitic nematode *P. penetrans* and the larvae of the cabbage root fly *D. radicum*), Van Dam et al. (2005) indicated that root feeding can significantly alter the nutritional quality of shoots by changes in secondary metabolite levels and hence the performance of a specialist shoot feeder. Moreover, it was observed that *P. rapae* larvae grew more slowly and produced fewer pupae on plants that were infested with root feeders, especially on plants infested with the endoparasitic nematode *P. penetrans* and that both glucosinolate as well as phenolic levels were affected by root feeding.

4 Brassicas and Their Potential for Integrated Pest Management

Integrated pest management (IPM) is an ecological approach to managing insect pests, by using different pest control methods, that are aimed at the entire pest complex of a crop ecosystem and finally ensures high-quality agricultural production in a sustainable, environmentally safe, and economically sound manner (Bajwa and Kogan, 2002 as cited by Guerena, 2006). The insect pest management strategies include pest monitoring, that allows accurate timing of pesticide applications; combined use of semiochemicals, host-plant resistance and trap crops to manipulate pest behaviour, employing biological control approaches or selective insecticides to reduce pest populations and developing insect-resistant crops (Pickett et al., 1997).

From these perspectives of pest management aiming at an ecochemical control, we have briefly reviewed the use of brassicas as biocontrol, cover crops and trap crops in order to control or manipulate the insect-pest complex not only for brassicas, but also insect pests and diseases for other economically important crops. In addition, we have described in brief the role of plant breeding in developing insect resistance in *Brassica* crops.

4.1 Brassicas as Biocontrol Agents

Brassicas as biocontrol agents generally employ the process of biofumigation. Biofumigation is referred to as the process of suppression of pests and pathogens through release of volatile substances from degradation of biomass into the soil. For the biofumigation process, *Brassica* plants are incorporated into the soil and due to the significant amounts of glucosinolates, toxic breakdown products, especially isothiocyanates, are produced after tissue maceration (Brown and Morra, 1997; Kirkegaard and Sarwar, 1998; Gardiner et al., 1999; Smith et al., 2004). Isothiocyanates carry fumigant properties similar to metham-sodium (Sarwar et al., 1998; Warton et al., 2003). Metham-sodium (sodium N-methyldithiocarbamate) is a compound that is used globally to control noxious soil-borne organisms in intensive cropping systems as it generates the wide-spectrum fumigant-like compound methyl isothiocyanate after coming into contact with the wet soil (Matthiessen and Shackleton, 2005). Methyl isothiocyanate (not present in brassicas) is the only isothiocyanate which is used as a pesticide on a commercial basis but other isothiocyanates have also become the subject of interest due to their biofumigation properties (Angus et al., 1994). However, in a cross-enhancement study, Warton et al. (2003) suggested that in soil suffering from enhanced biodegradation of methyl isothiocyanate, biofumigation using isothiocyanate-producing *Brassica* plants is unlikely to be an effective alternative.

Methyl bromide was used as a soil fumigant with wide-spectrum potential to control soil-borne pests and diseases, nematodes and weeds for economically important crops such as tomato, strawberries and cucurbits (Ploeg, 2008). The phasing out of the ozone-depleting soil fumigant methyl bromide and a higher interest in developing safe and economically viable insect pest management strategies has led to the initiation of alternative approaches. One of these approaches was the process of biofumigation by using macerated *Brassica* tissues as biofumigant agents and as cover crops (Noble et al., 2002; Matthiessen and Shackleton, 2005; Monfort et al., 2007). The biofumigation process potentially fulfils the requirements of a safe and economical pest management strategy and is included as a non-chemical alternative to methyl bromide by the Methyl Bromide Technical Options Committee (MBTOC, 1997), as documented by Ploeg (2008). Novel approaches to disease control, for example incorporating brassicas to biofumigate soil and engineering solutions to disease control has made The Scottish Agricultural College's (SAC) contribution to supporting the potato industry unique (Smith, 2002).

Smith and Kirkegaard (2002) tested the effect of 2-phenylethyl isothiocyanate under in vitro conditions towards a range of fungi, oomycetes and bacteria. *Trichoderma* spp. was the most tolerant, while *Aphanomyces*, *Gaeumannomyces*, *Phytophthora* and *Thielaviopsis* were very sensitive to 2-phenylethyl isothiocyanate. Matthiessen and Shackleton (2005) tested four pure isothiocyanates (methyl, 2-propenyl, benzyl and 2-phenylethyl isothiocyanate), in vapour exposure tests for biological activity against the model soil insect whitefringed weevil both in vitro and in the presence of three contrasting soils and under four temperatures. The results indicated that brassicas rich in aliphatic isothiocyanates are more likely to have the potential to exert stronger isothiocyanate-based biofumigation effects than those similarly rich in aromatic isothiocyanates. The potential of tissue amendments from wild and cultivated *Brassica* species was assessed to kill the root lesion nematode *Pratylenchus neglectus*. The amendment of soils with equimolar levels of purified 2-phenylethyl isothiocyanate resulted in comparable levels of nematode mortality, suggesting that 2-phenylethyl glucosinolate has a role in the suppressive impact of *Brassica* spp. root tissues (Potter et al., 1998). Cox et al. (2006) used *B. juncea* cv. *Pacific Gold* (BSM) to control sting nematodes and observed that BSM seed meal with irrigation provided 92% control, while non-irrigated BSM provided 99.5% control. Monfort et al. (2007) evaluated the potential of *Brassica* species as an alternative control measure for root-knot nematode (*M. incognita*) by using them as green manure amendments prior to planting. The results of their trial, although variable, showed some promise of *Brassica* spp. as a biological control option in vegetable production in Georgia. In another study the effects of soil amendment with rapeseed meal from *B. napus* cv. *Dwarf Essex* (high glucosinolate concentrations) and *Stonewall* (low glucosinolate concentrations) on the biological control activity of *Trichoderma harzianum* towards *Sclerotinia sclerotiorum* and *Aphanomyces euteiches*, Dandurand et al. (2000) demonstrated that both Dwarf Essex and Stonewall meals inhibited colonisation of *S. sclerotiorum* in soil by *T. harzianum*, from 100% to 0% and 8%, respectively. *A. euteiches* was significantly reduced by *T. harzianum* alone (100%), by amendment with *Dwarf Essex* meal alone (77%), and by *T. harzianum* in combination with *Dwarf Essex* meal (100%). Klingen et al. (2002a) analysed the effect of brassicaceous plants on the survival and infectivity of insect pathogenic fungi and their in vitro studies showed that 100 ppm of 2-phenylethyl isothiocyanate completely inhibited growth of *Metarhiziumanisopliae* and *Tolypocladium cylindrosporum*. In a study of oilseed rape *B. napus* extracts in the laboratory for toxicity against adults of *Sitophilus oryzae* and *Rhizopertha dominica* at different concentrations, data showed that the surface treatment of wheat seeds with acetone or petroleum ether extracts of *B. napus* proved to be very effective as they gave 51.8 and 45.0% mortality among *S. oryzae* adults at 4.0% conc. level, respectively (Salem et al., 2007). While all tested extracts *of B. napus* showed various toxicities to *R. dominica* adults, acetone extract was found to have to have the highest effect as it gave 92.4% mortality at 4.0% concentration level, followed by the diethyl ether extract (90%). In a study by Noble et al. (2002), larvae of masked chafer beetles (*Cyclocephala* spp.) were placed in soil amended with *B. juncea* (PI 458934) tissue. Allyl isothiocyanate levels were observed to be positively correlated with larval mortality, with the 8% *B. juncea* treatment resulting in 100% larval mortality with an average allyl isothiocyanate concentration of 11.4 mg L^{-1} of soil atmosphere.

4.2 Brassicas as Cover Crops

"Cover crops slow erosion, improve soil, smother weeds, enhance nutrient and moisture availability, help to control many pests and bring a host of other benefits to farms, and simultaneously can reduce costs, increase profits, and even create new sources of income" (Clark, 2007).

Brassicaceae cover crops are considered to play a role in controlling nematodes, fungi, weeds and diseases by releasing chemical compounds from decomposing residue with promising but variable results among different species, varieties, geographical locations and differences due to the planting dates (Clark, 2007). *B. hirta*, syn. *S. alba*, *B. juncea*, *Raphanus sativus* and *B. napus* have been used as cover crops with varying success (Boydston, 2004). Mustard cover crops have been used in Europe and the Pacific Northwest to suppress soil-borne nematodes, pathogens and weeds. *Brassica* crops such as *B. juncea* have been reported to improve root health in a subsequent cash crop, such as potatoes, grown after a green incorporated Brassica cover crop (Snapp et al., 2006). Brassica cover crop incorporation as a green manure suppresses some pathogens, including *Verticillium* in potato; *Pythium, Fusarium* and *Rhizoctonia* root rots in beans; *Pythium* in lettuce; pink rot in onion; *Aphanomyces, Pythium, Rhizoctonia* and *Fusarium* root rot in peas; and cavity spot and *Fusarium* in carrot (summarised by Sanders, 2005 as cited by Snapp et al., 2006). *B. juncea*, having high levels of glucosinolates along with fungal inhibition properties, was the most effective Brassica crop for reducing powdery scab and common scab disease problems in the field trials conducted (Larkin and Griffin, 2007).

4.3 Brassicas as Trap Crops

The practice of trap cropping, i.e. using a sacrificial resource for the pest to attack, in order to protect a valued resource, has been known for centuries (Foster and Harris, 1997). The use of *Brassica* as trap crops has gained interest in recent years. In order to decrease loss to the main crop from insects, trap crops are deployed to attract or catch targeted insects (Shelton and Badenes-Perez, 2006). The trap crop, bearing highly attractive host plants preferred by pests, is planted close to the main crop to protect it from pests. Being attractive, the trap crop captures pests and concentrates them on itself, where they get destroyed before reaching the main crop (Hokkanen, 1991). Recently, the '*push-pull strategy*' based on an attractive trap crop has been developed to protect oilseed rape (*B. napus*) from its specialist pests (as reviewed by Cook et al., 2007a). The push-pull strategy "*involves the behavioural manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive or unsuitable to the pests (push) while luring them toward an attractive source (pull) from where the pests are subsequently removed*" (Cook et al., 2007a). The term push-pull was first accepted in Australia in 1987, as a strategy for insect pest management (IPM) (Pyke et al., 1987 as cited by Cook et al., 2007a).

In order to protect spring-sown oilseed rape from two major inflorescence pests, the pollen beetle (*M. aeneus*) and the seed weevil (*C. assimilis*), Cook et al. (2006) followed a strategy of using *Brassica* as a trap crop. The strategy comprised *Starlight*, an oilseed rape cultivar with relatively low proportions of alkenyl glucosinolates in the leaves and thereby releasing lower levels of attractive isothiocyanates than conventional cultivars as the main crop, and turnip rape as a trap crop. Turnip rape showed good potential as a trap crop for oilseed rape pests, particularly the pollen beetle, as its odour was more attractive to pests than that of oilseed rape (Cook et al., 2006). A study by Barari et al. (2005) examining turnip rape as a trap crop to reduce oilseed rape infestation, and the effects of insecticide treatment on pest incidence and larval parasitism, showed that the turnip rape trap crop borders reduced *P. chrysocephala* but not *C. pallidactylus* infestation of oilseed rape plots. Treatment of the trap crop with insecticide had little effect on either pest or parasitoid incidence in the oilseed rape.

Understanding the mechanisms underlying host-plant preferences of herbivorous pests can lead to improved effectiveness and reliability of the trap crop. With this perspective, Cook et al. (2007b) investigated the behavioural and chemical ecology underlying the success of turnip rape trap crops in protecting oilseed rape from the pollen beetle (*M. aeneus*), which feeds in the flowers and lays its eggs in the buds. Phenylacetaldehyde and $(E,E)-\alpha$-farnesene were found to be present in air entrainment samples of both plant species at the flowering growth stage, but only in those of *B. rapa* at the bud stage. The former two compounds were behaviourally active in olfactometer tests. These compounds were suggested to be involved in host location by *M. aeneus* and, at least partially, responsible for the attractiveness of turnip rape and its success as a trap crop to protect oilseed rape from this pest.

Shelton et al. (2008) observed that several types of trap crops have been recommended to control diamondback moth, including *B. oleracea* and *B. juncea*. However, the results varied as populations of *P. xylostella* develop on these trap crops and spill over to the cash crop. In order to overcome this problem, Shelton et al. (2008) sought to develop "dead-end" trap crops that were more attractive for oviposition than the cash crop and on which *P. xylostella* larvae cannot survive. Transgenic collard and Indian mustard lines expressing the *Bt* gene (cry1C) were produced to be used as a "dead-end" trap crop for *P. xylostella*. The use of *Bt* Indian mustard as a trap crop significantly reduced the number of larvae that appeared on a cabbage cash crop, compared with using a non-*Bt* Indian mustard trap crop.

4.4 Plant Breeding Towards Insect Resistance in Brassica Crops

Plant breeding has undergone major changes throughout the past 30 years and moved from the traditional phenotype-based via phenotype-protein-based breeding to the utilisation of so-called molecular markers. The development of PCR techniques has given rise to marker technologies and the generation of detailed molecular maps for selection purposes and breeding programmes for important crop plants, also including crucifers. Additionally, the utilisation of model plants, and in particular *A. thaliana*, has opened up for comparative investigations, not least since *Arabidopsis* is a close relative to *Brassica* species, and thus, facilitated and pushed efforts toward molecular breeding (Snowdon and Friedt, 2004) and state-of-the-art metabolomics-assisted breeding (Fernie and Schauer, 2008). Recent approaches for potential genotype selection and cultivar development in the Brassicaceae (Snowdon, 2007; Duran et al., 2009; Nicolas et al., 2009; Riaño-Pachón et al., 2009) include:

- re-synthesis of *Brassica* species through somatic or interspecific hybridisation;
- genetic markers, mapping and identification of *quantitative trait loci* (QTLs);
- plant model (*Arabidopsis*)-based breeding through intergenome knowledge transfer;

- haploid techniques based on anther and microspore cultures;
- ‘omics technologies for global elucidation of genetic, protein and/or metabolite information.

At least two or several of the described approaches are normally applied at the same time, in order to efficiently improve and accelerate the selection process. General breeding goals in *Brassica* crops comprise quite different traits ranging from morphology and yield, oil content and quality, male sterility, and abiotic stress tolerance, to pathogen and insect resistance (Leckband et al., 2002; Snowdon and Friedt, 2004; Sarfraz et al., 2006).

Nevertheless, today’s situation in *Brassica* breeding with regard to insect resistance traits has to be informed on the background of gene modification (GM) of crop plants, on the one hand. Due to successful introduction of genes coding for *Bt* toxins against insect pests in important crops such as cotton, maize and potato (AGBIOS, 2009), R&D activities in GM *Brassica* species toward insect resistance traits have been forced in recent years. In cabbage and oilseed rape, the expression of *Bt* toxins (Jin et al., 2000; Liu et al., 2008), harmful enzymes (Wang et al., 2005; Mulligan et al., 2006) and the regulation of insect gene expression through RNA interference (RNAi) (Baum et al., 2007) have been addressed. However, insect-resistant GM *Brassica* crops are not commercially available so far. On the other hand, biological premises and limitations challenge the breeding efforts toward insect resistance. Evolutionary-evolved plant responses upon feeding insect species have resulted in specific adapted and partly, multitrophic defence mechanisms, depending on whether generalists or specialists are involved. Thus, insect–plant relationships rely on a complex interplay of factors related to attraction, recognition and oviposition, which makes breeding efforts difficult since several traits have to be taken into account. Rather few examples on breeding Brassicaceae toward insect resistance traits exist. Both the (1) biochemical basis of resistance (glucosinolates; host-plant volatiles; other secondary metabolites) and/or the (2) morphological basis of resistance (leaf colour, size and position; epicuticular waxes; trichome density) might be addressed as reviewed by (Sarfraz et al., 2006). In the case of the glucosinolates and derivatives, intensive research in the past decades has led to new knowledge about both the potential toxicity of single biochemical compounds and potential induced resistance and thus, opened up new breeding purposes in the brassicas in general (Lou et al., 2008; Hopkins et al., 2009). Although glucosinolates, and specifically their breakdown products, have been shown to play a major role in defence mechanisms against generalist and specialist insects (stressed in Sects. 2 and 3), such interactions have to be studied on a case-by-case basis for a purposeful plant genotype selection, as pointed out for the diamondback moth in oilseed rape (Sarfraz et al., 2006, 2007). Promising biochemical traits other than glucosinolates have been pointed out by Silverstein and co-authors regarding defensins and smaller cysteine-rich peptides for the establishment of constitutive resistance against insect pests (Silverstein et al., 2005, 2007). Trait selections in less important species, e.g. kale (*B. oleracea* var. *acephala*) and nabicol (*B. napus* var. *pabularia*) (Rodriguez et al., 2005; Picoaga et al., 2003) have shown that the morphological characteristics (glossy leaves) are connected to resistance toward lepidopterous species. In the case of generalist insects, e.g. the cabbage maggot, cross-species approaches using different crucifers might help to identify sources and mechanisms of resistance and thus, to select suitable plant genotypes for hybridisation breeding (Jyoti et al., 2001).

To what extent insect resistance as a newly introduced trait interferes in multitrophic relationships between the host plant, insect pests and their parasitoids has specifically been studied in gene-modified *B. napus* expressing the *Bt* toxin, with negligible effects on beneficial species (Schuler et al., 2001, 2004; Ferry et al., 2006; Mulligan et al., 2006). Recent studies on the glucosinolate content and composition of host plants with differing insect resistance potential have shown that changed plant chemistry possibly impairs parasitoid fitness, underscoring how fine-tuned evolutionary-developed plant herbivore-parasitoid interactions are (Gols et al., 2008b; Bukovinszky et al., 2009; Hopkins et al., 2009).

5 Conclusions and Future Directions

This review binds together broadly scattered literature on the chemical ecology of *Brassica* plants towards different insects, multiple defence mechanisms of these plants, their role towards the insect-pest complex that attacks brassicas, their potential in insect-pest management and plant breeding towards insect resistance in *Brassica*. Several chemical ecology studies on different *Brassica* plants and insects attacking brassicas emphasise the importance of glucosinolates, breakdown products (isothiocyanates and nitriles), volatile compounds (terpenes, green leaf volatiles, aromatic compounds) and phytoalexins as key components of plant defence against insects. In addition, these studies show that the *Brassica* crops exhibiting multiple defence responses in response to different insects along with insect behaviour represent a complex system. In order to understand this complex system further and to identify differential responses triggered by different insects (herbivores, parasitoids/predators) as well as the underlying regulatory networks and signalling pathways, there is a need to perform gene expression studies on brassicas. Due to the availability of the *A. thaliana* genome, a lot of transcriptional/ecogenomics studies have been performed on *Arabidopsis* in recent years. Using a full genome microarray platform for studying early transcriptional responses in *A. thaliana* against *B. brassicae* infestation, Kuśnierczyk et al. (2008) found strong indications that camalexin is a hitherto unknown insecticide. This finding was further supported by the analysis of camalexin induction and aphid fecundity experiments.

Since *Arabidopsis* and Brassicas belong to the same family, *Arabidopsis* microarrays have been employed for the transcriptional studies in *Brassica* spp. (Dong et al., 2004; Carlsson et al., 2007; Hudson et al., 2007), and can also be used to perform ecogenomics studies to analyse insect infestation responses in *Brassica* crops. Moreover, with the availability of *Brassica* microarrays, it is now possible to perform more complete global transcription profiling studies of insect infestation of *Brassica* plants, providing another gateway towards insect pest management. Future studies could also aim at manipulating plant secondary metabolites such as camalexin, glucosinolates and their breakdown products, volatile compounds, plant allelochemicals or other semiochemicals in order to control pests. This can be applicable through the production of transgenics, through a combination of breeding methods and tissue culture techniques, and through the exploitation of wild *Brassica* germplasm.

We end this review with a hope that future studies might also discover other aspects of plant-insect interactions, their chemistries in combination with ecogenomics studies, and utilisation of brassicas towards insect-pest management, and will provide us with even more information and clues to understand the plant-insect world and its mysterious trophic interactions. This field of research has gained more and more attention in the past decade and attracted a huge number of scientists, who have spent years exploring the plant-insect world, and have already provided us with excellent information, sources and some natural solutions to overcome insect-pest problems.

Acknowledgements We are grateful to Ingeborg Klingen, Ralph Kissen, Carolyn Baggerud and Sankalp Ahuja for critical reading of the article and providing constructive comments. We further thank three anonymous reviewers for their suggestions. The authors thank Drs. Jean-Marie Bossennec (France), Ian Kimber and Ben Smart (UK), Paolo Mazzei (Italy), and Anna Kusnierczyk (from our group) for providing insect pictures or giving permission to use insect images from homepages: (http://www.inra.fr/hyppz/pests.htm), (http://ukmoths.org.uk/show.php) and (http://www.leps.it/index.htm) to make Fig. 3. This work was supported by the grants No. 185173 (*Plant innate immunity*), No. 184146 (*A systems biology approach for modelling of plant signalling and host defence*) and No. 175691 (NKJ 126 - *Genomic analyses and modelling of plant secondary metabolism for development of oilseed rape with improved pest resistance*) from the Norwegian Research Council (RCN).

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Ionising Radiation and Area-Wide Management of Insect Pests to Promote Sustainable Agriculture

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Contents

Abstract Despite the liberal use of broad-spectrum insecticides to keep many insect pests of agricultural and veterinary importance at bay, food losses, both pre- and post-harvest, due to these insect pests contribute significantly to the high prevalence of undernourishment in the world. New, innovative pest control tactics and strategies are therefore needed that are both effective and not detrimental to the environment. As part of the arsenal of environmentally-friendly control tactics, the sterile insect technique (SIT) has proven to be a very effective tool against selected insect pests when used as part of an area-wide integrated pest management (AW-IPM) approach. Likewise, the use of natural enemies for augmentative or inundative biological control is now a major component of pest control in many parts of the world. Both control tactics are complementary and even synergistic under certain circumstances, but their combined use has so far not been applied on an operational scale. Ionising radiation can be readily employed to effectively and safely induce sexual sterility in insects. Although the sterile insect technique has often been associated with an eradication strategy, major advances in rearing efficiency, and improved handling and release methods, have made the use of sterile insects economically feasible for insect pest suppression, prevention or containment. Recently, more emphasis has been placed on the quality of the sterile insect once released in the field rather than mainly assessing quality in the rearing facility. This combined with other innovations such as the development of genetic sexing strains, better understanding the impact of radiation on radio-resistant species such as Lepidoptera and the development of the F_1 sterility concept, advances in monitoring the induced sterility, etc. have significantly increased the

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_29,

from Vreysen and Robinson, Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010009

efficiency of the sterile insect technique for several insect species. The action of sterile insects is inversely dependent on the density of the target population, and sterile insects have the intrinsic capacity to actively search for and mate with the last individuals of a pest population. These two characteristics make them ideal to deal with outbreaks of invasive insect pests. The use of sterile insects presents no threat to the environment, but aspects such as diet and waste disposal in large rearing facilities or bio-security in cases where the rearing facility is located in an area that is already free of the pest require the necessary attention. Ionising radiation can also be applied to greatly improve the efficiency of mass-rearing, handling and shipment of insect parasitoids and predators. Area-wide integrated pest management programmes that use sterile insects or natural enemies are complex and management-intensive, and require a management structure that is exclusively dedicated to the programme. Past and current examples have shown the enormous benefit-cost ratios that these programmes can generate and their importance for enhanced agriculture is increasing in significance.

Keywords Natural enemies • Sterile insect technique • Area-wide integrated pest management • Economic benefits

1 Introduction: Insecticide Use and Food Security

Although the prevalence of undernourishment (i.e. those people whose dietary energy consumption is continuously below a minimum dietary energy requirement for maintaining a healthy life and carrying out light physical activity with an acceptable minimum body-weight for attained height) has declined worldwide from 18% in 1990–1992 to 13% in 2003–2005, the total number of undernourished people in the world has remained constant, or has even slightly increased (841 million in 1990–1992 to 848 million in 2003–2005) (FAO, 2008). Even in the developed world, the Food and Agriculture Organization of the United Nations (FAO) estimates that more than 15 million people remain undernourished, and the US Department of Agriculture (USDA) estimates that 36.2 million US citizens, including 12.4 million children, were food-insecure in 2007 (US Working Group on the Food Crisis, 2009). In 2003–2005, the food deficit of the undernourished population was 90–110 kcal/person/day in countries such as Israel, Luxembourg, Canada, Norway, etc., but between 350 and 440 kcal/person/day in Liberia, Eritrea, Burundi, Sierra Leone, Haiti and the Democratic Republic of Congo (FAO, 2008).

Insect pests that harm livestock and damage crops, both pre- and post-harvest, are often at the root of food insecurity, with more than 10 000 insect and mite pests described. To keep these pests at bay, farmers use three million metric tons of insecticides annually at a cost of USD 35 000 million (Pimentel, 2007). In Europe alone, more than 140 000 tonnes of pesticides are sprayed on food crops every year, which corresponds to 280 grams/EU citizen/year (Reuters, 2008). Rising concerns about pesticide residues in food commodities, as well as increased resistance of many pest insects to insecticides, contamination of the environment and outbreaks of secondary pests, have compelled the EU to call for a ban on aerial crop-spraying of 22 toxic chemicals by 2016 (United Press International, 2008). Therefore, enhancing more efficient livestock and crop production systems requires the development and implementation of insect pest control methods and strategies that are more effective, more target-specific, and which have minimal impact on the environment. During the last decades, numerous environmentally-friendly surrogates for the use of broad-spectrum synthetic insecticides have been developed, but their wide application has not always been straightforward as some of these techniques, such as mating disruption for lepidopteran pests (Cardé and Minks, 1995) or the sterile insect technique (SIT) (Dyck et al., 2005), require application on an area-wide basis (see below) (Vreysen et al., 2007a; Koul et al., 2008). Especially in Europe, farmers are very individualistic in their approach to pest control, and area-wide practices have not gained the same level of importance as in other continents. In the last decades, it has become clear that the careful selection of control tactics and their appropriate application and combination in space and time results in most cases in more effective pest control and thus enhanced sustainable agriculture (Vreysen et al., 2007a).

2 Area-Wide Integrated Pest Management (AW-IPM)

Many years ago, the innovative Knipling (1959, 1972, 1979) used simple mathematical models to show the importance of applying pest control techniques on an area-wide basis, targeting the entire pest population within a defined area, as opposed to a field-by-field approach where portions of the pest population are left untouched. In one of his models, he compared two areas with equivalent populations undergoing a five-fold natural rate of increase per year. In one area, 99% of 90% of the pest population is destroyed each year (but no control is conducted on the remaining 10%) while in the other area the control is less intense, eliminating only 90% of the pest population but from the total area (100%) each year. Following five years of these treatments in the two areas the model indicates that there would be 100 times more pests in the first area than in the second (Fig. 1). Exercising less intensive control against the total pest population is therefore more effective than subjecting only part of the population to more intensive control efforts (Klassen, 2005). These calculations, however oversimplified, illustrate how critical it is to consider the total pest population (area-wide population control or total population control) in operational planning of pest control activities and how devastating the recruitment of individuals from relic foci can be for the control effort (Knipling, 1979; Hendrichs et al., 2007). This paper will focus on the use of biological control agents, namely sterile insects and natural enemies, in area-wide integrated pest management programmes and illustrate the use of ionising radiation in the application of these biological control agents. Sterile insects were not previously considered biological control agents under the International Plant Protection Convention (IPPC) as they were not self-replicating organisms, and were thus excluded from regulatory approval within the relevant International Standards for Phytosanitary Measures (ISPM). This situation changed in 2005 with the adoption by the IPPC of ISPM 3 "Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms" which recognised sterile insects as biological control agents (FAO, 2005).

Although sterile insects and natural enemies interact with the pest population in different ways, natural enemies by increasing daily mortality and sterile insects by reducing fertility, both kinds of agents must be distributed in the field on an area-wide basis. As well as sharing a similarity in the way they are applied, the biological control agents also have other components in common. The use of both biological control agents and sterile insects relies on efficient mass-rearing

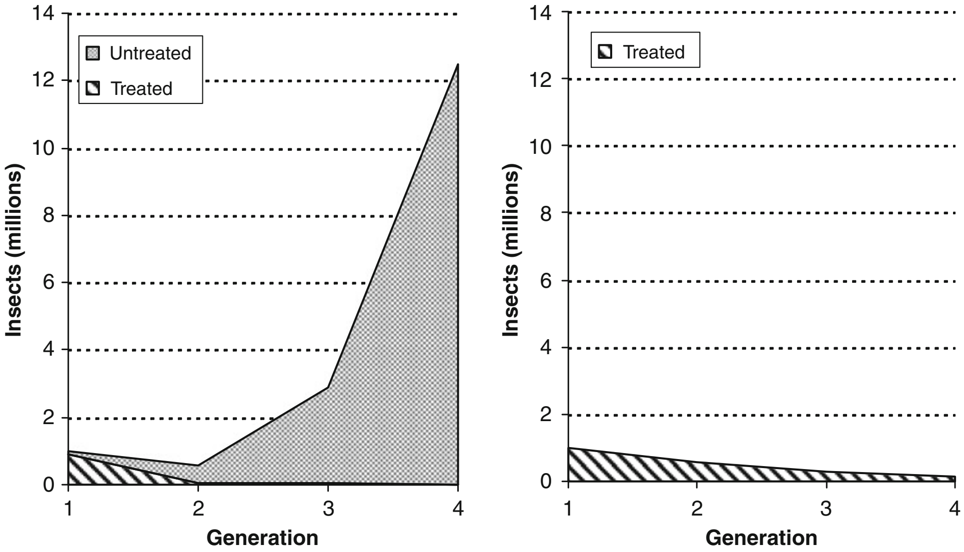

Fig. 1 (Right) the effect of total population control (less intense control (90%) on the total (100%) population) on the size and growth of a hypothetical insect population as compared with (left) a strategy where only part of the population is subjected to control (99% control on 90% of the population) (Modified after Knipling, 1979)

technology and on protocols to maintain strain quality. Both types of agents must be released in large numbers at regular intervals. Use of both kinds of agents requires effective monitoring of released and wild insects before, during and after releases. Finally, the use of both kinds of agents benefits from an ability to manipulate the sex ratio of the released insects. As well as these similarities, they also have the potential to act synergistically to reduce pest numbers when used in combination with each other (Knipling, 1966, 1972; Carpenter et al., 2005).

3 Sterile Insects

The use of sterile insects for pest control was conceived in 1937 and first applied in 1954, when there was little concern for environmental issues, sustainability and invasive species in relation to the control of agricultural pests (Knipling, 1955). In fact, the motivation to develop this technology came from the observation that a major pest of cattle in the USA, the New World screwworm *Cochliomyia hominivorax* (Coquerel), was present in the field at very low densities compared with other insect pests, which would make it vulnerable to introduction of sterility. Knipling's conceptual breakthrough was to realise that insect pests could be controlled by compromising their hereditary machinery and that the integrity of this machinery is critical for the survival of the insect. As is the case with many new concepts, "this approach to screwworm control was proposed to a number of scientists, but little enthusiasm for its possibilities was generated" (Knipling, 1959). Although the approach has gained much credibility in the intervening years (Krafsur, 1998) there are still many scientists and administrators who do not fully appreciate both the limitations and the potential of the technique (e.g. Vale and Torr, 2005). The concept was simple but revolutionary and, like most paradigm shifts, is obvious with hindsight.

The approach was a radical departure from the traditional methods of pest control, which usually relied on compromising the survival of insect pest populations through manipulation of the insect's environment using insecticides (Courschee, 1960), removal of hosts (Kovaleski, 2007), or other aspects critical for the survival of the insect (Ford et al., 1970). Also, in contrast to the traditional approaches, sterile insects have no immediate impact on the pest population as they do not directly kill the insects in the field. It was also the first time that a population of a given species was used to control or suppress a field population of the same species. The sterile insect technique is truly species-specific, in that it exerts its effect through conspecific matings of released sterile insects and their wild counterparts. Simply stated, the sterile insect technique is a form of birth control.

When sterile insects were first released on the island of Curaçao, they were used in the absence of other control methods as a stand-alone technique, in part to be able to measure their effectiveness without the complication of other confounding treatments (Baumhover et al., 1955). Subsequent experience has shown that this is rarely the case and to be effective, sterile insects must be integrated with other control tactics as part of area-wide integrated pest management programmes (see Fig. 2, inverse density dependence) (Vreysen et al., 2007a).

Each pest control tactic has its limitations, be they biological, operational, ethical, social, environmental, economic, etc., and in that respect, the use of sterile insects is not an exception. Sterile insects cannot be used for species where the stage to be released, usually the adult, is the one responsible for damage to the crop or livestock, i.e. insect vectors of diseases (e.g. horn flies - but see below), species where the adults are plant herbivores (e.g. locusts) and species where adults cause nuisance (e.g. house fly, cockroaches). A second limitation of the sterile insect technique is its substantial start-up and operational costs. There-

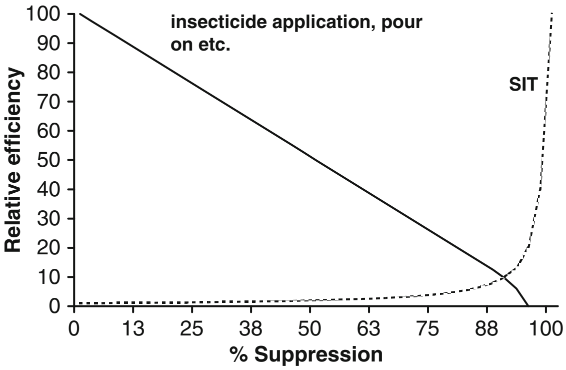

Fig. 2 The effectiveness of various control tactics in relation to the density of the pest population (pour on = application of residual insecticides on livestock). SIT: sterile insect technique

fore, the application of the sterile insect technique is largely limited to use against very important key insect pests for which effective and affordable alternative controls are not available (Lance and McInnis, 2005). In some cases there is a value-added component, as controlling the key pest without the use of chemicals provides the opportunity for the secondary pest species to be regulated by their natural enemies. Economics will also play a key role related to the cost of rearing and releasing large numbers of sterile insects. Methods to artificially rear many species of insects have not yet been developed, including for many species of beetles and weevils, the human botfly *Dermatobia hominis* (Linnaeus Jr.) and other parasites of mammals, and some insects with an obligatory diapause such as the western cherry fruit fly *Rhagoletis indifferens* Curran (Lance and McInnis, 2005).

Sterile insects can be used in several different types of programmes (see boxes) but their use has been – and in many instances still is – associated with the concept of eradication/elimination of pest populations from ecologically isolated regions (Hendrichs et al., 2005). This is perhaps logical and a direct result of the successful eradication of New World screwworm populations from the USA, Mexico and Central America (Meyer, 1994; Wyss, 2000; Vargas-Terán, 2005). Sterile insects have a unique biological advantage that matches them very well to the concept of eradication, i.e. their effectiveness increases as the pest population declines in numbers: their action is inversely dependent on the density of the target population (Dame, 1970). Once an isolated pest population begins to decline in numbers, following initial releases of a given number of sterile insects, continued releases at the same rate will result in increased ratios of sterile to wild males, which will not only accelerate that decline but will inevitably lead to elimination (Knipling, 1979). This is in contrast to many other pest control methods – such as insecticides and many biological control agents – where each treatment eliminates a constant proportion of the target population and which makes them much more effective at high pest population densities (Dame, 1970) than sterile insects. Consequently, the optimal efficiency in an area-wide integrated pest management programme is therefore obtained when control tactics are specifically chosen for their effectiveness at different pest population densities and used in sequence in order to maximise their effectiveness (Fig. 2).

Complete ecological isolation of a target population is not a requirement to achieve eradication providing the appropriate operational strategy is chosen, i.e. the rolling carpet principle (Hendrichs et al., 2005). In this strategy, the target area is divided into different treatment blocks, which are selected so as to minimise reinvasion pressure. Control is exercised along a unidirectional front and the four operational phases of area-wide integrated pest management (pre-intervention, population reduction, sterile male releases and maintenance/verification of low-prevalence/pest-free areas) are carried out simultaneously and in a phased manner. Therefore, once the programme has started, it cannot be interrupted until a pest-free area (in an eradication strategy) or the maintenance phase to sustain an area of low pest prevalence (in a suppression strategy) has been reached (Hendrichs et al., 2005). The New World screwworm programme in the Americas is an example of the rolling carpet principle. The programme started in the southern part of the USA in the 1950s and the control front advanced in the next 40 years through Mexico and the various countries of Central America, reaching Panama in the late 1990s (Wyss, 2000). Following the declaration of eradication in Panama, a permanent buffer zone of 30 000 km^2 was established in the Darien gap of Panama to protect the screwworm-free areas. Forty million sterile flies are released per week in the buffer zone to prevent reinvasion of the fly from South America (APHIS/USDA, 2001).

The intrinsic capability of sterile insects to actively search for and mate with the last individuals of a pest population and their increased effectiveness at low pest population densities makes them an ideal tool to deal with outbreaks of invasive pests (Vreysen et al., in preparation). Sterile insects were a component of programmes to eliminate biological incursions of the New World screwworm in Libya (Lindquist et al., 1992), the invasive Australian painted apple moth *Teia anartoides* Walker in New Zealand (Suckling et al., 2007), melon fly *Bactrocera cucurbitae* (Coquillett) in Okinawa, Japan (Koyama et al., 2004), and cactus moth *Cactoblastis cactorum* (Berg) in Mexico (NAPPO, 2008). New pest incursions are characterised, initially at least, by low population densities, and adequate overflooding ratios of sterile to wild insects can often be easily obtained if adequate laboratory rearing protocols can be established. As new pest introductions usually have a patchy or fragmented distribution, the release of sterile insects is amongst the most effective ways of finding and eradicating these populations; much more effective than other control tactics (e.g. deployment of toxic baits) that depend on the judgement of entomologists or field technicians (Vreysen et al., in preparation).

Sustainability is a key concern for all pest control programmes. The state of eradication is, by definition, a sustainable end point as there are no individuals of the targeted pest species left in the ecologically isolated target area. The question is whether the sustainable end point can be sustained. In certain circumstances, this is not easy to achieve as areas can be re-infested either accidentally or deliberately and an extensive monitoring system is required to confirm that the area remains pest-free (Barclay et al., 2005). This is not only expensive, but maintaining support and funding over time can be difficult, leading to a reduction in vigilance and an increasing chance of re-infestation. However, programmes such as the elimination of the tsetse fly *Glossina austeni* Newstead from Unguja Island, Zanzibar (Vreysen et al., 2000) or the removal of the New World screwworm from Libya (Lindquist et al., 1992) have demonstrated that eradication programmes can be sustainable. Permanent vigilance is required to prevent re-introduction and sometimes, stringent measures such as the continuous release of sterile flies on the islands close to Taiwan in the Okinawa archipelago or in the Darien gap in Panama are needed to protect the cleared areas (Koyama et al., 2004; Hendrichs et al., 2005).

In the last decade, sterile insects have increasingly been considered as part of area-wide integrated pest management suppression programmes against certain strategic pest insects. This is due to the fact that the rearing and release of sterile insects such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) has become much more cost-effective, and the use of insecticides has become much more restricted and more complex due to intermingling of commercial production areas with human settlements, increased demand for organic food, and difficulties in establishing effective quarantine measures to maintain an area pest-free (Hendrichs et al., 2005). A suppression strategy requires significantly lower investment in monitoring and quarantine measures need not be so stringent as compared to those required for eradication. These trends have resulted in more frequent use of sterile insects for suppressing populations of the Mediterranean fruit fly as an alternative to the use of insecticides (Barnes et al., 2004). This strategy has mainly gained acceptance for use against crop pests, as a certain level of crop damage can be tolerated; something that is much more difficult with pests of veterinary or human health importance (Hendrichs et al., 2005). The various strategies are described in more detail in Section 3.6 and in Box 1.

3.1 Rearing Insects

The ability to rear the target insect using an artificial diet was one of the essential factors that enabled the use of sterile insects for New World screwworm control (Brown, 1984; Marroquin, 1985). Rearing insects for sterile release requires firstly the development of an artificial diet followed by upscaling and automation of the processes to, in some cases, an industrial level (Parker, 2005). For example, the Mediterranean fruit fly mass-rearing facility in El Pino, Guatemala, currently produces 2000 million sterile male insects per week but has the capacity to produce 3500 million/week (Fig. 3, Table 1) (Tween, 2002; IAEA, 2008).

As the scale of rearing increases, the selection pressure on the insect to adapt also increases and this can lead to behavioural changes in the colony that impact on the ability of the sterile insects to perform adequately in the field. The quality of insects for use in programmes has to be defined in terms of "fit for purpose". In the rearing facility, high productivity and survival are paramount, whereas in the field, the only quality aspects of relevance are the ability of the sterile males to find, mate with and effectively inseminate wild females (Lance et al., 2000; Vreysen, 2005). These differing quality requirements are not always readily attained, but the importance of the quality of the released insect in the field rather than performance in the rearing facility is receiving more and more attention (Simmons et al., 2010).

3.2 Producing Sterile Males

Successful use of sterile insects requires that the sterile males locate, mate with and effectively fertilise the wild females (Knipling, 1979). The basic reproductive biology of most insects would indicate that only males

Fig. 3 (Upper, left) the Mediterranean fruit fly mass-rearing facility in El Pino, Guatemala, (lower, left) heat treatment of eggs to remove the female sex – each water bath contains 390 million eggs, (upper, right) racks of cages with adult flies, and (lower, right) racks with larval rearing trays (photos courtesy of Gerald Franz, reproduced with permission)

need to be released for the sterile insect technique to be effective (Franz, 2005). What, if any, is the role of the released sterile females in the field? At best they can be considered neutral but at worst they may provide an opportunity for assortative mating with the released sterile males and so divert the males from seeking out and mating with wild females (Rendón et al., 2000, 2004).

Mathematical modelling has indicated that in species where there is a male-choice mating system (such as the screwworm), asymmetric mating due to colonisation processes (sterile males mate at random with wild and sterile females, whereas wild male screwworms mate preferably with wild females) requires a doubling of the amount of sterile insects released per unit surface area to get the same level of suppression as compared with a situation where there is random mating. The release of only sterile males of a species that has a female-choice mating system – such as tropical fruit fly species – is more efficient in population suppression than the release of both sterile males and sterile females. In such species the number of multiple matings per male needs to be minimised because both sperm quality and quantity diminish with successive matings. In addition, sterile males distribute themselves more widely when sterile females are absent (Vreysen et al., 2006a).

Whatever the situation in the field, the production and release of sterile females incur an economic cost to the programme as they have to be reared, marked, irradiated and released along with the males. Rearing and releasing insects is a major cost factor and the removal of females would increase effectiveness (McInnis et al., 1994; Hendrichs et al., 1995; Rendón et al., 2004) and deliver significant economic benefits (LaChance, 1979). For human disease vectors such as mosquitoes where only the female is the obligatory blood feeder, the removal of females from the release insects is essential (Lance and McInnis, 2005).

Very few sex-linked differences in insects have been discovered thus far, which might facilitate large-scale separation of males and females. Consequently, New World screwworm (Baumhover, 1966) and Lepidoptera (Bloem et al., 2005) sterile insect technique programmes have been implemented by releasing both sexes. Discrete differences between the sexes can be produced if certain mutations (e.g. pupal colour, resistance to insecticides, sensitivity to temperature) are linked to either sex (Robinson, 2002). In the case of the Mediterranean fruit fly, a genetic sexing

Table 1 Selected rearing facilities and production capacity of various fruit flies, screwworm flies and moths

Insect/s	Name	Country	City	Operated by	Capacity/week
Ceratitis capitata	Infruitec Medfly Facilities	South Africa	Stellenbosch	Agricultural Research Council	5 million
	USDA Pacific Basin Area Research Center	United States of America	Honolulu	USDA/ARS	5 million
	Medfly facility	Tunisia	Tunis	National Center of Nuclear Sciences and Technologies (CNSTN)	10 million
	UOC-Fruit Flies	Greece	Heraklion, Crete	Biology Department	10 million
	Bio-Fly	Israel	Sde Eliyahu	Bio-Fly	25 million
	Centro Produccion Insectos Esteriles	Chile	ARICA	Servicio Agricola y Ganadero (SAG)	30 million
	Florida Department of Agriculture	United States of America	Gainesville	FDACS, DPI	50 million
	Biofábrica do Programa Madeira-Med Centro de Producción y Esterilización	Portugal	Camacha	Madeira Regional Governement	50 million
	Mosca de la Fruta La Molina - CPELM	Peru	Lima	Servicio Nacional de Sanidad Agraria	160 million
	Bioplanta KM8	Argentina	Mendoza	ISCAMEN	200 million
	Planta Moscamed, Metapa	Mexico	Metapa de Dominguez	Senasica/USDA	550 million
	El Pino	Guatemala	Santa Rosa	USDA/MOSCAMED	3000 million
Bactrocera tryoni	Queensland fruit fly production facility	Australia	Camden	New South Wales Agriculture	15 million
Bactrocera philippinensis	Philippine Fruit Fly Mass Rearing Facility	Philippines	Quezon City	Philippine Nuclear Research Institute	15 million
Bactrocera dorsalis	Irradiation Center for Agricultural Development	Thailand	Bangkok	Department of Agricultural Extension	30 million
Anastrepah ludens	Mexican Fruit Fly Rearing Facility	United States of America	Edinburg	USDA APHIS PPQ	160 million
Bactrocera cucurbitae	Okinawa Prefectural Agricultural Experiment Station	Japan	Okinawa	Okinawa Prefectural Government	200 million
Anastrepha ludens, A. obliqua	MOSCAFRUT	Mexico	Chiapas	Mexico Government	600 million
Bactrocera oleae	Bio-Fly	Israel	Sde Eliyahu	Bio-Fly	0.04 million
Pectinophora gossypiella	Pink Bollworm Rearing Facility	United States of America	Phoenix	USDA-CDFA	196 million
Cydia pomonella	Okanagan-Kootenay Sterile Insect Release Comisión México Americana para la	Canada	Osoyoos	Regional Districts (local government)	15 million
Cochliomyia hominivorax	Erradicación del Gusano Barrenador del Ganado.	Mexico	Chiapas	USDA-SAGARPA	500 million

strain that carries two mutations (*temperature-sensitive lethal (tsl)* (Franz et al., 1994) and *white pupae (wp)* (Rössler, 1979)) is now routinely used in all sterile insect release programmes for this species, the global weekly production being 3500 million (Franz, 2005).

3.3 Inducing Sterility

A major obstacle facing the entomologists who pioneered the use of sterile insects was that they did not know of any means to induce sexual sterility in insects.

Although X-rays had been shown in the early part of the century to be able to sterilise insects (Runner, 1916) it was not until 1950 that, following communications between Müller and Knipling's group, the use of ionising radiation was considered as a potential technique for insect sterilisation (Bushland and Hopkins, 1951, 1953). Geneticists understood very well that ionising radiation induced sterility, i.e. dominant lethal mutations in germ cells of irradiated insects (Van Borstel, 1962). These mutations are caused by breakage of chromosomes in the germ cells and when sperm carrying these mutations fertilise eggs, the resulting embryo dies (LaChance et al., 1967). The word "sterility" suggests a single state or condition; however, ionising radiation induces levels of sterility, which are dependent on the dose of radiation used: the higher the dose, the higher the level of sterility (LaChance et al., 1967; Van der Vloedt et al., 1978; Vreysen, 1995; Vreysen et al., 1996). Ionising radiation not only induces dominant lethal mutations in the germ cells but also causes mutations in somatic cells, and in some species this impacts on the overall quality of the insect after radiation (Sakurai et al., 2000), expressed as the development of abnormalities, a reduction in lifespan, flight ability, mating propensity, etc. (Bakri et al., 2005a). In general, doses of ionising radiation have been used which lead to full sterility and this impacts negatively on insect quality, although recent publications (Toledo et al., 2004; Parker and Mehta, 2007) suggest that using a lower dose with less sterility but increased quality is more efficient at introducing sterility into the field population. Using a mathematical model, Klassen and Creech (1973) found that the rate of increase in a population to be suppressed determines the effectiveness of the combination of levels of male sterility and release ratios. When the rate of increase is low (e.g. five-fold), 90% male sterility can be effective at fairly low release ratios. By contrast, when the rate of increase is high (ten-fold or more) the level of sterility must be near 99% to induce a steep downward trend in the population (Klassen and Creech, 1973).

Sensitivity levels of insects to ionising radiation are affected by the level of oxygen present during irradiation (Economopoulos, 1977; Fisher, 1997). In an oxygen-reduced environment, the formation of free radicals during irradiation is reduced as compared with air, which lowers the damage induced by the ionising radiation. This entails the need for higher doses to obtain the same level of sterility (Bakri et al., 2005). This effect tends to be higher for somatic damage than for sterility and therefore, the use of hypoxia (a deficiency of oxygen reaching the tissues of the body) can be a strategy used to increase the competitiveness of an insect without compromising the level of sterility (Calkins and Parker, 2005; Lance and McInnis, 2005). Tephritid pupae are normally sealed in airtight plastic bags, with as little air as possible, and are left for about one hour before irradiation. During this period, the pupae exhaust most of the oxygen in the container. Saturating the atmosphere with helium or nitrogen before and during irradiation is another way of creating hypoxia (Ashraf et al., 1975; Hooper, 1989; Vreysen and Van der Vloedt, 1995).

Insect species vary in their sensitivity to the induction of sterility by ionising radiation, sometimes by an order of magnitude; for example, less than five Gy is required to sterilise Acrididae (Orthoptera) and Blaberidae (Dictyoptera), whereas doses above 200 Gy are in most cases required to sterilise pupae or adult Lepidoptera (Bakri et al., 2005a, b). However, in general insects can be divided into two groups depending on the type of chromosomes they possess. Some orders such as Lepidoptera and Hemiptera have holokinetic chromosomes (properties of the centromere are distributed over the entire chromosome), which are very radiation-resistant and high doses of ionising radiation are required to induce sterility. However, the majority of insect Orders have monokinetic chromosomes and are considerably more radiosensitive. The high radiation doses needed to sterilise Lepidoptera reduced quality and hence their usefulness in field programmes. However, Proverbs (1962) discovered quite serendipitously that if codling moths *Cydia pomonella* (L.) were irradiated with a lower dose, the females were sterilised but not the males and the progeny from these males, both males and females, were fully sterile. This phenomenon has been termed F_1 sterility or inherited sterility (North, 1967, 1975; LaChance, 1985).

Field releases of several lepidopteran pests such as the cabbage looper *Trichoplusia ni* (Hübner) (North and Holt, 1969), the corn earworm *Helicoverpa zea* (Boddie) (Carpenter and Gross, 1993), the gypsy moth *Lymantra dispar* (L.) (Mastro, 1993), the pink bollworm *Pectinophora gossypiella* (Saunders) (Staten, 1993; Walters, 2000) and the codling moth *C. pomonella* (Bloem et al., 2001) have demonstrated the efficacy of the inherited sterility.

3.4 Sterile Insect Quality Management

The success of an area-wide integrated pest management programme that incorporates the release of sterile insects is dependent on many factors (Vreysen et al., 2007b), with adequate sterile insect quality being one of the most important. Released males have to intermingle rapidly with the wild population, locate the wild virgin females and be competitive with their wild counterparts in mating with wild females (Vreysen, 2005). The quality of the released insect can easily be impaired by aspects related to colonisation and mass-rearing processes (Parker, 2005), sterilisation with ionising radiation (Bakri et al., 2005a), and marking, handling, transport and release procedures (Calkins and Parker, 2005; Dowell et al., 2005). The quality of the reared insect has often mainly been assessed in the rearing facility, with little attention being given to its competitiveness once released in the target area (Vreysen, 2005). Quality management parameters in the mass-rearing facilities were more related to production processes (quantity) than to competitiveness (quality) in the field (Simmons et al., 2010). The sterile males' ability to seek out the native virgin females and to transfer the sterile sperm is a vital component of a sterile insect technique programme (Miyatake and Yamagishi, 1993). The monitoring in the field of the sterile males' sexual competitiveness, their mobility and dispersal capacity, and their dispersion (spatial distribution within the habitat) needs to be carried out rigorously, frequently and accurately to provide the programme managers with the data needed to make the necessary programme decisions (Vreysen, 2005).

3.5 Monitoring Insects

As in most pest control programmes, the effectiveness of the applied control tactics is determined by monitoring the target insect population before, during and after a particular treatment. When sterile insects are used it is also necessary to monitor this component of the insect population in the field and to be able to differentiate released insects from wild insects. In some programmes it is also possible to monitor directly the induced sterility in wild females (Vreysen et al., 2000). Monitoring adult density or abundance is carried out using traps, which sample the insect population and provide some estimates of relative abundance. Trap design, placement, efficiency and insect activity all interact to provide on a regular basis some data on the numerical fluctuations of the wild population. This direct assessment can be complemented by indirect methods, e.g. fruit sampling to assess larval infestation levels for fruit flies and parasite levels in the case of insect vectors of disease (Vreysen, 2005).

Sterile insects are generally marked in the rearing facility with a fluorescent dust before release to be able to distinguish them from the wild insects when caught in traps but also to be able to monitor their survival and dispersal in the field (Parker, 2005). The ratio of wild males to sterile males is a key parameter in determining progress in the programme. An increasing ratio in favour of sterile males to wild males indicates that progress is being made, and if this can be combined with direct measurements of sterility levels in wild females then very important estimates of the competitiveness of the sterile males can be obtained (Vreysen, 2005).

A key question in eradication programmes is establishing absence of the pest: absence of evidence is not evidence of absence. This question is compounded by trap efficiency, which may very well change when field populations are driven to very low absolute densities. This problem can to a large extent be solved by knowing the generation time of the population in the field, having some estimates of trap efficiency and accepting some level of probability (Barclay et al., 2005). A recent mathematical model calculates, given a certain amount of traps in an area, an estimate of the efficiency of the used traps, the number of trapping days to catch an insect with a certain statistically acceptable level of probability (Barclay and Hargrove, 2005). This user-friendly model (written in Excel) can be a great cost-saving factor, as it will not only give information of absence of the pest with a certain level of confidence, it will also provide guidance when to stop the releases (Clift and Meats, 2002, Vreysen, 2005).

In operational programmes that include the release of sterile males, the decision to stop releases is often influenced by political and financial motives. As an example, the dispersal of sterile New World screwworms in the Central American programme continued for 6-18 months after the last case had been detected (FAO, 1992; Wyss, 2000). In fruit fly programmes, it is standard procedure that releases continue for at least three fly generations after the last wild fly has been trapped (IAEA/FAO, 1997).

3.6 Using Sterile Insects

Although historically the use of sterile insects has been associated with the elimination of local pest populations, and although sterile insects always function in the field by introducing sterility into wild females, they can be deployed in several different ways depending on the ecological situation, the pest threat and the programme goals. Sterile insects have been used successfully in eradication, suppression, containment and prevention programmes (Hendrichs et al., 2005) (see boxes 1, 2, 3, 4). These options illustrate the flexibility with which sterile insects can be used to protect agricultural commodities from pests, be they endemic or exotic.

Box 1 Suppression

The FAO (2005) defines suppression as the application of phytosanitary measures in an infested area to reduce pest populations. Using this strategy, sterile insects are deployed simply as a biological insecticide in order to maintain the pest population below an agreed and acceptable economic injury level. This strategy has the advantage of decreased pesticide use, lower investment needed for monitoring and no need for rigorous quarantine measures, but it requires pre-export treatment (as part of a systems approach to develop Areas of Low Pest Prevalence), active participation of the growers, and the control effort has to be permanent (Hendrichs et al., 2005). This strategy has been applied successfully against the codling moth *Cydia pomonella* (L.) (Canada), the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Israel/Jordan, South Africa, Madeira, Spain and Tunisia), the oriental fruit fly *Bactrocera dorsalis* Hendel (Thailand), and the false codling moth *Thaumatotibia leucotreta* (Meyrick) (South Africa).

Box 2 Eradication

Eradication is defined as the application of phytosanitary measures to eliminate a pest from an area (FAO, 2005). This clearly implies the elimination of a local population, contrary to the definition used by the World Health Organization (WHO) where the term is restricted to global extinction (e.g. smallpox). The control efforts for the local eradication of a pest insect population are limited in time and cost, will eliminate all insecticide use in the target area eventually, and provide access to specific lucrative pest-free export markets. The rigorous quarantine setup needed to maintain the area pest-free is costly, but this also benefits quarantine procedures against other pest insects. An eradication strategy usually requires a high, but short-term investment, and long-term good monitoring networks (Hendrichs et al., 2005).

Box 3 Containment

Containment programmes are adopted in and around an infested area to prevent spread of the pest (FAO, 2005). Classical examples are (1) the containment programme of the pink bollworm *Pectinophora gossypiella* (Saunders) in the San Joaquin Valley, where sterile insect releases have, since 1968, prevented the establishment of the pink bollworm in the valley, protecting a cotton crop with an annual value of USD 1000 million (Bloem et al., 2005), and (2) the containment programme of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) on the Mexico-Guatemala border, that has protected the horticultural industry in Mexico and the USA since 1983. It is estimated that for every dollar invested in this programme, there has been a return of USD 167 in terms of crop revenue and savings in control costs (Enkerlin, 2005).

Box 4 Prevention

Prevention is defined as the application of phytosanitary measures in and around a pest-free area to avoid the introduction of a pest. This strategy is the most desirable in terms of cost as it is always cheaper to prevent outbreaks than control them, as is exemplified by the preventive sterile release programme in California. Periodic outbreaks of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in California were previously controlled by unpopular aerial applications of Malathion. Outbreaks in 1980 and 1982 required more than USD 100 million to bring the situation under control. In 1994, a preventive release programme was started which releases 400 million sterile male fruit flies per week over the 2500-square-mile target area. The programme costs about USD 15 million/year, which compares favourably with the cost of USD 30 million that was spent annually to control the outbreaks (Vreysen et al., 2006b).

4 Ionising Radiation and Natural Enemies

During the past 120 years, more than 2000 species of exotic arthropods have been introduced on more than 5000 occasions into 196 countries for the control of arthropod pests. Many new exotic pests have established themselves in Europe, temporarily or permanently, as a result of increased acreage of protected grown crops. Biological control by augmentation or inundation is now a major component of pest control in protected crops in Europe (Loomans, 2006). The number of exotic natural enemies introduced and released has increased the last few decades, with about 90 species of natural enemies currently used and commercialised around Europe (EPPO, 2002). The increase in natural enemies shipped and released to control native and exotic pests may result in an increase in risks imposed on the environment, which has been the topic of several recent publications (e.g. Bigler et al., 2006). However, when appropriately applied, natural enemies are one of the most promising, environmentally-sound and effective tools for sustainable control of arthropod pests of agriculture, and biological control has been an accepted method of pest management for over 120 years in agriculture, forestry and rangeland areas and for medical and veterinary pests (van Lenteren et al., 2006; van Driesche et al., 2008).

In classical or inoculative biological control, non-indigenous biological control agents, usually selected from the suite of parasitoids, predators and diseases that co-evolved with the pest are introduced into the target area. One of the key concerns of this approach is the host specificity and host range of the natural enemies following release. There is always the possibility that introduced biological control agents "jump hosts" (Follet and Duan, 2000; Wajnberg et al., 2001), leading to criticisms (Hamilton, 2000; Louda et al., 2003) and ever more stringent environmental risk assessment protocols (van Lenteren et al., 2006). In cases where doubts remain about very promising natural enemies of weeds or insect pests, they can be radiation-sterilised before release to enable a more definite assessment to be made under natural conditions of host specificity without any risk of permanent establishment (Hendrichs et al., 2009).

Ionising radiation has other roles to play in the area of augmentative or inundative biological control where natural enemies are mass-reared and released in very large numbers, often several times each season. Augmentative biological control faces challenges including the cost of production, quality control and quality assurance, shipping and regulation, and radiation can facilitate some of these issues (Hendrichs et al., 2009).

4.1 Ionising Radiation for Improved Rearing

Ionising radiation can sometimes suppress the immune response of the hosts, and thereby make older larval instars suitable for parasitoid development. A radiation-impaired immune system would also allow the production of natural enemies on easier to rear factitious or non-habitual hosts (Genchev et al., 2007) or on those that are more economical to mass-rear (Hamed et al., 2009). Certain complex, difficult to study physiological processes in the host (e.g. defence mechanisms, hormone metabolism), which are not well understood in relation to improved rearing efficiency, can be selectively modified by ionising radiation, thereby facilitating the study of particular host-parasitoid interactions. Ionising radiation can arrest development of certain species of hosts and prey, thereby increasing their shelf life and allowing for storage and stockpiling (Celmer-Warda, 2004; Seth et al., 2009; Zapater et al., 2009). It can also delay normal insect development and thus may extend the time window for host parasitisation or modify the internal host environment to the benefit of the natural enemy (Cancino et al., 2009; Fatima et al., 2009; Hamed et al., 2009; Zapater et al., 2009). In most insect mass-rearing facilities, excess production of particular insect life stages or batches of sub-standard insects are normally discarded, which requires an investment for their disposal. These excess products may be processed instead (Nakashima et al., 1996) or irradiated, if still alive, to support the production of natural enemies (Cancino et al., 2009).

4.2 Ionising Radiation to Facilitate Handling, Shipment, Trade and Release

During mass-production of natural enemies, a certain proportion of the hosts or prey insects may not be parasitised or eaten and will therefore develop normally, which requires additional handling steps to remove these fertile individuals from the rearing process. This decreases efficiency in large-scale mass-rearing, and can be avoided by irradiating the hosts and prey to inhibit further development and prevent the need for costly separation procedures (Cancino et al., 2009).

4.3 Integrating the Sterile Insect Technique (SIT) or F_1 Sterility and Natural Enemies

The release of sterile or semi-sterile insects together with natural enemies has been known to have synergistic effects for population suppression when applied simultaneously (Knipling, 1992; Wong et al., 1992; Bloem et al., 1998). This synergy results from the sterile insects curtailing reproduction of the adult stage of the target pest, while natural enemies destroy one or more of its immature stages, including reproducing on the F_1 offspring in F_1 sterility releases. However, successful integration of these two control tactics is only possible if the natural enemies do not negatively impact the irradiated insects and their progeny more than the wild population, and if F_1 sterility does not negatively impact the efficacy and reproduction of natural enemies. The possibility of parasitisation on progeny from irradiated males and untreated females was demonstrated in studies in the laboratory, field cages and in the wild with irradiated and untreated (1) male *Spodoptera exigua* (Hubner) as a host for the braconid larval parasitoid *Cotesia marginiventris* (Cresson) (Carpenter et al., 1996), (2) male *H. zea* as a host for the tachinid *Archytas marmoratus* (Townsend) (Mannion et al., 1994), and (3) *C. pomonella* as a host for the egg parasitoids *Trichogramma cacoeciae* (Marchal) and *Trichogramma nerudai* (Pintureau and Gerding) (Botto and Glaz, 2010).

5 Protecting the Environment

The presence of sterile insects in the environment presents little or no threat (Nagel and Peveling, 2005). They are biodegradable, they act in a species-specific manner and they do not persist when releases are terminated. Consequently, the sterile insect technique is considered to be amongst the most environmentally-friendly insect pest control tactics. However, prior to arriving in the environment they have to be reared, sterilised and released, all processes that impact the environment in some way, but probably in a similar way to the manufacture of other products for pest control. Large rearing facilities have to be built and they produce many tons of spent diet a day that has to be treated before being used for other purposes, and waste water management is a priority (IAEA, 2008). Bio-security is an issue where facilities find themselves in eradicated areas through the course of a successful programme, e.g. the New World screwworm mass-rearing facility in Tuxtla Gutièrrez in Chiapas, Mexico (Wyss, 2000). For insect sterilisation, conventional self-shielded irradiators (Fig. 4) are used and the dose received by the irradiated insects is quantified by standard dosimetry (Parker and Mehta, 2007).

Fig. 4 The three ^{137}Ce Husman gamma irradiators at the New World screwworm mass-rearing facility in Tuxtla Gutièrrez in Chiapas, Mexico

The transport and importation of this type of gamma cell is governed by international safety procedures and standards and its operation is under strict control by competent national authorities to ensure its safety and security. Releasing insects in large programmes is frequently carried out by aircraft, which again is well regulated and poses no environmental concerns. Many of these same concerns also apply to the mass-production of natural enemies, although biosecurity is probably less of a concern, except where pest species are used as hosts.

6 Enhancing Sustainable Agriculture

Area-wide integrated pest management programmes using sterile insects and natural enemies are complex and management-intensive, and there is no guarantee of success (see Vreysen et al., 2007b). It is beyond doubt that the use of these biological control agents in area-wide integrated pest management programmes has resulted in high benefit-cost ratios (Mumford, 2005) and these programmes have played and continue to play a significant role in reducing the total amount of insecticides applied (Vreysen et al., 2006b).

The highest benefit-cost ratios are usually obtained in programmes that are operated to protect a high-value crop and its export market (e.g. export of fruits, vegetables, etc. to large markets of industrialised nations such as the EU, USA and Canada) or to protect an extensive livestock industry (e.g. cattle industry in southern USA). In those cases, it has been relatively easy to convince federal or regional governments, and farmers' associations to invest the necessary funds to operate these programmes as the benefits are very obvious. In the case of protecting subsistence farming in sub-Saharan Africa, it becomes much more difficult to find the funding for these programmes as there are no big industries or export markets to protect: what is protected are individual animals or crops, herds, and the livelihood of farmers. However, the very impressive programme that controlled the cassava mealy bug in large areas of Africa using a natural enemy showed the potential, even under very difficult conditions (Herren and Neuenschwander, 1991).

Insect pests cause both direct and some indirect losses, which translate into direct and indirect benefits when the pest is managed in an appropriate way. Direct benefits are fairly easy to account for and are measured in terms of increase in yield (e.g. number of fruits produced, milk and meat yield, reduced mortality, reduced abortions, increased longevity, etc.), improved quality of the produced commodities, and reduced production costs through more effective management of the pest (Enkerlin, 2005). Indirect benefits are more difficult to incorporate in the benefit-cost equations and can include an increase in export volumes, increase in yield through reduced secondary pest outbreaks, savings in medical costs as a result of exposure to insecticides, reduced legal costs arising from damage to private and public property due to insecticide misuse, increased human health due to reduced insecticide residues in food commodities, new jobs created, better human nutrition, etc. (Enkerlin, 2005).

The following examples provide ample evidence of the significant contribution area-wide integrated pest management has made to sustainable agriculture.

6.1 New World Screwworm in the Americas

Using an area-wide integrated pest management approach and sterile insects, the New World screwworm was eradicated from the USA during the period 1957–1966 (Knipling, 1960) although there were several reintroductions, with the last six cases in Texas in 1982 (Jim Novy, unpublished report to the FAO). Mexico was declared free of the New World screwworm in 1991, and the remainder of Central America was declared New World screwworm-free over a period of 12 years (from 1988 to 2000) (Vargas-Terán, 2005). The annual direct producers' benefits from the eradication of screwworm were estimated at USD 896 million for the USA, USD 328 million for Mexico and USD 87.8 million for Central America (Wyss, 2000). The benefits were derived from decreases in death losses, decreases in veterinary services, veterinary medicines, insecticides and inspections, and increases in meat and milk production. These effects have, however, an indirect impact on the general economy of each country and the multiplier effect is estimated at 3 (Wyss, 2000). Since the first sterile New World screwworms were released on Curaçao, an estimated USD 1000 million has been spent on the eradication campaign over 50 years. The annual direct benefits that accrue every year from this programme are therefore equal to or more than the total project cost over five decades. Whereas in 1934 screwworm infestations killed 1.3 million cattle in the south-eastern USA (Dove, 1937) and in 1935, in Texas alone, 230 000 screwworm cases were detected (FAO, 1992), the screwworm saga has been erased from the memories of today's US livestock owners.

6.2 Chile's National Fruit Fly Programme

Export of fruit and vegetables is an important component of the economy of Chile. Chile's most important trading partners are the USA and Japan, who impose stringent import conditions: the produce needs to be produced in a fruit fly-free area. Only the northern province of Arica was infested with the Mediterranean fruit fly, and following the use of bait sprays and sterile insects, Chile was declared a fruit fly-free country in 1995 (MAG/SAG, 1995). Since then, Chile's National Fruit Fly programme has been able to prevent the introduction and the establishment of fruit flies of economic importance. The programme uses two main strategic activities, i.e. (1) a preventive programme based on effective quarantine measures and an extensive trapping network, and (2) in collaboration with Peru and Argentina, a containment programme in Arica province using twice-weekly releases of 50 million sterile males (Gonzalez and Troncoso, 2007). Since Chile was declared a fruit fly-free country, annual fruit exports have grown to two million tonnes of mainly grapes, apples, stone fruits, kiwis and avocados with an estimated value of USD 1600 million. The annual export of fruit from the area that could harbour the Mediterranean fruit fly amounts to USD 250 million. However, the Government of Chile spends only USD 4 million each year to keep the country free of fruit flies (Enkerlin, 2005).

6.3 Melon Fly in Okinawa

The melon fly *B. cucurbitae* is one of the most destructive pests of cucurbit crops. This pest invaded the Okinawa Islands of Japan from 1910 to 1970, thereby preventing the export of 40 important vegetables and fruits to fruit fly-free areas, such as the main islands of Japan. In 1979, an area-wide integrated pest management programme was initiated that integrated the use of the male annihilation technique with the release of sterile insects. All islands were declared free of melon fly in 1993 and this status is maintained through a continuous detection programme, rigorous quarantine measures and preventive releases on the island closest to Taiwan that is still infested. As a result of the programme, between 1990 and 2000, melon production increased 2.3 times (from 2720 to 6220 tonnes) and the production of mango 4.6 times (from 278 to 1290 tonnes), or an equivalent of USD 335 million. A total of USD 172 million was invested for the main eradication campaign between 1982 and 1991. However, six years after achieving eradication, the break-even point had been reached, and between 1997 and 2000, revenues from the sale of commodities to mainland Japan totalled USD 169 million. For the same period, only USD 31 million was required to keep the islands free of new invasions (Enkerlin, 2005).

6.4 Cassava Mealy Bug in Africa

Although the theoretical basis for the combined use of sterile insects and natural enemies was already established by Knipling (1992) and numerous laboratory, semi-field and field experiments have indicated the synergistic benefits (e.g. Wong et al., 1992; Bloem et al., 1998), the principle has not found a practical follow-up "as yet" on a large operational scale. This is remarkable as both sterile insects (Dyck et al., 2005) and natural enemies (Hokkanen and Lynch, 1996) have been used successfully to tackle major insect pests. One of the most impressive classical biological control programmes was undoubtedly the one directed against the cassava mealy bug *Phenacoccus manhoti* Mat.-Ferr. (Homoptera: Pseudococcidae) in Africa. The mealy bug was first detected in Zaire and Congo in the early 1970s (Herren and Neuenschwander, 1991) and spread the following 10 years over the entire cassava belt of Africa, with the exception of Madagascar, causing root yield losses of up to 84% (Herren et al., 1987). A parasitoid wasp *Apoanagyrus* (*Epidinocarsis*) *lopesi* (DeSantis), originating from the mealy bug's native home Paraguay, was selected as the prime candidate for a release programme. Aerial releases of the parasitoid wasp were initiated in 1981 and by 1991 the biological control agent had been spread to 26 African countries covering 3 million km^2 (Neuenschwander, 1993), bringing the pest under control in 95% of all the cassava fields (Herren and Neuenschwander, 1991). An economic analysis that tabulated the benefits and costs over a period of 40 years (1974–2013) indicated a benefit-cost ratio of the programme of 200 when cassava was listed at world market prices and of about 370-740 when inter-African prices were considered (Zeddies et al., 2001).

6.5 The Private Sector Enters the Fruit Fly Industry in Israel

Suppression of the Mediterranean fruit fly in the Arava/Araba Valley (Israel/Jordan) was initiated in 1998. Initially, the programme imported 15 million sterile male pupae weekly from the El Pino Moscamed mass-rearing facility in Guatemala. After September 11, 2001, airfreight regulations changed drastically, which significantly increased the duration of the pupal shipments, with increased pupal mortality and a general decline in fly quality as a consequence. As a result, Bio-Bee, an Israeli company that mass-produces and markets biological control agents and bumblebees, conducted in 2003 a study to assess the economic feasibility of establishing a rearing facility. A new company named Bio-Fly was established in the Kibbutz Sde Eliyahu and the rearing facility was inaugurated in March 2005. The facility currently produces 20–25 million sterile male flies every week (Franz, 2005), although its maximum production capacity is estimated at 50 million sterile males per week using the existing infrastructure. Bio-Fly delivers sterile male flies to projects in the Golan Heights (800 ha. of deciduous fruits), the Sea of Galilee (800 ha. of mangos and citrus), to Beit-Shean (300 ha. of citrus), to Lachish Tali (600 ha. of grapes), to the West Negev (200 ha. of citrus), and to the Arava-Araba Valley (4000 ha. of commercial plots and backyards) (Dael Levy, General Manager, Bio-Fly, pers. commun.). Whereas before 1998 the Arava Valley was not able to export any agricultural commodity to the USA, export of bell peppers and tomatoes to the USA had increased to USD 500 000 in 1999 and to USD 50 million in 2003–2004.

6.6 Codling Moth in Canada

The codling moth *C. pomonella* is the most important pest of pears and apples in British Columbia, Canada (Bloem and Bloem, 2000). A pilot programme, conducted from 1976 to 1978 in the Similkameen Valley, BC, demonstrated that a combination of suppressive action with chemicals and the release of sterile moths could eliminate codling moth (Proverbs et al., 1982). Operational activities were initiated in the Okanagan Valley in 1995 and since then, the apparent density of the wild codling moth population in the first intervention zone (as revealed through adult moth catches in pheromone traps) was reduced from an average of 2.5–13 moths/trap/week to an average of 0.08 moths/trap/week in 2000 (Bloem et al., 2005). In the first zone, the proportion of orchards with no detectable codling moth damage increased from 42% in 1995 to 91% in 2000, while the amount of organophosphate insecticides purchased decreased from 18 903 kg in 1991 to 3403 kg in 2001 (Bloem et al., 2005).

7 The Future

There are several areas where the sterile insect technique could be improved and made more efficient. The quality of released insects and hence, their ability to locate virgin females and transfer the sperm is of prime importance for success of the technique. The use of field-based protocols that rigorously assess the impact of changes in the rearing and handling on the quality of the insect should be standard procedure (Vreysen, 2005). One can compensate for low insect quality to a certain extent by releasing a larger number of insects, but there are limits to this both economically and biologically, i.e. below a certain quality factor, further increases will simply not be effective. Genetic studies need to shed light on the amount of insects needed to initiate insect colonies with adequate genetic variability and how this variability can be maintained throughout the rearing process. Insects adapt rapidly to artificial conditions and successful colonisation results in the selection for adapted genotypes that can negatively impact on their competitiveness. Strain replacement at regular intervals has been used in the past to compensate for this but is logistically very demanding. The filter rearing system concept, where a mother colony is maintained under relaxed, more natural conditions, has shown great potential for some fruit fly species. The mother colony is checked at every generation and unwanted individuals are removed. Eggs from the mother colony are used to produce a large colony from which insects are taken for release following 3–4 generations of amplification. This strategy not only reduced selection pressure but can also maintain the integrity of genetic sexing strains (Fisher and Cáceres, 2000; Cáceres et al., 2004). Other areas that have great potential to increase the efficiency of the sterile insect technique are improvement of artificial diets through the inclusion of micro-organisms that contribute nutrients, the addition of hormonal, nutritional, semio-chemical supplements to enhance sterile male performance, increased fitness through hybrid strains, increased shelf life of certain life stages that would make sterile insects available in large numbers when needed in the season, improved shipping procedures to reduce negative effects of transport, reducing the negative impact of ionising radiation, improved release procedures, monitoring techniques, etc. Modern biotechnology will most likely also contribute to increased efficiency and programmes could benefit from transgenic techniques in terms of insect marking, genetic sexing, molecular sterilisation and disease refractoriness (Robinson and Hendrichs, 2005).

8 Conclusions

The suppression, containment, prevention or eradication of pest insects will in most cases result in more sustainable pest control when the various control tactics are applied on an area-wide basis, i.e. against an entire pest population within a delimited geographical area. Both sterile insects and natural enemies have been used very successfully in the past to control important insect pests. Although both control tactics are complementary and in some circumstances even synergistic, their combined use has not yet found a practical follow-up. Ionising radiation is not only a very suitable, effective and safe way to produce sterile insects, it can also be used in many ways to increase the usefulness of natural enemies for insect control. Both natural enemies and sterile insects, when appropriately used, have minimal negative effects on the environment. There are numerous examples of past and ongoing operational programmes that have successfully deployed sterile insects or natural enemies that have resulted in enormous economic benefits and in many cases have opened up very lucrative export markets of agricultural commodities. There is great potential to use these control tactics and strategic approaches against major insect pests in the future and the potential for economic growth and enhanced agriculture could motivate farmers to apply these approaches more in an area-wide context.

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Biodiversity and Pest Management in Orchard Systems

Sylvaine Simon, Jean-Charles Bouvier, Jean-François Debras, and Benoît Sauphanor

Contents

Abstract Conventional agriculture is based on a high level of chemical inputs such as pesticides and fertilisers, leading to serious environmental impacts, health risks and loss of biodiversity in agrosystems. The reduction of pesticide use is a priority for intensively sprayed agricultural systems such as orchards. The preservation and promotion of biodiversity within orchards and their boundaries is therefore an issue to explore. Indeed, orchard systems contain high plant diversity and perennial multi-strata designs that provide wealthy resources and habitats to living communities such as beneficial organisms. Orchards thus offer favourable areas to maintain food-webs within the agrosystem, provided that favourable situations are not altered by cultural practices such as applying an excess of pesticides. Here, we analysed literature on the effects of the manipulation of plant diversity and habitats on the control of pests by arthropod and bird communities in apple, pear and peach orchards. Many investigations focus on the role of plant management to enhance biodiversity in orchards but only 22 research reports presenting 30 case studies were dedicated to the study of the ecosystem service provided by plant diversity for orchard pest control. The underlying mechanisms were seldom demonstrated, and the tested grass covers and tree assemblages aimed at favouring either the beneficial complex or only some beneficial species to control one or a few pests. The effect of plant management on pest control was mostly positive (16 cases) or null (9), but also negative in some cases (5). This finding reveals the difficulties of identifying selected plants or plant assemblages for the control of key pests. We conclude that further research is needed to identify the processes involved on different scales for biological control. Orchard systems

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_30,

from Simon et al., Agron. Sustain. Dev. 30 (2010) 139–152. DOI: 10.1051/agro:2009013

should be re-designed to optimise ecosystem services provided by biodiversity.

Keywords Biodiversity • Orchard • Fruit tree • Plant • Arthropod • Bird • Community • Pest management • Hedgerow • Plant cover

1 Introduction

Since the 90s and the Rio summit on biodiversity in 1992, there has been increasing concern about the environment, and a consciousness of the impact of production and service human activities on the environment and on biodiversity. Conventional agricultural production which is based on a high level of chemical inputs, e.g. pesticides and fertilisers, is in the focus. Political actions at national and European Community levels aim at reducing the number and amount of pesticides used (PIRRP, 2006; Commission Européenne, 2008), and to promote research programmes to reduce their use or the risks due to their use (Aubertot et al., 2005; Sauphanor et al., 2009). Indeed, many environmental risks are related to the use of conventional insecticides, e.g. their aerial dissemination and the contamination of soil and water, with negative effects on animal communities directly or indirectly exposed to these chemicals (Aubertot et al., 2005), and on human health (Baldi et al., 1998). Moreover, besides the loss of habitats, pesticides also contribute to the decrease in plant and animal biodiversity in the agrosystem (Krebs et al., 1999). Reduction in the use of plant protection products is thus crucial for the implementation of sustainable agricultural systems, and especially in systems based on a high pesticide use such as orchards. Indeed, in temperate areas, orchards are among the most intensively sprayed agricultural systems to impair pest and disease damage and produce fruits with no visible fault to satisfy international commercial quality standards. Whereas French orchards only represent 1% of the utilised agricultural area, they make up 21% of the insecticide sales in France (Codron et al., 2003). Recently, information on the pesticide residues in fruits has altered the perception of fruits by consumers as fresh and healthy food, leading in several countries to the implementation of zero residue programmes (Berrie and Cross, 2006). There is thus a challenge in satisfying a societal demand for environmentally friendly systems and healthy fruits, and keeping pests and diseases below economic thresholds to maintain the growers' income in an evolving regulation context. The preservation and promotion of biodiversity within agricultural landscapes could be a key issue to answer both ecological and agronomic purposes.

Cultural systems have, on different scales, a dualistic relationship with biodiversity. They often reduce, or alter, biodiversity through simplified systems or cultural practices. However, cultural systems also contribute to the agrosystem richness and to the occurrence of some plant and animal species that would otherwise have disappeared (Le Roux et al., 2008). Besides, cultural systems are dependent on several ecosystem processes provided by biodiversity that contribute to soil fertility, pollination and pest control (Zhang et al., 2007). If there is a consensus on the role of ecosystem services for crop production, then strategies to maintain, favour and preserve biodiversity are more debated. These strategies can range from surface areas dedicated to biodiversity conservation ('land-sparing agriculture') to biodiversity preservation within agricultural areas ('eco-friendly agriculture') (Clergue et al., 2005). Agroecology (Altieri, 1995) represents the challenge to match production and biodiversity conservation within agricultural landscapes, especially in areas where cultivated lands occupy a large surface area.

Among cultivated crops, orchards are particularly suitable systems to study the level of ecosystem services provided by biodiversity, because they are perennial systems and present a complex multi-strata design. In most European orchards, plant components associated with fruit trees are planted and/or preserved and managed within orchards and/or in their boundaries for agronomic purposes, e.g. prevention of soil compaction due to machinery traffic, windbreaks and physical barriers. Orchard plant design thus contributes to plant diversity within agricultural areas and therefore to an increase in resources for animal communities such as arthropods and birds (Boller et al., 2004), among which are pest antagonists, provided that cultural practices, namely pesticide use, are not disruptive. Moreover, the importance of the ecosystem service provided by natural enemies for pest control has been pointed out for decades in orchards, with a focus on the control of many fruit pests such as mites, aphids, leafminers and psyllids by natural enemies (e.g. Wildbolz, 1988; Boller et al., 2004). The key role of natural enemies has been demonstrated for psyllids in pear orchards (Shaltiel and Coll, 2004) and mites in apple orchards (Solomon et al., 2000). There is thus a need to evaluate in orchards the role of functional diversity, i.e. the ecosystem service for pest control, but also the importance of disservices due to agricultural and ecosystemic management (Zhang et al., 2007).

The aim of the present review is to analyse the complex relationships between orchard systems, i.e. orchard design and practices, and functional biodiversity with a focus on plant, arthropod and bird communities. Our work is based on a thorough investigation of the ISI Web of Knowledge database from 1992 to January 2008, and on former articles cited in this literature, complemented by recently accepted articles. Orchards were considered to be agricultural surface areas dedicated to fruit production, which excluded several types of agroforestry or pastoral systems planted with fruit trees. Only pome and stone fruit productions in temperate areas were studied. Lastly, biodiversity was defined according to Noss (1990) and comprised compositional, structural and functional biodiversity on different scales. We develop two main points: (i) the contribution of orchard systems to plant and animal diversity against adverse effects of orchard practices on biodiversity, and (ii) the benefits for the orchard pest management of biological control through the conservation of habitats.

2 Orchard Systems and Biodiversity

The potential contribution of orchard systems to biodiversity is based on the analysis of their main specific features: permanency of the system, multi-strata design and adjacent plant management (Table 1) as favourable aspects, and the need for intensive pest management, including a recurrent use of pesticides, as a detrimental factor.

2.1 Perennial Habitats

Orchards are planted for several years or even decades in temperate areas: the diversity in time due to the successive crops of the cultural rotation is thus low in orchard systems. However, such a permanency in the host plant and the associated cultural practices is likely to enhance the stability of the system (Brown and Welker, 1992) and its resilience (Kozár, 1992). The permanency of the host plant favours the presence of some herbivores (which include pests) to the benefit of the permanency of food-webs. The entomofauna richness measured in apple orchards is higher than in annual crops in Hungary (Kozár, 1992), and the control provided by the natural enemies of pests is also reported to be higher in perennial than in annual crops (Hall and Ehler, 1979; Risch et al., 1983). Food and living conditions in organic apple orchards are favourable to the presence and the nesting of insectivore birds such as the Great Tit *Parus major* (Paridae),

Table 1 Contribution of annual and orchard systems to plant biodiversity

Plant diversity provided by the agrosystem	Annual systems	Standard orchard systems
Diversity in time	Crop rotation: turnover of host plants	Perennial crop: permanency of host plants
Diversity in space:		
- Plant architecture	-	Fruit tree complex branching structures
- Spatial distribution of plants within the field	Homogeneous plant cover and mono-stratum systems	Heterogeneous distribution (fruit tree rows, alleys) and multi-strata (arboreal, understorey) systems
- Adjacent diversity	Large fields adapted to machinery	Need for windbreaks in windy regions: planting of lining hedgerows

with reproduction rates equal to that of pesticide-free forests (Bouvier et al., 2005). Lastly, the soil litter is also likely to develop, to the benefit of the scavenger biomass which favours the abundance of some natural enemies (Longcore, 2003). As permanent habitats, orchards therefore contribute to the presence of a diversified arthropod community including scavengers, herbivores, predators and parasitoids, and to the permanency of food-webs including high trophic levels, namely, the insectivorous bird community.

2.2 Multi-Strata Habitats

In most cases both understorey and arboreal habitats are present within orchards. A grassy ground cover is generally sown or naturally occurs between rows and in the turning ends of the orchard to prevent soil compaction by machinery traffic, to limit erosion and/or pesticide transfer (Lacas et al., 2005). The arboreal habitat mainly consists of planted fruit trees. Various species of arthropods can live in one or more of these strata. The surface area of the orchard thus consists of a meadow interplanted with fruit tree rows where the soil arthropod community is more related to that of a meadow than of a forest (Fazekas et al., 1992). The effect of the orchard plant design on arthropod diversity is analysed on different spatial scales: (i) arboreal strata and within-tree structure, (ii) additional grass cover as understorey strata, and (iii) orchard system.

Fruit trees have a characteristic architecture, partly due to tree-training performed to ensure regular fruit bearing. Indeed, branching structures of the branches and patterns of distribution and growth of fruiting and vegetative shoots contribute to a complex within-tree architecture. Moreover, different scales, including leaf structure and infra-structures such as domatia and trichomes (Cortesero et al., 2000) are present. This structural complexity favours the richness of the entomocenosis (Price et al., 1980; Lawton, 1983) and the abundance of natural enemies (Langellotto and Denno, 2004; Finke and Denno, 2006). Even though underlying processes affecting tree arthropods within complex structures are not always disentangled by authors the most plausible are: (i) the diversity of plant resources benefits specialised herbivores which are the prey or the host of various natural enemies, being themselves the prey or host of other predatory or parasitoid insects; and (ii) intra-guild predation decreases in complex structures (Finke and Denno, 2006) and ‘enemy-free spaces’ are more important (Lawton, 1983). The structural complexity and heterogeneity of the fruit tree thus favours the diversity of the canopy arthropod community. However, complex structures are also detrimental to the foraging of some beneficial insects, through an increase in the time needed to locate their prey or host (Gingras and Boivin, 2002; Skirvin, 2004). Consequently, pest control through parasitism or predation is not always higher in complex than in simpler structures despite higher entomological diversity or abundance (Langellotto and Denno, 2004; Simon et al., 2007a).

As additional plant strata to productive trees, the plant cover in the alleys is generally composed of grasses (Poaceae), mixed with weeds and sometimes with other sown species such as leguminous plants. The presence of an understorey cover generally provides a benefit for the orchard pest control. A three-species plant cover sown in the alleys provides a higher richness and diversity of the pear canopy entomocenosis compared with a bare ground (Rieux et al., 1999). The beneficial aphidiphagous complex of the apple orchard is favoured by flowering strips to the benefit of aphid control (Wyss, 1995). Plant covers are only reported to be detrimental compared with a bare ground in peach orchards where they favour leafhoppers (McClure et al., 1982) and phytophagous mites (Meagher and Meyer, 1990a).

The co-existence of different strata creates a diversity of habitats and resources for animals: shelters, e.g. resting, diapause or hibernating sites, reproduction areas and refuge areas to escape disruptive agricultural practices, as well as food, e.g. alternate preys and hosts, nectar and pollen (Greaves and Marshall, 1987). Arthropod communities exploiting the soil, the grass and the canopy (Miliczky et al., 2000) cohabit within the orchard and contribute to its richness. Some species are likely to exploit more than one of these strata and are therefore likely to stay and multiply through higher levels of resources. As high levels of beneficial arthropod richness are displayed within the grass cover, whereas low levels of predation and pest control are observed within the arboreal strata (Simon et al., 2007b), strong interactions among strata are not always established in field experiments. The hypothesis of a structural rather than a functional assemblage is promoted by some authors (Vogt et al., 1998; Miliczky et al., 2000; Horton et al., 2002; Simon et al., 2007b).

It cannot be excluded that the beneficial complex of the fruit tree canopy does not benefit the grass cover richness or diversity. Although the intrinsic complexity of fruit trees and the diversity in resources provided by the orchard plant design are high, the resulting arthropod diversity is not always highly functional for pest control.

2.3 Plant Diversity in the Boundaries of Orchards

As fruits are delicate high value products and orchards perennial systems, they easily support the installation of windbreaks in windy regions (Prokopy, 1994). The most common ones are planted hedgerows. Some of the planted hedgerows in the orchard boundaries are multi-species hedgerows, for instance, composed after the recommendations by the IDF (Institut pour le Développement Forestier, 1981). As the diversity of planted fruit species or cultivars is low within the orchards, e.g. the most common cases are one or a few clones, these hedgerows improve the orchard system plant diversity. Although hedgerows may impair crop protection by harbouring potential pests and diseases (Solomon, 1981; Jeanneret, 2000), they are also physical barriers that stop drifts from adjacent pesticide applications and thus minimise side effects of pesticide use. If the hedgerow contributes by itself to the local plant biodiversity through the same mechanisms as plant covers, the association of orchards and hedgerows within the landscape creates and favours specific habitats and ecosystems. This contributes to the increase in global and landscape biodiversity (Pollard and Holland, 2006). A mosaic landscape consisting of orchards separated by hedgerows and/or ditches favours a specific flora and fauna through a higher availability of habitats and resources (Rands, 1986). Such areas are also hibernating sites for many insect species (Harwood et al., 1992; Lys and Nentwig, 1994). The communities of both adjacent plantings and local surroundings interfere with the orchard (Krebs et al., 1999; Simon, 1999; Benton et al., 2003). On a local scale, the biodiversity of the orchard system is improved by lining hedgerows and ditches (Green et al., 1994; Parish et al., 1994; Moles and Breen, 1995), as the biodiversity on the landscape scale is improved (Benton et al., 2003) through an increase in available biotopes (Rosenzweig, 1995). This latter aspect is especially emphasised for mobile taxa such as Lepidoptera (Jonsen and Fahrig, 1997) and birds (Robertson et al., 1990). Orchard systems and their boundaries are thus highly relevant candidates to contribute plant and animal diversity on different scales.

However, it is necessary to minimise such potentially favourable situations: the widespread use of the mating disruption method to control Lepidoptera requires large surface areas without interplanted hedgerows (Witzgall et al., 2008; Sauphanor, 2009). Italian and Northern American studies (Neumann, 1993) indicate an optimal efficiency for continuous surface areas of homogeneous orchards of tens of hectares protected by this method, which favours pheromone diffusion and minimises the vulnerability of borders. A recent study (Ricci et al., 2009) also indicates that the codling moth *Cydia pomonella* populations of a given orchard are negatively correlated with the surrounding surface areas planted with apple and treated with chemicals, which promote the production of apples within large surface areas whatever the pest control method (if efficient) against codling moth. The planting of large surface area orchards excluding hedgerows, which are replaced by hail nets as windbreaks and shields for the physical control of *C. pomonella* and tortricids (Tasin et al., 2008), are likely to develop in Southern France. There is an antagonism between the optimal use of various pest control methods and the management of plant diversity in the boundaries of the orchard system.

2.4 Pesticide Applications

Because of their host-tree permanency pests and diseases may remain present in the orchard throughout the year. This favours the increase in infestation or infection levels from one year to another, with the need for a continuous protection, namely, a recurrent use of pesticides to control them. Fruit tree protection is highly intensive and requires far more pesticide amounts than other crops. In 2006, an average of 36.5 treatments were sprayed in French apple orchards (Sauphanor et al., 2009). In all producing countries, current apple production systems resort to such intensive use of pesticides (Eurostat, 2002). Moreover, the trend is for an increase in the yearly number of treatments because of the development of resistant strains in some pests (Sauphanor et al., 2000; Reyes et al., 2008), low surface areas planted with resistant or low-susceptibility cultivars, and 'zero default fruit' market standards. Global warming is also likely to increase voltinism and the period of risks for some pests (Sauphanor, 2004), and to introduce new pests. From green tip to harvest, i.e. during a 6- to 8-month period, apple orchards are thus under pest and disease management regimes based on the use of pesticides. The side effects of their use on organisms living or foraging within the orchard may be direct through mortality and/or lower fecundity, or indirect through biomass (i.e. prey or host) reduction or host-plant suppression in the food-web.

In orchards the effect of pesticides and pest management regimes on arthropods is well documented for a few taxonomic groups, amongst which are spiders (Pekár, 1999; Bogya and Markó, 1999; Bogya et al., 1999; Miliczky et al., 2000; Brown et al., 2003) and ground-living beetles (Pearsall and Walde, 1995; Labrie et al., 2003) but it is more seldom studied for the total arthropod community (Sauphanor et al., 1993, 2005; Suckling et al., 1999; Brown and Schmitt, 2001; Debras et al., 2006; Simon et al., 2007b). The use of pesticides has a negative effect on hunting spiders (Pekár, 1999), ground-living arthropods (Epstein et al., 2000) and insects parasitising leaf miners (Prokopy et al., 1996) but, surprisingly, the total arthropod diversity or richness of the tree canopy is not or very little affected by the use of broad-spectrum insecticide programmes compared with more environmentally friendly methods (Suckling et al., 1999; Brown and Schmitt, 2001; Simon et al., 2007b). Hypotheses that are likely to explain such results may be related to: the resilience of the orchard system (Brown, 1993); a high immigrating rate of arthropods in small-sized orchards within mosaic landscapes (Liss et al., 1986; Whalon and Croft, 1986; Brown, 1993; Kozár, 1992; Bengtsson et al., 2005; Miliczky and Horton, 2005); and/or the inadequacy of synthetic diversity indices to give information on a whole community composed of groups with inconsistent responses (Suckling et al., 1999; Hole et al., 2005). However, even though the diversity measured by classical ecological indices such as the Shannon index is not always affected, the abundance of arthropods is always negatively affected by intensive pest management regimes (Suckling et al., 1999; Brown and Schmitt, 2001; Simon et al., 2007b), as are soil micro-arthropods (Doles et al., 2001).

The structure of the arthropod community also differs among high- and low-intensity pest management regimes (Andreev et al., 2006), and the natural control of some apple pests may be altered under intensive management regimes (Brown and Adler, 1989; Balázs et al., 1996; Suckling et al., 1999; Simon et al., 2007b): the beneficial arthropod complex is thus no longer present for ecosystem services. Enhanced ecosystem services for pest control permitted by a reduction in pesticide exposition such as in organic or low-input orchards illustrate the mutual benefits between conservation biological control and a reduced pesticide use (Sauphanor and Audemard, 1983; Brown, 2001a; Zehnder et al., 2007).

Birds constitute bio-indicators which are used to assess the effect of cultural practices on the environment (Ormerod and Watkinson, 2000). Because they occupy a high or top position in the food-web, they are relevant indicators of its global alterations (Furness and Greenwood, 1993). Besides, some of their biological requirements such as reproduction are concomitant with the period of pesticide applications in fields (Chamberlain et al., 2000). The documentation on the effect of pest management strategies on birds in apple orchards is still incomplete. Most of the studies focus on the reproduction rate of passerine birds, which is lower in intensively managed orchards compared with organic ones, in Northern America (Powell, 1984; Fluetsch and Sparling, 1994; Bishop et al., 2000) as well as in Europe (Bouvier et al., 2005). The effect of pesticides on bird communities is less studied. The bird diversity and abundance in German orchards was higher in organic than in Integrated Pest Management (IPM) orchards (Rösler, 2003). Consistently, the two research teams working on the subject have assessed that bird communities are more abundant and diversified in organic apple orchards, or to a lesser extent in IPM orchards, than in conventional intensive ones where the number of insectivore species is also lower (Bouvier, 2004; Genghini et al., 2006).

Local and regional environments, cultural practices and manipulations of the orchard plant diversity widely interfere. Only a few recent studies (Debras et al., 2006; Agerberg, 2007; Monteiro et al., 2008) have quantified the weight of these external factors. The weight of environmental variables to explain the composition of the orchard arthropod community is 28.7%, whereas it is 12.4% and 2.2% for cultural practices and lining hedgerows, respectively (Debras et al., 2006). For bird communities, both pesticide applications and the local environment account for 25% in the results, whereas the landscape effect contributes 15% to the total variance in apple orchards in Southern France (Agerberg, 2007). This latter result is consistent with the study by Monteiro et al. (2008) on the parasitoid community of pome fruit orchards in Southern France that displayed a similar contribution of local (27%) and landscape (16%) factors. However, further research is needed to validate such results within various contexts and regions, to assess the potential contribution of local and/or landscape diversity to explain the structure of bird and arthropod communities, and to identify local and landscape managements maximising the abundance of natural enemies.

3 Benefits of Biodiversity for the Control of Orchard Pests

The most studied benefits of biodiversity for fruit tree production are related to crop protection and are mainly based on an increase in plant diversity that favours the increase in animal diversity, including birds, mammals and arthropods. A higher level of pest control is thus expected, at least for some pests, through an increase in the abundance and the richness of their natural enemies. Within this framework of conservation biological control (Barbosa, 1999), we develop the effect on orchard pest control of (i) two plant assemblages associated with the orchard, i.e. plant ground covers and lining hedgerows, and (ii) the local land uses in the agricultural landscape. Lastly, the role of insectivore birds, favoured by nesting-boxes, will be discussed. Diversity is understood as measurements by classical ecological indices such as the Shannon index, but also by richness and abundance of the studied groups. All taxonomic levels are taken into account.

3.1 Manipulation of Plant Diversity to Enhance Orchard Pest Control

The effect of plant diversity on the arthropod populations of pests and natural enemies relies on several complex mechanisms (Russell, 1989): plant-insect relationships, prey-predator and host-parasitoid interactions, population dynamics, and structure and organisation of arthropod communities (Liss et al., 1986). The mechanisms involved are seldom demonstrated by authors. Both bottom-up and top-down effects are promoted to explain a reduced herbivory in complex compared with simple environments (Russell, 1989). The following mechanisms are either simultaneously (Bugg and Waddington, 1994) or individually proposed:

- within a diversified system, the decrease in pest damage is related to greater difficulties in localising their host plant(s) and to lower resources (Risch et al., 1983); structural and chemical complexities of plant assemblages are thus the cause of such decrease in herbivory (Brown, 1998);
- plant associations may alter the microclimate, the physiological stage or even the pest biology, contributing to pest control (Parfait and Jarry, 1987; Andow, 1991);
- due to a diversified vegetation, the predatory and parasitoid complex likely to control pests is maintained and made perennial (Risch et al., 1983; Szentkiràlyi and Kozár, 1991; Chaubet, 1993; Wyss, 1996; Brown, 2001a). The longevity or fecundity of some species may also be increased (Irvin et al., 2006).

The manipulation of the orchard plant diversity may affect communities living within or near the orchard through an increase in the resource range, i.e. habitat, shelter and food. Herbivores, including orchard pests, polyphagous and disease vector arthropods, pollinators, and predatory and parasitoid arthropods are involved, and the manipulation can result in beneficial or detrimental effects for the orchard pest control (Grison and Biliotti, 1953; Van Emden and Williams, 1974; Gruys, 1982; Fye, 1983; Solomon, 1981; Bugg and Waddington, 1994; Prokopy, 1994; Rieux, 1994; Schoemans, 1995; Simon, 1999; Boller et al., 2004; Debras, 2007). Very few studies address the economic benefit of such manipulation of plant diversity in the orchard or its boundaries. Besides several studies on the arthropod community of understorey plants (e.g. Westigard et al., 1990; Flexner et al., 1991; Meyer et al., 1992; Coli et al., 1994 on mites), pest control resulting from the introduction of plant assemblages is seldom directly assessed. The results may

Table 2 Effects of plant diversity on the control of orchard pests

Fruit tree production	Target pest(s)	Plant manipulation(s) or presence	Effect on pest control[1]	Source/Region
Apple	Apple aphids *Aphis pomi* *Dysaphis plantaginea*	Flower strips	Positive Positive	Wyss (1995); Wyss et al. (1995), Switzerland
Apple	Apple aphids *D. plantaginea*	Flower strips	Positive	Pfammater and Vuignier (1998), Switzerland
Apple	Apple aphids *A. pomi* *D. plantaginea*	Flower strips Negative	Null	Vogt et al. (1998); Vogt and Weigel (1999), Germany
Apple	Tent caterpillar and codling moth	Understorey plants	Positive Positive	Leius (1967), USA
Apple	Leafroller (Tortricidae)	Buckwheat	Positive	Stephens et al. (1998), New Zealand
Apple	Leafroller (Tortricidae)	Buckwheat Alyssum Phacelia	Positive Positive Null	Irvin et al. (2006), New Zealand
Apple	Leafroller (Tortricidae)	Peach nectaries	Null	Brown et al. (2008), USA
Apple	Apple aphids *Aphis spiraecola*	Peach nectaries Buckwheat	Negative Null	Spellman et al. (2006), USA (greenhouse experiment)
Apple Peach	Apple pests Peach pests	Plant cover and/or interplanted fruit-tree	Null or variable according to years and pests	Brown (2001b), USA
Apple	Apple pests	Plant cover	Null	Jenser et al. (1999)
Apple	Spider mites	Understorey plants	Positive under conditions	Croft B.A. (1982), USA
Apple	Spider mite *Tetranychus* spp.	Understorey plants	Positive under conditions	Alston D. (1994), USA
Apple	Spider mite *Panonychus ulmi*	Plant cover	Null	Nyrop et al. (1994), USA
Apple	Apple pests	Plant cover	Globally positive	Altieri and Schmidt (1985), USA
Apple	Spider mite *P. ulmi*	Adjacent bushes	Positive	Tuovinen (1994), Finland
Apple	Spider mites *Tetranychus spp.*	Plant cover	Positive	Yan et al. (1997), China
Apple Pear	Spider mite *P. ulmi* *Cacopsylla pyri*	Flower plant mixture	Null Null	Fitzgerald and Solomon (2004), UK
Peach	leafhoppers	Plant cover	Negative	McClure et al. (1982), USA
Peach	Spider mite *T. urticae*	Plant cover	Negative	Meagher and Meyer (1990a), USA
Peach	Hemiptera species	Plant cover	Negative	Meagher and Meyer (1990b), USA
Pear	*C. pyri*	Plant cover	Positive	Rieux et al. (1999), France
Pear	*C. pyri*	Hedgerow	Positive	Debras (2001, 2007), France

[1] The effect of plant manipulation on pest control is considered to be positive, null or negative when either the density of the pest arthropod of the fruit tree, fruit damage and/or the number of pesticide applications against the target pest is lower, equal or higher, respectively, compared with control.

vary according to the host fruit species, the pest and the tested plant assemblage (Table 2). Among the 22 listed articles presenting 30 case studies on the subject, the effect on pest control was positive in 16 cases, 5 plant assemblages had a negative effect and 9 others were indifferent. Plant manipulations generally aimed at favouring either predator or parasitoid beneficial taxonomic groups or species. The total beneficial complex is more seldom targeted. Most of the plant manipulations were based on the manipulation of understorey plants or plant assemblages, or on the analysis of naturally occurring plant ground covers. Only two of them were related to arboreal plant assemblages (adjacent bushes or lining hedgerows), attesting to the difficulties of carrying out field experiments on perennial plant assemblages. With the exception of one case in orchards, i.e. the detrimental effect of flower strips on apple aphid (Vogt and Weigel, 1999), negative effects were mainly due to the development in weeds of spider mites migrating into fruit trees when weeds are chemically or mechanically removed.

Several aspects can explain such variability in the results: the studied "pest-antagonist" couple, local context, composition, age and management of the tested plant assemblage, and orchard design. Cultivar and age of producing orchards are reported to be of little importance to explain the structure of the orchard arthropod community, providing the cultivars are not insect-resistant and tree architecture is similar (Brown and Adler, 1989). Only juvenile orchards, which are seldom experimented on, differ from older ones (Pekár, 2003). More generally, the effect of a plant manipulation largely relies on the biology of each targeted pest and each natural enemy, and on their interactions, between them and with other species of the arthropod community. We present below understorey and arboreal plant manipulations dedicated to enhancing pest control in orchards. Only plant-based approaches were considered. The manipulation of the habitat of ground-dwelling arthropods, for instance, by mulching the groundcover (Miñarro and Dapena, 2003; Mathews et al., 2004) was not reviewed.

3.1.1 Lining Hedgerows

Hedgerows lining the orchard are plant assemblages comprising tree species that may constitute a reservoir, or a source, of natural enemies, and also the source of infestation or infection by pests and diseases (Solomon, 1981; Prokopy, 1994; Schoemans, 1995; Maudsley, 2000; Boller et al., 2004). Studies or reviews on the specific entomocenosis of many tree species planted in hedgerows are available in Southern France (Barthelet, 1982; Defrance et al., 1987; Campo, 1992; Carraretto, 1992; Gauthier, 1993; Simon et al., 1993; Rieux, 1994; Delmas, 1995; Sarthou, 1995; Reboulet, 1996; Simon, 1999; Baudry et al., 2000; Debras, 2001; Debras et al., 2002), but precise and comprehensive information is still missing because of local specificity, climatic variations and time-consuming assessments by experts trained in arthropod systematics.

Very few hedgerows dedicated to crop protection have been experimented on. The mixed hedgerow proposed by Rieux (1994) for the control of the pear psyllid *Cacopsylla pyri* in pear orchards and experimented on since 1992 in Southern France has been built up according to the following principles and experimentally assessed (Simon et al., 2009):

- exclude tree species hosting orchard or quarantine pests and diseases, i.e. hawthorn, which is the host of fireblight;
- provide some natural enemies, i.e. the one(s) active against the main orchard pest(s) with various habitats and resources: shelter, hibernating site, and areas to escape within-crop cultural practices. These consist of hollow stems of herbaceous plants, bark crevices, evergreen leaves of bush or tree species, intertwine stems of creeping species; food such as pollen, nectar, alternate preys or hosts;
- organise all year long successive resources in order to maintain and multiply beneficial arthropods in the vicinity of the orchard;
- favour the motion of natural enemies from the hedgerow towards the orchard, using tree species hosting migrating alternate preys which induce natural enemies to search for new preys.

The presence of natural enemies is generally higher in the part of the orchard lining the hedgerow than in its centre (Altieri and Schmidt, 1986; Reboulet, 1996; Paoletti et al., 1998) and aphid abundance is correlatively the lowest in orchard edges where beneficial numbers are the highest (Altieri and Schmidt, 1986). A gradient of density from the hedgerow towards the orchard is described for lacewings (Rodet, 1985; Simon et al., 1998). Earwigs issuing from the hedgerow are collected within the orchard in Southern France (Debras et al., 2007). Debras (2007) and Debras et al. (2008) also assessed that the distribution of natural enemies within the orchard is affected by the hedgerow: natural enemies actively move from the hedgerow to the orchard in relation to prey availability, even though cultural practices (among which the use of pesticides) alter this functional pattern in most orchards. However, a significant effect of the hedgerow on the orchard beneficial complex is not always displayed. Coli et al. (1994) did not relate high densities of predatory mites hosted by bush species to mite populations of the adjacent orchard. As an adverse effect, codling moth abundance was observed to be the highest along the hedgerow (Audemard, 1992). Lastly, no significant correlation was displayed between predator abundance due to plant environment and *C. pyri* control in a survey of 8 commercial pear orchards

(Simon, 1999). Patterns of pest distribution and patterns of predator densities introduced by the plant environment are thus not always correlated. Such discrepancies may be explained by the thermal and biological requirements of the considered arthropod species and by the climatic effect of the hedgerow on the distribution of both pests and natural enemies (Debras et al., 2008; Ricci et al., 2009). Lastly, the age (Burgio et al., 2006) and the cultural management of these hedgerows may alter their structural and plant diversity, therefore altering their functionality (Forman and Baudry, 1984). The benefit of the increase in the abundance and diversity of natural enemies induced by plant manipulation is, however, seldom measured; the benefit, if any, can be slight, and not sufficient to avoid pesticides against the most noxious pests. We noticed that only pests such as mites and psyllids, which can be tolerated at high population levels in the orchards, may benefit from such manipulations (Table 2). Beyond short-term pest control, the recolonisation of the orchard by natural enemies issuing from adjacent plant assemblages can contribute to the restoration of the community structure (e.g. the case of mites, see Tuovinen, 1994) and to a more stable system.

3.1.2 Plant Ground Covers and Interplanted Fruit Tree Species

A wide range of plant covers and interplanted peach trees (Brown, 2001b; Brown et al., 2008) were tested by the authors (Table 2). Most of the tested assemblages aimed at providing beneficial arthropods with pollen and nectar through flowers or peach nectaries. Grassy or flower strips sown in the orchard alleys (between rows) are proposed in apple orchards to help control the rosy apple aphid *Dysaphis plantaginea* (Wyss et al., 1995; Wyss, 1995; Pfammater and Vuignier, 1998; Vogt et al., 1998). Single species covers with buckwheat, phacelia or alyssum were experimented on in New Zealand to help control Tortricidae (Stephens et al., 1998; Irvin et al., 2006).Weeds are also companion plants in orchards and may shelter natural enemies (Kozár et al., 1994), especially flower weeds (Leius, 1967; Zandstra and Motooka, 1978; Wyss, 1995) and nettle *Urtica dioica* (Stary, 1983; Hérard, 1986). The plant cover not only shelters an abundant arthropod community likely to offer alternate preys or hosts, but also orchard pests: aphids, mites (Meagher and Meyer, 1990a), phytophagous mirids (Fye, 1980), leafhoppers (McClure et al., 1982; Meagher and Meyer, 1990b), tortricids (Brown, 2001b) and Coleoptera (Wyss, 1996). However, the migration of these pests towards the cultivated trees is often more affected by the management of the plant cover than by the plant cover itself: weeding leads the hosted pests to migrate towards another resource, i.e. orchard trees (Van Emden and Williams, 1974; McClure et al., 1982; Westigard et al., 1990; Flexner et al., 1991). Natural enemies hosted by the plant cover of the alleys are also negatively affected by frequent mechanical mowing (Horton et al., 2002).

For the total arthropod fauna and beneficial complex, the presence of a grassy ground cover within the orchard increases (Altieri and Schmidt, 1985) or not (Wyss, 1996) the diversity of beneficial arthropods. Responses in terms of pest control vary widely according to the pests (Altieri and Schmidt, 1985; Brown and Glenn, 1999), and most of the studied plant covers address one or a few key pests. Many studies in Northern America focus on the effect of understorey covers on mite populations in orchards. Information is provided on the conditions for optimal biological control of spider mites by predatory mites in terms of surface area to be covered by grass, distance to fruit trees, and composition of the plant assemblage (Nyrop et al., 1994; Croft B.A., 1982; Alston D., 1994). In pear orchards, both an increase in Anthocorid numbers and a decrease in *C. pyri* prey are assessed when a grassy ground cover is sown in the alleys compared with bare ground (Rieux et al., 1999). Flower strips in apple orchards enhance *D. plantaginea* control (Wyss et al., 1995; Pfammater and Vuignier, 1998). By providing a within-orchard higher density of preys they contribute to maintaining a high density of spiders and generalist predators, which prey on immigrating aphids in autumn, and can survive on, and control, low densities of preys. In spring, flower strips are also expected to favour aphidiphagous syrphids (Wyss, 1995), but this effect is not displayed in a second experiment (Vogt et al., 1998; Vogt and Weigel, 1999), most probably because of a delayed bloom in a Northern region and the mowing of the strips in winter. Lastly, very few studies (Irvin et al., 2006) address all the processes involved in the tri-trophic system targeted by plant manipulations: food preference of each orchard pest, effect of companion plants on the abundance and the fitness of the natural enemies to promote, predation or parasitism rates in the orchard and interactions with other natural enemies. The plant ground cover is therefore a plant component of the orchard which is easily manipulated and experimented on. A wide range of plant assemblages targeting various pests has already been tested with promising results. As for hedgerows, we notice that empirical, rather than scientific, knowledge is involved in most cases. Consequently, beyond adverse effects due to the management of the plant cover, failure or success cases in pest control cannot be explained and results are not always reproducible. Further research is needed to identify occurring processes and the ability of both pests and beneficial arthropod species to exploit both understorey and arboreal resources.

3.2 Effect of Surrounding Land Uses

The association of both agricultural and uncultivated areas has been considered to preserve biodiversity (Grison and Biliotti, 1953) and to favour natural enemies of crop pests (Chaubet, 1993; Sarthou, 1995; Landis et al., 2000; Deguine and Ferron, 2004; Tscharntke et al., 2007). Integrated Fruit Protection (OILB, 1977) and ecological compensation areas (Garnier, 1994; Herzog et al., 2005) rely on such association in order to enhance natural control of orchard pests and to increase biodiversity, respectively. The effect of local or regional landscape on the arthropod populations of orchards is reported by many authors (Liss et al., 1986; Altieri and Schmidt, 1986; Whalon and Croft, 1986; Szentkiràlyi and Kozár, 1991; Kozár, 1992; Bengtsson et al., 2005; Miliczky and Horton, 2005). Winged arthropods represent 50% of the total number of the orchard entomofauna and are strongly related to local and regional backgrounds (Szentkiràlyi and Kozár, 1991; Kozár, 1992). However, very few studies address the relationships or correlation between the features of the agricultural area and beneficial effects or pest control in orchards. The total surface area covered by uncultivated lands (woods, fallow fields) within 100 m around pear orchards is significantly and positively correlated with the total and beneficial arthropod diversity of the orchard, and negatively correlated with *C. pyri* pest numbers, whereas the types of crops and their relative importance within the same area are not significantly correlated. Hedgerows lining the orchards increase the above-mentioned correlation between surface areas of uncultivated lands and the orchard beneficial complex diversity (Simon, 1999). These results are consistent with the study by Gut et al. (1988), establishing that the development of *C. pyri* is low in plant diversified environments. Such approaches are similar to those based on landscape ecology and developed in vineyards (Van Helden et al., 2006).

The presence of alternate host plants of pests in the orchard surroundings permits the provision of refuge areas for insecticide-susceptible alleles. This is likely to contribute to the management of resistance to insecticides and therefore to the sustainability of crop protection methods, provided that susceptible alleles have a selective advantage on resistant ones when insecticides are no longer applied. For the rosy apple aphid whose primary host is the apple tree, as well as the codling moth hosted by cultivated fruit trees only (apple, pear, nut and quince trees), refuge zones for strains susceptible to insecticides are mainly unmanaged or organic orchards (Boivin et al., 2005). More than plant diversity, it is the diversity of cultural practices which is expected to be beneficial. Lastly, it is clear that the study and the management scales outstrip the orchard scale, due to the potential moving distances of various groups of arthropods (Lewis, 1969).

3.3 Predation by Insectivorous Birds

The integration of insectivorous birds into pest control patterns is of benefit with higher yields and income (Jones et al., 2005a). Such integration can be performed by increasing the number of artificial nesting sites within the orchard, especially for cavity-nesting passerine birds which lack natural cavities (Bishop et al., 2000). Sanz (2001) showed that such management of artificial nesting sites favours the installation of Tit populations. As all species of Tits feed their brood with Lepidoptera caterpillars, they may significantly reduce fruit damage caused by codling moth larvae (Mols et al., 2005). As birds generally avoid parasitised preys, the biological control of pests due to birds seems to be complementary to that of other bio-control agents (Jones et al., 2005b).

4 Conclusion

Beyond a global increase in the richness of the agrosystem, an increase in predation or parasitism favoured by the conservation of habitats of beneficial organisms is assessed in many studies. There are generally only partial effects for pest control which is insufficient to reduce the use of pesticides except for some pests, e.g. mites and psyllids, that can be tolerated at high levels of populations without any damage on fruits or reduction in yield. Further research is needed to investigate all of the processes involved in conservation biological control on different interconnected scales and to identify: (i) the most relevant beneficial candidates or association of candidates to be promoted among predators and parasitoids, generalists and specialists; and (ii) the species composition, age, density and design of plant assemblages that would maximise beneficial effects and minimise detrimental ones when considering the global orchard community. The reduction in pesticide exposition of orchard communities is certainly a key point to maximise ecosystem services for pest control. We also promote the redesign of orchard systems to meet such a purpose of an 'agroecologic' orchard. We particularly propose to investigate: (i) the effect of a decrease in the genetic (one clone) and spatial (linear arrangements) monotony of current orchard designs; (ii) the emphasis of some favourable traits of current orchard designs, i.e. multi-strata design, plant diversified environment and soil litter development. Options such as the interplanting of missing strata (i.e. bush layer), mix cropping coupled with the supervised management of the whole orchard plant diversity, and the management of the soil organic status are candidate issues to favour functional diversity for pest control; and (iii) the manipulation of the architectural and microclimatic traits of the fruit tree through genetics and tree training, as a tool to modify the habitat of orchard pests and the foraging area of their natural enemies, and therefore their development. Lastly, the challenge mainly relies on integrating all these tools on different interconnected scales, from fruit tree leaf infrastructures to orchard and landscape scales, in order to maximise ecosystem services on each scale and to implement synergistic effects.

Acknowledgements The authors wish to thank C. Bussi for his advice on an earlier draft of this manuscript. We thank Katie L. and David Bennett for improving the English of the manuscript.

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Pathogenic and Beneficial Microorganisms in Soilless Cultures

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Contents

Abstract Soilless cultures were originally developed to control soilborne diseases. Soilless cultures provide several advantages for growers such as greater production of crops, reduced energy consumption, better control of growth and independence of soil quality. However, diseases specific to hydroponics have been reported. For instance, zoospore-producing microorganisms such as *Pythium* and *Phytophthora* spp. are particularly well adapted to aquatic environments. Their growth in soilless substrates is favoured by the recirculation of the nutrient solution. These pathogenic microorganisms are usually controlled by disinfection methods but such methods are only effective as a preventive measure. Contrary to biofiltration, active treatments such as UV, heat and ozonisation have the disadvantage of eliminating not only the harmful microorganisms but also the beneficial indigenous microorganisms. Here, we review microbial populations that colonise ecological niches of hydroponic greenhouse systems. Three topics are discussed: (1) the general microflora; (2) the pathogenic microflora that are typical to hydroponic systems; and (3) the non-pathogenic and possibly beneficial microflora, and their use in the control of plant diseases in soilless greenhouse systems. Technical, economic and environmental concerns are forcing the adoption of new sustainable methods such as the use of microbial antagonists. Thus, increased attention is now focused on the role of natural microflora in suppressing certain diseases. Managing disease suppression in hydroponics represents a promising way of controlling pathogens. Three main strategies can be used: (1) increasing the level of suppressiveness by the addition of antagonistic microorganisms; (2) using a mix of microorganisms with complementary ecological traits

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_31,

from Vallance et al., Agron. Sustain. Dev. In press. DOI: 10.1051/agro:2010018

and antagonistic abilities, combined with disinfection techniques; and (3) amending substrates to favour the development of a suppressive microflora. Increasing our knowledge on beneficial microflora, their ecology and treatments that influence their composition will help to commercialise new, ready-to-use substrates microbiologically optimised to protect plants in sustainable management systems.

Keywords Antagonistic agents • Biological control • Microbial ecology • Disinfection methods • Hydroponics • Recirculating solutions • Root rots • Suppressive microflora • Wilting • Zoosporic pathogens

1 Introduction

Soilless cultures are used worldwide. Depending on the country, growers use a variety of complex technologies, all of which offer advantages making them appropriate alternatives to traditional soil culture (Fig. 1a). In cases where the soil is polluted by chemical residues or contaminated by pathogens which colonise and persist in the soil for years or when excessive salinity causes water problems, soilless cultures can be an alternative. The main advantage of soilless cultures is that plants grow in a controlled environment. For instance, nutrient solution supply, electrical conductivity, pH and temperature are monitored and regulated by the grower. It provides an ideal environment for growth and development of plants and a greater yield is frequently obtained than with traditional cultural methods. The majority of greenhouse crops are grown using artificial substrates (Fig. 1b), which improves control of water, aeration, nutrition and root distribution. These systems were originally developed as open systems and excess nutrient solution was disposed of outside the greenhouse. In recent years, closed hydroponic systems have been developed to minimise pollution. In a closed system, the nutrient solution is recovered, replenished with nutrients and water, depending on plant uptake, and the pH adjusted before resupplying to the plants.

Microbial contamination of the root system in these culture systems can arise from many sources: plant material, growth media, and water from lakes, rivers and wells (Stanghellini and Rasmussen, 1994). Root colonisation by fungi and bacteria is favoured by at least three factors: (i) genetically uniform host plants, (ii) environmental conditions, i.e. suitable temperature and moisture regime, and (iii) rapid dispersal of root-colonising agents throughout the cultural system via the recycled nutrient solution.

The activity of microorganisms, however, may be pathogenic or protective, so two scenarios are possible. (1) One of the reasons for developing soilless culture was to prevent root diseases caused by soil-pathogenic microorganisms. Although a decrease in the diversity of root-infecting microorganisms has been reported, root diseases still occur frequently in hydroponics and disease outbreaks are sometimes greater than in soil (Stanghellini and Rasmussen, 1994). Some minor infections have become threats in soilless culture, indicating that unique diseases are observed with this method of plant cultivation. (2) The role of natural microflora in suppressing certain diseases was demonstrated by comparing systems with and without their original microflora (Postma et al., 2000; Minuto et al., 2007). Indeed, it has been shown that the natural microflora can suppress diseases (Berger et al., 1996; Chen et al., 1998) and that a high density of bacteria in the rhizosphere can limit pathogenic attacks on roots (Tu et al., 1999). From these observations ensued the hypothesis that indigenous bacteria were involved in disease biosuppression.

In this study we focused on the microbial communities colonising the root systems of plants growing in soilless cultures and highlighted the specificity of microbes in this type of cultivation system. Three topics were reviewed: (i) the general microflora; (ii) the pathogenic microflora of typical diseases related to hydroponics; and (iii) the non-pathogenic and possibly beneficial microflora and their use in the control of plant diseases in soilless greenhouse systems.

2 Ecology of the Microflora in Soilless Systems

Soon after the start of a soilless culture, a microflora rapidly colonises three ecological niches: the substrate, the nutrient solutions and the rhizosphere of the cultivated plants. The density and diversity of this microflora are affected by the type of substrate (organic or inorganic), the nutrients in the solutions and the age and cultivar of the plant species.

Fig. 1 Tomato soilless culture and the main associated fungal pathogens. Suspended substrate in a tomato soilless culture (**a**), rockwool containing healthy and altered roots (**b**), *Phytophthora cryptogea* sporangia on the surface of a necrosed root (**c**), *Pythium aphanidermatum* oospores (round with a thick wall) in the root cortex cells (**d**), macroconidia of *Fusarium oxysporum* f. sp. *radicis-lycopersici* with chlamydospores in formation (**e**), *Colletotrichum coccodes* acervulus with black seta (**f**)

Cultural methods have been used to characterise this microflora, but in recent years other methods based on sole-carbon-source utilisation (Khalil and Alsanius, 2001; Khalil et al., 2001b), phospholipid fatty acid profiling (Waechter-Kristensen et al., 1996; Khalil and Alsanius, 2001; Khalil et al., 2001a, b) and molecular fingerprinting (Postma et al., 2000; Calvo-Bado et al., 2003, 2006) have provided structural and functional analysis of the soilless microflora. Recent studies on microflora have provided key information on the microbial diversity and dynamics of soilless systems.

2.1 Influence of the Kind of Substrate on Microflora

In soilless cultures a microflora rapidly develops soon after the start of the culture via the plants and the water supply, even though inorganic substrates contain few microbes. Once plants are introduced into greenhouses, extensive colonisation of rockwool substrates by bacteria and fungi rapidly occurs (Price, 1980; Carlile and Wilson, 1991). Inorganic substrates are mainly colonised by bacteria while organic substrates are colonised by fungi (Koohakan et al., 2004). In the case of crops of tomatoes, for instance, bacteria including fluorescent pseudomonads were higher in rockwool than in peat substrates and the reverse was observed for fungi, actinomycetes and *Trichoderma* spp. (Khalil and Alsanius, 2001). This might be due to the presence of available organic compounds within the organic substrates which may modify the microbial equilibrium through reduced competition (Koohakan et al., 2004). The level of conduciveness to the diseases caused by a given pathogenic agent might be determined by the nature (structure, composition) of the growth substrate of the crop. For instance, rockwool is more conducive to *Pythium* root rot and crown rot in cucumber culture than coir dust, pumice and perlite (van der Gaag and Wever, 2005). Temperature and oxygen concentration did not explain the differences between the media but the higher incidence of disease on rockwool was associated with a much greater water content than in the three others. When the height of the rockwool slabs was increased, the percentage of diseased plants decreased. These results indicated that water content plays a major role in the development of root and stem rot and that the type and height of substrate are important tools for decreasing yield losses.

2.2 Root System and Nutrient Solution Microflora

Microorganisms multiply rapidly on roots and in nutrient solutions. Large populations of heterotrophic bacteria (10^5–10^6 cfu mL^{-1}) developed in the circulating nutrient solutions 20 h after planting tomatoes (Berkelmann et al., 1994). The number of bacteria on young tomato roots can be as high as 10^{10} cfu g^{-1} of fresh roots (Waechter-Kristensen et al., 1994). However, there are differences between microbial communities colonising roots and nutrient solutions; more fungi and bacteria were detected on roots than in nutrient solutions (Koohakan et al., 2004). Besides the densities, the structure and the diversity of bacterial communities, as assessed by a molecular fingerprint method (Single-Strand Conformation Polymorphism, SSCP), were also different on roots and nutrient solutions (Renault, 2007).

The cultural systems (inorganic and organic media, deep flow technique and nutrient film technique) favoured in different ways the growth of unique indigenous microorganisms (Koohakan et al., 2004). Fungi and *Fusarium* spp. were found to colonise preferentially roots grown in a coconut-fibre system (organic medium) compared with a rockwool system (inorganic medium). *Pythium* spp. were mainly detected in nutrient solutions and on roots from the nutrient film technique system. Among the non-specific bacterial genera, aerobic bacteria seemed predominant on roots and in nutrient solutions, with only slight differences between the four systems (inorganic and organic media, deep flow technique and nutrient film technique). Whatever the system, fluorescent pseudomonads were frequently detected on roots and in nutrient solutions, which was consistent with previous findings showing that 40% of the cultivable bacteria belonged to the genus *Pseudomonas*, known to contain potentially antagonistic agents toward pathogens (Berkelmann et al., 1994). Similar results were obtained in the recycled nutrient solution during a six-month experiment in a soilless tomato greenhouse (Déniel et al., 2004). These findings might be explained by the fact that the temperature, high nitrogen content and oxygen concentration of the nutrient solutions offer an optimal growth environment for this genus.

2.3 Influence of the Rhizosphere on the Microbial Communities

There is a clear relationship between cultivated plants and the establishment of the rhizosphere microflora. In closed hydroponic systems, it results from the release of organic compounds by the roots (Waechter-Kristensen et al., 1997). Passive or active leakage of carbon sources from plant roots differs in quantity and quality depending on plant species, plant cultivar and environmental factors such as light, climate, nutrient source, pH, humidity, etc. Whatever the hydroponic habitat, the diversity of microorganisms depends on their ability to metabolise the available carbon sources. Although a nutrient film technique system is much simpler than a soil-based culture system, SSCP analyses showed the bacterial diversity of the rhizoplane to be as high as that of the rhizosphere in soil (Chave et al., 2008). However, further studies comparing the microorganisms colonising soil and soilless cultures are needed to draw any conclusion.

2.4 *Evolution of Microbial Communities*

As mentioned above, biological processes in the rhizosphere are strongly affected by plant root exudates that attract specific microbial populations and stimulate their growth and evolution. Based on viable counts, aerobic bacteria colonising the rhizosphere of four types of soilless tomato production systems (inorganic substrate: rockwool; organic substrate: coconut-fibre; deep flow technique, nutrient film technique) were found to become stable at 10^{10} cfu g^{-1} (of fresh roots) in all systems investigated, contrary to fungi, that tended to increase throughout the experiment (Koohakan et al., 2004). However, changes in the composition of the microflora have been demonstrated by molecular and biochemical analyses. For instance, Khalil et al. (2001b) highlighted the differences between the microflora of two supposedly identical hydroponic cultivations by comparing sole-carbon-source utilisation (SCSU) patterns and phospholipid fatty acid profiles (PLFA). In tomato soilless cultures, Renault et al. (2008) also observed a temporal shift over a cropping season in the bacterial composition both in the nutrient solution and on the roots. Indeed, community-level physiological profiles (CLPPs) indicated that bacterial metabolism in nutrient solutions progressively shifted from carbohydrates towards the degradation of specific amino acids and carboxylic acids.

There is no consensus about whether shifts in the rhizosphere microflora can result from pathogenic attacks. Indeed, changes in the microbial communities of the rhizosphere could be a consequence of both root damage caused by pathogens such as *P. ultimum* and secondary colonisation due to the resulting nutrient leakage (Naseby et al., 2000; Hagn et al., 2008). On the other hand, it has been reported that the microbial communities established early on the roots of tomatoes grown in soilless systems were robust and resistant to the effect(s) of the introduction of oomycete pathogens or of switching from a recirculating to a run-to-waste nutrient supply (Calvo-Bado et al., 2006). However, this assumption, arising from experiments conducted over only 1.5 months, is contradicted by the observation of changes in the microbial communities of tomato plants grown hydroponically over the 6-month experiments of Vallance et al. (2009). SSCP analyses of three different DNA regions indicated increases in the complexity and size of the fungal microflora as the cropping season progressed. Nevertheless, both studies suggest that there are no substantial changes in the genetic structure of the indigenous rhizospheric fungal community after root inoculation with the non-pathogenic oomycete *P. oligandrum* or the pathogenic oomycetes *Pythium* group F, *P. aphanidermatum* and *P. cryptogea*.

3 Unique Disease Problems in Soilless Cultures

3.1 *Infections by Zoosporic Oomycetes*

Among the pathogenic microorganisms frequently detected in hydroponic cultures, those producing zoospores, i.e. *Pythium* spp. and *Phytophthora* spp., are particularly well adapted to these cultivation systems (Favrin et al., 1988; Rafin and Tirilly, 1995) (Figs. 1c, 1d). As zoospores can swim, recycling facilitates rapid dissemination and subsequent root infection of the whole culture (McPherson et al., 1995). Disease epidemics can occur, particularly in periods of stress, because of high temperatures and the low concentrations of dissolved oxygen in the nutrient solution (Gold and Stanghellini 1985; Stanghellini and Rasmussen 1994; Chérif et al., 1997). Highly pathogenic *Pythium* species, i.e. *Pythium ultimum*, *P. irregulare* and *P. aphanidermatum* (Blancard et al., 1992; Jenkins and Averre, 1983; Linde et al., 1994), caused root rot and wilting.

In Brittany (France), two stages in root infection by *Pythium* spp. in commercial tomato greenhouses were observed by Rey et al. (2001). The first is generally from the start of the winter crop (February) to June. A small population of *Pythium* spp. is frequently detected. The population then dramatically increases between July-August and the end of the cropping season (October–November); this increase is sometimes associated with root necrosis and root rot, but generally infections are limited to root necroses and are even symptomless. This pattern was particularly observed in greenhouses with organic (peat) and, to a lesser extent, inorganic substrates (rockwool). With a nutrient film technique system, *Pythium* spp. invasion was earlier and more severe than in other cultures, but with no amplification of symptoms.

A DNA macroarray for the detection and identification of more than 100 *Pythium* species was developed to assess the number and diversity of *Pythium* species on a single root sample (Tambong et al., 2006). This technology has the advantage of combining DNA amplification with the screening capability of DNA arrays, resulting in a high degree of sensitivity and multiple species specificity. The results of the DNA array tests confirmed that the substrate was almost free of oomycetes at the start of the plant culture. *P. dissotocum* (or *Pythium* group F) was spontaneously detected on roots throughout the growing period but other *Pythium* species (*P. intermedium*, *P. ultimum* and *P. sylvaticum*) were sporadically detected (Le Floch et al., 2007). The relative predominance of *P. dissotocum* (or *Pythium* group F) and the low diversity of *Pythium* species confirm the results of previous studies conducted in soilless cultures (Herrero et al., 2003; Moorman et al., 2002; Moulin et al., 1994; Rafin and Tirilly, 1995; Rey et al., 1997).

3.2 Complex of Pathogens on Necrotic Roots

A variety of fungal complexes and oomycetes are responsible for root necroses. A three-year experiment in tomato soilless cultures in France revealed that the distribution of the fungi and of the oomycetes was region-dependent (Blancard, unpubl. data). In the South-West, between two and five fungi and oomycetes were frequently found on roots, whereas in the five other regions (Brittany, the Eastern Pyrenees, Nantes region, Orleans region, the South-East), up to three different microorganisms were isolated from the samples. Some fungi, including *Fusarium oxysporum* f. sp. *radicis lycopersici*, and oomycetes, such as *Pythium* species, were found in all the greenhouses investigated in the six French regions (Fig. 1e). Other fungi, i.e. *Colletotrichum coccodes, Rhizoctonia solani* and *Thielaviopsis basicola*, or oomycetes such as *Phythophthora* spp. were only found on roots in some of the greenhouses (Fig. 1f).

3.3 Symptomless and Minor Pathogen Infections on Roots

Asymptomatic root colonisation in hydroponic cultures can be correlated with yield loss (Rey et al., 1997; Stanghellini and Kronland, 1986). *Pythium dissotocum* caused yield reductions of up to 54% in hydroponically grown lettuce although there was no visible damage (Stanghellini and Kronland, 1986). Such infections might be more common in soilless greenhouse systems than originally thought, because of the lack of root symptoms (Favrin et al., 1988). Immunoenzymatic staining procedures showed that *Pythium* spp. were the most frequent fungal invaders in asymptomatic roots of hydroponically grown tomato plants. *Pythium* spp. represented around 40% of the colonised segments as opposed to 12% for the other fungi. *Pythium* group F accounted for 75 to 90% of all the *Pythium* isolates from the loose or dense mycelia of *Pythium* spp. on the root epidermis (Rafin and Tirilly, 1995; Rey et al., 1997). Certain strains produce large numbers of zoospores (Rafin, 1993), possibly facilitating the spread and the development of *Pythium* group F in soilless cultures. When plants were grown under optimal conditions *Pythium* group F-infected roots were symptomless. However, roots looked generally macroscopically healthy but the oomycete caused limited changes in the root cortex (Rey et al., 1998) and produced metabolites that may facilitate *Pythium* group F infections (Rey et al., 2001). Moreover, due to high *Pythium* group F populations over the cropping season, limited but repeated damage to root cortexes could lead to slight yield reductions (Rey et al., 1997). Severe damage, such as root rot, only occurs when plants are placed under physiological stress conditions, i.e. lack of oxygen in nutrient solutions (Chérif et al., 1997). The nature of *Pythium* group F is still unclear. The taxonomic position of this oomycete has only become clearer in recent years with the increased interest in *Pythium* group F. Van der Plaats-Niterink (1981) used the term group F because oomycetes of this group only produce non-inflated filamentous sporangia on traditional culture media and sexual structures are not observed. However, after molecular characterisation of *Pythium* group F isolates by ribosomal and intermicrosatellite-DNA regions analysis, Vasseur et al. (2005) suggested that *Pythium* group F isolates could be *P. dissotocum*-like isolates unable to form sexual structures on traditional media. Moreover, Lévesque and De Cock (2004) suggested that *Pythium* group F could be related to *P. dissotocum* because of the complete homology of ITS sequences.

3.4 Other Potentially Pathogenic Microorganisms in Soilless Cultures

The pathogenicity of a few microorganisms (*Humicola* sp., *Olpidium brassicae* and *Plectosporium tabacinum*) (Figs. 2a–2e) needs to be determined because some root microorganisms of minor importance in soils have become of major economic importance in hydroponic cultures (Stanghellini and Rasmussen, 1994). Hydroponics, for instance, have favoured the development of *Phytophthora cryptogea* on lettuce, whereas, in the field, no attacks by this fungus have been reported. *Plectosporium tabacinum* (formerly *Fusarium tabacinum*), frequently isolated from soilless tomato cultures in France (Blancard, unpublished data) is a possible pathogen. It is detected on a variety of soil-grown plants, i.e. melon (Soran and Ozel, 1985), sunflower (Mirza et al., 1995) and basil (Minuto et al., 1997). Matta (1978) and Pascoe et al. (1984) reported that it caused necrotic lesions on young leaves in tomato plants and El-Gindy (1991) noticed necrosis and root rot in plantlets. Such symptoms have never been observed on tomato plants grown hydroponically. However, considering the pathogenic potential of *P. tabacinum* and its frequency in greenhouses, its pathogenicity in hydroponics needs to be assessed. Another example is *Humicola fuscoatra*. Gruyter et al. (1992) reported the association of *H. fuscoatra* with corky root symptoms in wilted glasshouse tomatoes. However, Menzies et al. (1998) pointed out that *Humicola fuscoatra* colonised roots, but did not cause necrosis and was, therefore, not pathogenic in tomato plants. These findings highlight the difficulties in distinguishing minor pathogens from other fungi, as both frequently colonise roots in soilless cultures.

4 Effect of Disinfection Techniques on the Microflora of Soilless Systems

Closed hydroponic systems increase the risk of pathogen attack by using water contaminated with pathogenic microorganisms (McPherson et al., 1995; van Os et al., 1999). Therefore, prevention of these infections has become a major challenge in the last decade (Runia, 1995; Ehret et al., 2001).

4.1 Active Methods

The so-called "active" methods disinfect the nutrient solutions and are very effective (Ehret et al., 2001; Goldberg et al., 1992; Rey et al., 2001; Runia, 1995; Steinberg et al., 1994); for example, UV radiation and heat treatment can eliminate up to 99% of the microflora colonising the flowing solutions. UV irradiation of recirculating solution was effective in controlling *Pythium* spp.-induced root rot in tomato and cucumber plants (Postma et al., 2001; Zhang and Tu, 2000). Tirilly and Letard (1997) reported a delay in *Pythium* root infection in soilless culture with this method; however, in several cases there was no difference in root colonisation from non-disinfected greenhouses. Moreover, re-contamination of the disinfected nutrient solution nullified the effect of disinfection (Déniel et al., 1999). Such drastic treatments create a microbiological vacuum in which microbial pioneers spread more easily because of the lack of competition (Paulitz and Bélanger, 2001; Postma, 2004). The microbial differences in solutions treated with UV and slow filtration often disappeared once they flowed through the rockwool slabs containing plant roots (van Os et al., 2004). Chlorination is effective in disinfecting water in storage tanks and reduces and

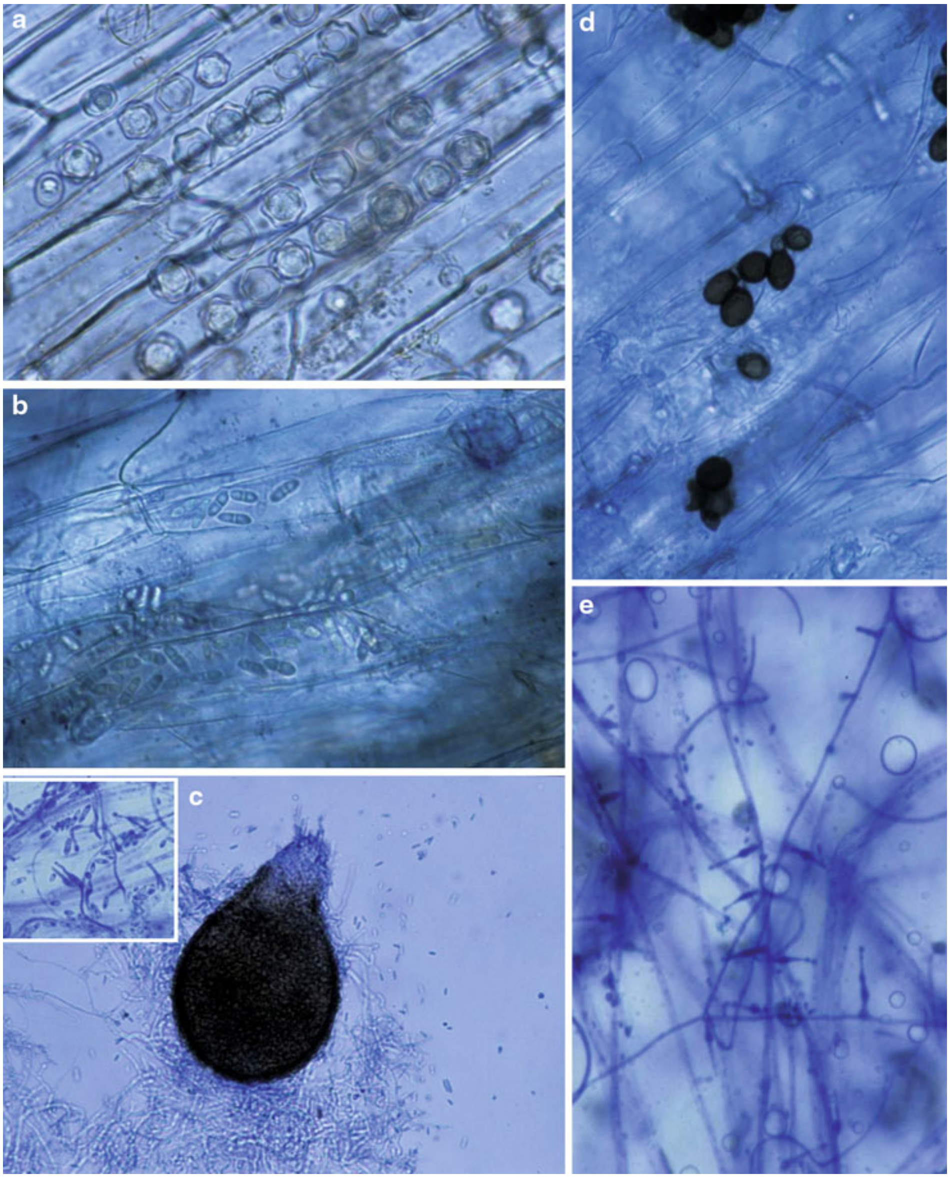

Fig. 2 In situ and in vitro appearance of three fungi sometimes associated with root rot in tomato soilless culture but whose aggressiveness has never been proven on this Solanaceae. *Olpidium brassicae* resting spores aligned in several root cells (**a**), *Plectosporium tabacinum* bicellular conidia within root cortex cells (**b**), phialides of *Plectosporium tabacinum* (**c**), aleuriospores (dark brown) of *Humicola* sp. (**d**), phialides of *Humicola* sp. perpendicular to the mycelium; they form chains of conidia (**e**).

delays root colonisation by *Pythium* spp. (Déniel et al., in press). However, this treatment has the disadvantage of eliminating not only harmful but also beneficial indigenous microorganisms; a weakness of "active" methods of disinfection. Zhang and Tu (2000) imputed the lack of control of *P. aphanidermatum* on tomato roots to the reduction of bacterial communities caused by UV radiation.

4.2 Passive Method: Slow Filtration

The traditional technique of slow filtration, used for more than 100 years for water disinfection (Graham and Collins, 1996; Ellis, 1985), has been adapted for horticulture over the last decade (Ehret et al., 2001). Water flows slowly through a bed of substrate, i.e. sand, rockwool or pozzolana; mechanical and biological factors are thought to be responsible for the efficacy of the system (Ellis, 1985; Weber-Shirk and Dick, 1997). Experiments to improve slow filtration efficacy have focused on the determination of flow rates through the filter unit as well as on the nature and the optimal depth of substrates in filter tubes (Wohanka et al., 1999). Further investigations showed that the formation of bacterial microcolonies or biofilms on substrates enhanced efficiency. Indeed, after sterilising a filtering column, a dramatic loss in *Xanthomonas campestris* pv. *pelargonii* elimination has been reported (Brand and Wohanka, 2001). *Pseudomonas* was the predominant genus (50%) from the cultivable bacteria colonising the filtering media, especially the top layers of sand filters, and 10% of isolates were identified as *Bacillus* (Brand, 2000; Calvo-Bado et al., 2003). The *Bacillus* and *Pseudomonas* genera were recently reported to account for 42 to 86% of the total cultivable bacterial flora in a biocenosis film of pozzolana grains used as filtering medium (Déniel et al., 2004).

Pathogens eliminated efficiently by this technique include zoosporic fungi, i.e. *Phytophthora* spp., bacteria, i.e. *Xanthomonas campestris*, nematodes and even viruses (Ehret et al., 2001; van Os et al., 1999). During a 3-year experiment in a commercial greenhouse, Déniel et al. (2006) reported that a biofilter eliminated more fungi than bacteria under tomato production conditions. The efficiency of elimination of pathogenic fungi was genus-dependent. *Pythium* spp. were more effectively eliminated (99%) than *Fusarium oxysporum* (92.7 to 99.3%). The high percentage of *Pythium* spp. elimination was correlated with low root colonisation by these pathogens. Effluents of filtering columns have been shown to be colonised by a considerable natural bacterial microflora (10^2–10^4 cfu mL^{-1}) (Déniel et al., 2004, 2006; Renault, 2007). Moreover, molecular fingerprinting analyses of the total microflora (denaturing gradient gel electrophoresis, DGGE, and SSCP) pointed out clear changes in bacterial communities after the passage of the nutrient solution through the filter unit (Postma et al., 1999; Renault, 2007). Thus, slow filtration preserved part of the natural microflora, because it is harmless to specific groups of bacteria which are assumed to preserve microbial ecosystems in the plant rhizosphere. Furthermore, resident bacteria of nutrient solutions were shown to reduce *Pythium* root rot in closed soilless systems (Tu et al., 1999). The potential benefit of microflora in soilless cultures thus has to be taken into account.

5 Disease Suppression in Soilless Systems

Pathogen-suppressive soils have been defined as "soils in which (i) the pathogen does not establish or persist; (ii) establishes but causes little or no damage; or (iii) establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil" (Borneman and Becker, 2007). Soils suppressive to several pathogens have been widely described and investigated (Alabouvette et al., 1979; Jager et al., 1979; Lifshitz et al., 1984; Garibaldi et al., 1989; Whipps and Lumsden, 1991), while the first studies of suppressiveness in soilless systems were by McPherson et al. (1995) and Tu et al. (1999). Both studies demonstrated the potential of the indigenous microflora to inhibit root diseases in hydroponic cultures. In soilless cultures, the term "suppressiveness" referred to the cases where (i) the pathogen does not establish or persist; or (ii) establishes but causes little or no damage. McPherson et al. (1995) described the spread of *Phytophthora cryptogea* in tomato nutrient film technique systems. In closed systems, the pathogen caused less damage than in the parallel run-to-waste ones; they therefore suggested that the potentially beneficial microflora colonising the recycled nutrient solution were responsible for disease suppression. They also suggested that the method of disinfection, i.e. "active" or "passive" (by total or partial elimination of the microflora) could be important in the maintenance of the disease suppression. Tu et al. (1999) also showed that *Pythium* root rot disease was less severe in closed rockwool systems than in open culture due to the greater numbers of bacteria in closed systems. They found a strong correlation between the resident bacteria and the biosuppression of *Pythium*.

The presence of microflora suppressing *Pythium aphanidermatum* in cucumber rockwool substrate has been reported and some of the microorganisms involved in the suppressiveness identified (Postma et al., 2000, 2004, 2005). *Pythium* damage was lower in non-autoclaved than in autoclaved rockwool; the disease incidence was reduced by 50 to 100%. Suppressiveness could be restored in sterilised rockwool substrates by re-introducing the original microflora through contact with untreated rockwool or through the nutrient solution taken from untreated slabs. These results indicate that disease suppression is of biological origin and is transferable. Experiments on the microbial communities of rockwool showed a positive association between disease suppressiveness and the composition and diversity of bacteria and culturable filamentous actinomycetes. Actinomycetes may prevent the colonisation of dead root fragments by *Pythium* zoospores, whilst bacteria may secrete antibiotics, surfactants, etc. preventing colonisation of fresh root fragments.

Suppression of *Fusarium oxysporum* f. sp. *radicis lycopersici* has also been demonstrated. The incidence of *Fusarium oxysporum* f. sp. *radicis lycopersici* on tomato seedlings was significantly reduced with recycled, non-disinfected rockwool compared with new rockwool (Minuto et al., 2007); and in tomato soilless culture, by the re-use of perlite and perlite-peat substrates (Clematis et al., 2008). The indigenous microorganisms colonising these recycled substrates were considered responsible for the suppressive effects.

How the suppressive microflora becomes established is relatively unknown, but it has been suggested that pathogens themselves might influence suppressiveness. For instance, a study showed that *P. ultimum* induced shifts in cucumber indigenous microflora, favouring groups known to include potential biocontrol agents (Hagn et al., 2008). However, knowledge of structural and functional interactions and synergisms between the microorganisms of the suppressive microflora is limited and the influence of the plant and the pathogens on the whole system needs further investigation (Weller et al., 2002; Burdon et al., 2006).

6 Management of the Soilless Microflora for Disease Suppression

Factors influencing disease suppression such as the activity of the total microflora, the diversity of the microbial communities and the presence of specific antagonists are not fully understood (Postma, 2004). Nevertheless, managing disease suppression in hydroponics represents a promising way of controlling pathogens. Three main strategies can be used: (i) increasing the level of suppressiveness by the addition of antagonistic microorganisms; (ii) using a mixed culture of microorganisms with complementary ecological traits and antagonistic abilities combined with disinfection techniques; and (iii) amending substrates to favour the development of the suppressive microflora.

6.1 Increasing the Level of Suppressiveness by the Addition of Antagonistic Microorganisms

Environmental conditions in greenhouses are controlled and can be optimised to suit antagonistic agents. The biological vacuum and the limited volume of the matrix of the soilless substrates are thought to facilitate the introduction, establishment and interaction of the biocontrol agent with the root environment (Paulitz and Bélanger, 2001; Postma, 2004). Thus, representatives of a range of bacterial (*Pseudomonas*, *Burkholderia*, *Bacillus*, *Serratia*, Actinomycetes), fungal (*Trichoderma*, *Penicillium*, *Gliocladium*, non-pathogenic *Fusarium*) and oomycete (*Pythium*) groups have been tested as biocontrol agents in soilless cropping systems. The antagonistic activities of these microorganisms can be divided into several categories: competition for nutrients and space, parasitism, antibiosis and systemic induced resistance (Garbeva et al., 2004; Alabouvette et al., 2006; Lemanceau et al., 2006). Nevertheless, biocontrol of root diseases is often inefficient and only a few antagonists are available commercially.

The lack of efficiency is due to unsuitable methods of selection of antagonistic microorganisms. Results from in vitro studies did not always correlate with the antagonistic activity of the biocontrol agent once they were introduced into greenhouses (Fravel, 2005; Alabouvette et al., 2006; Georgakopoulos et al., 2002). These results also demonstrated the importance of the medium used for doing the in vitro tests; it has to be as close as possible to the environment into which the antagonists will be introduced. Even then, the colonisation, survival and antagonistic activity of the biocontrol agent may be insufficient and/or inconsistent at the infection site because the antagonist is not adapted to the soilless environment. The use of microorganisms selected from the indigenous suppressive microflora and not from a suppressive soil or a different crop might solve this problem: the microorganisms would be better adapted to the soilless crop environment and the ecological niche where their interaction with the pathogens will take place.

For example, the pathogenic fungi or oomycetes most frequently involved in root diseases in soilless cultures are those producing zoospores, such as *Pythium* spp. and *Phytophthora* spp., making them particularly well adapted to the aquatic environment of hydroponics. The use of an antagonist belonging to the same taxonomic group (i.e. oomycetes), with the same life cycle and similar properties, is of particular interest. An example of such an antagonist is the oomycete *P. oligandrum* (Rey et al., 2008; Vallance et al., 2009); it has been widely reported as an effective biocontrol agent (Foley et al. 1986; Jones and Deacon, 1995; Benhamou et al., 1997; Rey et al., 1998, 2005; Wulff et al., 1998). The beneficial effects of *P. oligandrum* are due to its potential to colonise roots without damaging the host plant cells and to survive in the rhizosphere. *P. oligandrum* biocontrol in the rhizosphere is a complex process including direct control of pathogens by mycoparasitism, antibiosis or competition for nutrients and space; and/or indirect control via the plant, i.e. induction of resistance and growth promotion (Le Floch et al., 2005; Rey et al., 2008). Persistent root colonisation by *P. oligandrum* strains may be associated with an increase in tomato yield in soilless cultures (Le Floch et al., 2003), a transient increase (Le Floch et al., 2007) or not (Vallance et al., 2009).

When root colonisation by *P. oligandrum* is assessed, results from molecular (DNA macroarray and real-time PCR) and culture-dependent methods may be contradictory. Indeed, in the experiment of Le Floch et al. (2007), *P. oligandrum* was detected throughout the growing season (6 months) with molecular methods, but only for three months with plate counting on semi-selective media. These findings have important implications for biocontrol strategies aimed at protecting plants. Indeed, two different strategies could be envisaged: (i) based on cultural data, *P. oligandrum* inoculation on roots should be repeated three months after the first application; or (ii) conversely, based on molecular results, reinoculation is unnecessary because *P. oligandrum* is still present. In conclusion, the second strategy probably represents the true pattern of root colonisation by the antagonist, because detection by DNA array and real-time PCR is more accurate. Appropriate methods should therefore be used to detect the antagonistic agent(s) in assessment of biocontrol.

A strategy for increasing suppressiveness and therefore making biocontrol more successful might be to associate several antagonistic agents with complementary and/or synergistic modes of action against one or several pathogens (Spadaro et al. 2005). This is the case in naturally suppressive soils, where suppression is the result of complex interactions between several microorganisms acting together. Known examples are soils suppressive to *Fusarium* wilts where non-pathogenic *Fusarium* and fluorescent *Pseudomonas* were identified as the main antagonists (Alabouvette and Lemanceau, 1999). The non-pathogenic *Fusarium* competes for carbon sources while bacterial antagonists produce siderophores competing for iron. In soilless cropping systems, the association of the non-pathogenic *Fusarium* strain Fo47 and fluorescent *Pseudomonas* strain C7R12 controlled fusarium diseases better than single inoculations of each antagonistic microorganism (Eparvier et al., 1991). Another strategy was to combine inoculation of *Lysobacter enzymogenes* with chitosan. Chitosan enhanced the biocontrol efficacy of *L. enzymogenes* in the control of *P. apahidermatum* in cucumber soilless greenhouse systems. Chitosan either served as a C and N source for the antagonist, induced antagonistic gene expression, or both (Postma et al., 2009).

6.2 Use of a Mixed Culture of Antagonistic Microorganisms with Disinfection Techniques

A more complex strategy consists of combining nutrient solution disinfection methods with biocontrol agents to colonise and protect the roots from pathogenic attack. One of the first experiments of this type combined slow filtration and *P. oligandrum* inoculation on roots in a tomato soilless greenhouse system (Rey et al., 1999). Then, the association of slow sand filtration and antagonistic strains of *Fusarium* spp. and *Trichoderma* spp. isolated from a gerbera rhizosphere was successfully tested (Garibaldi et al., 2003). A similar experiment also reported that slow filtration and antagonistic fungi (*Fusarium* spp. and *Trichoderma* spp.) operated synergistically to significantly reduce the incidence of *P. cryptogea* root rot in gerbera crops (Grasso et al., 2003). Another strategy with slow filtration is to enhance efficiency by biological activation of the filtering columns with bacteria with suppressive traits, i.e. antagonistic activities, or siderophore and auxin production (Déniel et al., 2004). These bacteria, i.e. *Bacillus* and *Pseudomonas* strains, were isolated from a mature tomato hydroponic slow filtration unit and then inoculated into a new filter (Renault et al. 2007). Further investigations showed that the six-month period for the control filter to reach maximum efficiency against *F. oxysporum* was shortened in the bacteria-amended filter; in addition, filtration was highly efficient from the first month. Fast colonisation of pozzolana grains by selected bacteria and their subsequent interaction with *F. oxysporum* is probably responsible for filter efficiency. *Pseudomonas* spp. are supposed to act by competing for nutrients and *Bacillus* spp. by antibiosis and/or direct parasitism (Déniel et al., 2004). However, after nine months of operation, bacteria from the genera *Pseudomonas* and *Bacillus*, used to inoculate the filters, were not recovered in significant numbers from substrates in these filtering columns (Renault, 2007). Therefore, although early bacterial inoculations promote filter efficacy and induce a significant shift in microbial communities, the inoculated bacteria do not colonise the filtering substrates for long periods.

6.3 Nutritional Amendments

Although physico-chemical factors influence the prevalence of *Pythium* diseases in certain substrates (van der Gaag and Wever, 2005), the main factor regulating disease suppression in hydroponic cultures is the microflora. The rhizosphere competency of potential biocontrol agents is often limited due to a lack of available organic nutrients in soilless growth media. Indeed, the main source of nutrients for the microflora on inorganic substrates is the plant roots, i.e. exudates, mucigel, sloughed root cells, etc. In conventional agriculture many other sources are available: organic amendments such as compost can be used as fertilisers or to improve the physical structure of the soil. Composted organic amendments are also substrates capable of suppressing plant diseases caused by a wide range of pathogens and pests, including bacteria, fungi and nematode species (Hoitink and Boehm, 1999; Alabouvette et al., 2006; Termorshuizen et al., 2006). Therefore, to maintain a critical threshold population of antagonistic microorganisms in soilless substrates, two approaches (similar to those in conventional cultures) could be considered: (a) the use of a different organic material, i.e. compost, as an alternative substrate for greenhouse production, and (b) the introduction of a food base for the biocontrol agent to sustain its antagonistic activity without stimulating that of the pathogen.

(a) Composted organic amendments have been tested as alternative substrates to peat in soilless systems to preserve peat bogs. Two different types of citrus compost and their water extracts were investigated as partial peat substitutes for melon seedlings in greenhouse nurseries. Compared with peat, both composts (containing plant nutrients and auxin- and cytokinin-like compounds) enhanced the plant growth; biocontrol of *Fusarium oxysporum* was also achieved due to the biotic component. Water extracts had no effect on plant yield but their biocontrol ability was similar to that of their solid matrices (Bernal-Vicente et al., 2008). Another study showed that the suppressiveness of compost is related to the ability of its microflora to degrade organic compounds. The microbial communities associated with three substrates with varying capacities of *Fusarium* wilt suppression were characterised: peat (conducive to wilt), cork (moderately suppressive) and grape marc (very suppressive). The nature and composition of the plant growth medium determined the microbial communities: in suppressive media, the microflora preferentially metabolised less easily biodegradable compounds such as carboxylic acids, amino acids, amines, phenolic compounds and polymers; while the microflora of peat used mostly sugars (Borrero et al., 2006).

(b) As the availability of nutrients is a limiting factor for the growth of the microbial communities in various plant habitats, the use of nutritional amendments has been studied to selectively increase the communities' size and the biocontrol efficacy of a target biocontrol agent. The feasibility of selective enhancement and maintenance of desired populations of naturally-occurring biocontrol agents such as *Pseudomonas putida* by amending the nutrient solution with a nitrogen stabiliser, N-Serve®, has been demonstrated. Both active and inert ingredients in N-Serve® were involved in the suppression of root disease of pepper and cucumber caused by *Phytophthora capsici* and *P. aphanidermatum*. Xylene and 1,2,4-trimethylbenzene, the constituents of the inert fraction of N-Serve®, served as carbon sources for the selective enhancement of the pseudomonad populations, and nitrapyin, the active ingredient, reduced the vegetative growth of both pathogens (Pagliaccia et al., 2007, 2008).

7 Conclusion

The last three decades have convincingly shown that, in soilless culture, the initial goal of growing plants free of soilborne microorganism attacks was not realistic. Diseases specific to this type of cultivation have been frequently reported; indeed, the elimination of the soil did not remove the pathogenic issue but has simply moved it. For instance, in comparison with soil, some diseases are only observed or have taken on a greater importance in soilless cultures. In that context, control methods have to be adapted to soilless greenhouses. One of the main options that has gradually emerged in recent years has been the use of non-pathogenic microflora. This assumption was based on the finding that if hydroponics is a "solution" for the development and spread of pathogenic zoosporic fungi and oomycetes, much evidence indicates that it can also be one for the management of the plant protective microflora. The development of sustainable control methods such as classical biological control but also new kinds of experiments, i.e. the re-use of substrates (with their suppressive microflora) or the use of suppressive ready-to-use substrates, is a must for soilless cultures. As numerous environmental parameters are controlled, managing the microflora is much easier in soilless culture than on soil. It will be a testing ground on which the results could be used for transfer to more complex systems such as soil.

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Allelopathy in Compositae Plants

S.-U. Chon and C.J. Nelson

Contents

Abstract Allelopathy plays an major role in agricultural management such as weed control, crop protection, and crop re-establishment. Compositae plants have potent allelopathic activity, and the activity is confirmed through (a) bioassays with aqueous or various solvent extracts and residues, (b) fractionation, identification, and quantification of causative allelochemicals, and (c) mechanism studies on the allelochemicals. Most assessments of allelopathy involve bioassays of plant or soil extracts, leachates, fractions, and residues based on seed germination and seedling growth in laboratory and greenhouse experiments. Plant growth may be stimulated below the allelopathic threshold, but severe growth reductions may be observed above the threshold concentration depending upon the sensitivity of the receiving species. Generally germination is less sensitive than is seedling growth, especially root growth. Some approaches showed that field soil collected under donor plants significantly reduced or somewhat promoted growth of the test plants. Petri-dish bioassays with methanol extracts or fractions and causative phenolic allelochemicals showed significant phytotoxic activities in concentration-dependent manner. Delayed seed germination and slow root growth due to the extracts could be confounded with osmotic effects on rate of imbibition, delayed initiation of germination, and especially cell elongation; the main factor that affects root growth before and after the tip penetrates the seed coat. Light and electron microscopic approaches extract evaluation at the ultrastructural level have been precisely investigated. Many Compositae plants have allelopathic potentials, and the activities and types and amount of causative compounds differ depending on the plant species. The incorporation of allelopathic substances

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_32,

from Chon and Nelson, Agron. Sustain. Dev. 30 (2010) 349–358. DOI: 10.1051/agro:2009027

into agricultural management may reduce the use of pesticides and lessen environmental deterioration.

Keywords Allelopathy • Compositae plants • Eco-friendly weed control • Bioassay • Extracts • Fractions • Residues • Water-soluble • Activation mechanism • Phenol compounds

1 Introduction

Allelopathy was defined by Molisch (1937) as a chemical interaction between plants or sometimes between microbes and higher plants that includes stimulatory as well as inhibitory influences. Later it was defined as any direct or indirect, harmful or beneficial effect of one plant as a donor plant on another as a recipient plant through the production of chemical compounds that escape into the environment (Rice, 1984). When a plant produces allelopathic compounds that are detrimental to establishment of new seedlings of the same species, the effect is called autotoxicity which is a specialized intraspecific form of allelopathy. Allelopathy and autotoxicity can play significant roles under both natural and manipulated ecosystems (Rice, 1984), mainly by adversely affecting seed germination and seedling growth. Allelopathic interactions among plants have been observed for centuries even though few specific allelochemicals have been identified.

Allelopathy plays a key role in weed control, crop protection, and crop re-establishment. Suitable manipulation of allelopathy towards improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases, conservation of nitrogen in crop land, and synthesis of novel agrochemicals based on natural products have gained prominent attention of scientists engaged in allelopathic research. The allelochemicals can affect physiological functions like respiration, photosynthesis and ion uptake. More recently, however, scientific attention has also been drawn to exploit the positive significant roles of allelopathy and what role this phenomenon can play in enhancing crop productivity.

A serious problem of modern agriculture is crop loss caused by weeds. Worldwide, weeds alone cause a 10% loss of agriculture production (Altieri and Liebman, 1988). Yet, allelopathic principles of crops can be used as an alternative mean of weed control based on natural products. Although allelopathy is often considered a problem for agriculture, there is now considerable evidence to suggest that it might be exploited to help manage weed problems in a variety of agroecosystems. In agroecosystems, several weeds, crops, agroforestry trees and fruit trees have been shown to exert allelopathic influence on associated or subsequent crops, thus, affecting their germination and growth adversely (Kohli et al., 1993). Most allelopathic evidence has been associated with the effect of weeds on crops and crops on crops, and crops on weeds. Of these, an important economical potential of allelopathy may be the ability of crops to suppress weeds. Allelopathic weeds also can affect crops by a number of ways like delaying or preventing seed germination and reducing seedling growth. Approaches that have already been explored are selection for allelopathic types within the germplasm of crops, use of allelopathic rotational or companion plants in cropping systems, and biosynthesis of useful natural herbicides from higher plants and microorganisms.

Alternatives to synthetic chemical herbicides need to be developed, especially for organic or eco-friendly farming operations, landscape management systems, home gardens, and for situations where public policies mandate reduced pesticide use. Most studies on allelopathy have focused on interference and allelopathic effects of several important weeds on crop yields. Several weeds have been shown to have allelopathic potentials and others are suspected to have allelopathic potential in agro-ecosystems (Rice, 1984). Plant seedlings of various crops possess allelopathic potential or weed-suppressing activity, including cucumber (*Cucumber sativs* L.) (Putnam and Duke, 1974), oat (*Avena spp.*) (Fay and Duke, 1977) and rice (*Oryza sativa* L.) (Dilday et al., 1994; Olofsdotter and Navarez, 1996). A total of 538 accessions of cultivated and wild cucumber were screened in pot and field tests with several accessions causing inhibited growth of *Brassica hirt*a and *Panicum miliaceum* (Putnam and Duke, 1974). Out of more than 3000 accessions of oat, several exuded a large amount of an allelochemical, scopoletin (Fay and Duke, 1977). Also oat suppressed the growth of *Erysimum cheiranthoides* in both laboratory and field tests due at least in part to an allelopathic mechanism (Markova, 1972).

Improving the competitive ability of crops also reduces dependency on herbicides. However, attempts to increase competitive ability while maintaining productivity have had limited success and no crop cultivar has been released with superior competing ability as a marketing argument. In crop competition including allelopathy, the importance of chemical interference has often been discussed (Rice, 1995). The incorporation of an allelopathic character into a crop cultivar could provide the plant with a means of gaining a competitive advantage over certain important weeds (Putnam and Duke, 1974). Wu et al. (1999) suggested that genetically improving crops with allelopathic potential and the allelopathy can play an important role in future weed management.

2 Allelopathic Compositae Plants

Twelve weed families include 68% of the 200 species that are the most important world weeds. Of them, Compositae plants account for 16% of the world's worst weeds are included (Holm, 1978). Allelopathy in weeds has been found in a number of plants employing various laboratory assays. Some of previous studies on allelopathic effects of weeds have been several Compositae plant species such as *Artemisia*, *Cirsium*, *Lantuca* and *Xanthium* species.

Inam et al. (1987) reported that aqueous extracts of *Xanthium strumarium* from different plant parts reduce germination, early growth and dry weight of *Brassica compestris*, *Lactuca sativa*, and *Pennisetum americanum*. *Parthenium hysterophours* is also known to be very allelopathic to wheat (Kanchan and Jayachandra, 1979), soybean, and corn (Mersie and Singh, 1978). Extracts and residues of these plants significantly reduced germination and root and shoot dry weight of the test plants. Bendall (1975) studied water and ethanol extracts and residues in soil and concluded that an allelopathic mechanism might be involved in the exclusion of some annual thistle (*Carduus crispus* L.), pasture, and crop species in areas infested with *Cirsium arvense* (L.) Scop. *C. arvense* litter reduced the growth of *Amaranthus retroflexus* L. and *Setaria viridis* L. more than that of cucumber (*Cucumis sativus* L.) or barley (*Hordeum vulgare* L.) in their greenhouse experiments (Stachon and Zimdal, 1980). In a field experiment, high densities of *C. arvense* reduced the incidence of annual weeds growing in the same vicinity of *C. arvense* (Stachon and Zimdal, 1980). More recently, Chon et al. (2003a) reported that aqueous leaf extracts from 16 Compositae plant species were bioassayed against alfalfa (*Medicago sativa*) to evaluate their allelopathic effects, and the results show the highest inhibition in the extracts from *C. japonicum, L. sativa*, and *X. occidentale*, all showing significant inhibition of seed germination and growth of alfalfa.

Einhellig (1986) noted that biological activity of allelochemicals, including the autotoxic factors in alfalfa, was concentration dependent with a response threshold. Plant growth may be stimulated below the threshold, with mild to severe growth reductions observed above the threshold; each depending on the sensitivity of the receiving species, the plant process affected and the existing environmental conditions. Many instances of stimulation effects of microorganisms on other organisms and of plants on microorganisms have been reported. Rice (1986) demonstrated growth stimulatory effects of volatile compounds, decaying leaves and root exudates of parasitic and nonparasitic plants on several other species. He showed that decaying ground ivy leaves stimulated seedling growth of downy brome and radish. Chon et al. (2003a) evaluted aqueous extracts of 16 Compositae plants and found *Lactuca sativa*, *Xanthium occidentale* and *Cirsium japonicum* showed the highest inhibition on alfalfa (*Medicago sativa*) seedlings. Conversely, extracts of *Chrysanthemum indicum, Youngia sonchifolia*, *Bidens frondosa*, and *Breea segeta* at concentrations below 20 g dry matter L^{-1} increased root length of alfalfa by 13–33%. In another study, alfalfa root growth was stimulated by very low concentrations from alfalfa leaf extracts (Chon et al., 2000). Our findings, however, indicated that this stimulatory effect for a reputed autotoxic chemical was less than those reported by Einhellig (1986) and Rice (1986) who evaluated stimulatory effects of allelochemicals. Chon et al. (2003a) reported that stimulatory as well as inhibitory effects of Compositae plant species were exhibited (Table 1).

2.1 *Artemisia sp.*

The genus *Artemisia* comprises over 400 species of perennial herbs and shrubs which are widely distributed throughout Europe, Asia, North America and South Africa (Stairs, 1986) and the plants are

Table 1 Effects of aqueous extracts from several *Compositae* plants on alfalfa root length (mm) 6 days after seeding. Root length of untreated control was 34.6 mm. Adapted from Chon et al. (2003a)

	Extract concentration, g L^{-1}			
Plant species	10	20	30	40
Bidens frondosa	44.3 (128)	39.7 (115)	31.4 (91)	7.1 (20)
Breea segetum	42.1 (122)	43.7 (126)	28.2 (81)	8.8 (25)
Chrysanthemum indicum	41.2 (119)	45.9 (133)	35.3 (102)	26.5 (76)
Youngia sonchifolia	43.5 (126)	40.1 (116)	36.5 (105)	27.4 (79)
Eclipta prostrata	28.9 (84)	19.5 (56)	4.1 (12)	1.7 (5)
Cirsium japonicum	31.5 (91)	22.0 (63)	7.6 (22)	0.0 (0)
Xanthium occidentale	33.3 (96)	2.9 (8)	0.0 (0)	0.0 (0)
Lactuca sativa	34.3 (99)	0.2 (1)	0.0 (0)	0.0 (0)

* Values in parentheses represent % of control.

considered weeds in many part of the world. Many weeds are ecologically important and contain bioactive compounds such as allelopathic and antifungal constituents in order to survive in ecosystem (Meepagala et al., 2003). Allelopathic constituents are cause of the symptom of the non-growth or growth retardation of neighbouring plant. The phenomena have been found in the ethereal oils and alkaloid absinthium excreted from *Artemisia absinthium* (Funke, 1943), natural and artificial rain drip from *Artemisia californica* (Halligan, 1976), aqueous extract and volatile substances of *Artemisia princeps* var. *orientalis* (Yun, 1991) and aqueous extract of *Artemisia campestris* ssp. *Caudate* (Yun and Maun, 1997). And the genus *Artemisia* is rich sources of biologically active natural products (Tan et al., 1998). Approximately 30 *Artemisia* species grown in Korea have been used in traditional Korean medicine. Of them *Artemisia princeps* var. *orientalis* is widly used as medicinal herb and is known as a strong weed, it is a Korean custom to uproot the plant in old tomb area because its water-soluble allelochemicals retard turf growth.

Kil and Yun (1992) reported that aqueous extracts from mature leaf, stem, and root of *Artemisia princeps* var. *orientalis* caused significant inhibition in germination and decreased seedling elongation of receptor plants, whereas germination of some species was not inhibited by extracts of stem and root. Dry weight growth was slightly increased at lower concentrations of the extract, whereas it was proportionally inhibited at higher concentrations. Kil et al. (1992) also reported, an in vitro study, that aqueous extract of *A. princeps* var. *orientalis* caused some reduction in concentration, induction, and growth of callus, although they looked normal, whereas the explants of most receptor plants did not develop callus at higher concentration. They also found that lettuce and *Eclipta prostrata* were the most sensitive species, and even A. *princeps* var. *orientalis* was affected by its own extracts. Yun and Kil (1992) reported differential phytotoxicity of residues of *A. princeps* var. *orientalis* using various plants as test species in their field and laboratory studies. In seedling growth tests with abandoned field soils (control) and soil underneath the *Artemisia* plant, the elongation and dry weight of seedlings grown in the soil from under the *Artemisia* plants were severely inhibited, thereby suggesting that certain growth inhibitors were released from the *Artemisia* plant and the inhibitor remained in the soil.

2.2 *Cirsium sp.*

Common thistle (*Cirsium japonicum*) is a noxious perennial weed which causes serious yield losses in spring sown small grains row crops, and pastures (Hodgson, 1963, 1968). Growth and germination of wheat (*Triticum aestivum* L.) and flax (*Linum usitatissimum* L.) were inhibited by aqueous extracts of Canada thistle (*Cirsium arvense* Scop.) roots and shoots (Helgeson and Konzak, 1950). Bendall (1975) studied water and ethanol extracts and residues in soil, and concluded that an allelopathic mechanism might be involved in the exclusion of some annual thistle, pasture, and crop species from Canada thistle areas. Stachon and Zimdal (1980) in their greenhouse experiments found that Canada thistle litter reduced the growth of redroot pigweed (*Amaranthus retroflexus* L.) and green foxtail (*Setaria viridis* L.)

more than that of cucumber (*Cucumis sativus* L.) or barley (*Hordeum vulgare* L.). Chon et al. (2003a) reported that the aqueous leaf extracts of 16 Compositae plant species, including common thistle, significantly inhibited hypocotyls and root lengths of alfalfa. In their earlier study, *trans*-cinnamic acid was found as the greatest amount at ethyl acetate fraction from methanol extracts of common thistle plant.

Chon (2004a) assayed phytotoxic effects of a series of aqueous extracts from leaves, stems, roots and flowers of common thistle (*Cirsium pendulum* Fisch.) against alfalfa (*Medicago sativa*) seedlings. The results showed highest inhibition in the extracts from flowers and leaves, and followed by stems, and roots. He found also that hexane and ethylacetate fractions of common thistle reduced alfalfa root growth more than did butanol and water fractions. Incorporation into soil with the leaf residues at 100 $\mathrm{g\,kg^{-1}}$ inhibited shoot fresh weights of barnyardgrass and eclipta (*Eclipta prostrate*) by 88 and 58%, respectively, showing higher sensitivity in grass species.

2.3 Lactuca sp.

Lettuce (*Lactuca sativa*) is an annual herbaceous plant of Compositae, one of the largest and most diverse families of flowering plants. Major weeds including barnyard grass (*Echinochloa colonum*), common purslane (*Portulaca oleracea*), smooth pigweed (*Amaranthus hybridus*), shepherd's purse (*Capsella bursa-pastoris*) and common lambsquaters (*Chenopodium album*) are known to interfere with lettuce (Haar and Fennimore, 2003; Santos et al., 2003; Fennimore and Umeda, 2003).

Chon et al. (2005) showed phytotoxic effects of aqueous or methanol extracts and residues from leaves of lettuce cultivars on alfalfa seed germination. Methanol extracts from hexane fraction of lettuce plants showed the most inhibition on alfalfa root growth and followed by ethylacetate, butanol and water fractions. Incorporation with leaf residues of 100 $\mathrm{g\,kg^{-1}}$ into soil significantly inhibited shoot- and root fresh weights of barnyard grass by 79 and 88%, respectively. Also, Chon et al. (2005) reported major allelopathic substances by means of HPLC were identified as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid and chlorogenic acid. Of them *p*-coumaric acid was found as the greatest amount (8.9 mg 100 $\mathrm{g^{-1}}$) in the EtOAc fraction. Hexane and EtOAc fractions of lettuce reduced alfalfa root growth more than did BuOH and water fractions.

2.4 Xanthiun sp.

Xanthium species is one of the most competitive weeds in crop fields as well as wastelands. Inam et al. (1987) found that aqueous extracts of *Xanthium strumarium* from different plant parts reduce germination, early growth and dry weight of *Brassica compestris*, *Lactuca sativa*, and *Pennisetum americanum*. Especially, *Parthenium hysterophours* is known to be very allelopathic to wheat (Kanchan and Jayachandra, 1979), soyabean, and corn (Mersie and Singh, 1978). Their extracts and residues significantly reduced germination and shoot and root dry weight of the test plants.

Chon (2004b) reported that aqueous extracts from *X. occidentale* completely inhibited the hypocotyl and root growth of alfalfa. Early seedling growth of both alfalfa barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.), and barnyard grass (*Echinochloa crus-galli*) was significantly reduced by methanol extracts. By means of high-performance liquid chromatography, chlorogenic acid and *trans*-cinnamic acid were quantified as the highest amounts from water and ethylacetate fractions, respectively. Butanol and ethylacetate fractions of *X. occidentale* reduced alfalfa root growth more than did hexane and water fractions.

3 From Petri-Dish Bioassays to Field Trials

3.1 Bioassays in Petri-Dish

Most assessments of allelopathy, especially in early stages, involve bioassays of plant or soil extracts based on seed germination and seedling growth. A reliable bioassay that is sensitive is needed for more in-depth studies of the growth mechanisms involved and for developing initial analytical procedures to determine the chemical (s) responsible. Generally germination is less sensitive to the allelopathic chemical than is seedling growth, especially root growth (Miller, 1996). Although many laboratory bioassays have proposed to demonstrate allelopathy, concerns have been raised that most of them have little relevance in terms of explaining behavior in the field (Connell, 1990; Inderjit and Olofsdotter, 1998; Inderjit and Dakshini, 1995, 1999).

It is a complex phenomenon involving a variety of inter-relationships among plants. Virtually all plant parts such as leaves (Kumari and Kohli, 1987), roots (Horsley, 1977), pollen (Cruz-Ortega et al., 1988), trichomes (Bansal, 1990), bark (Kohli, 1990) and seeds and fruits (Fredman et al., 1982) have allelopathic potential. It is generally accepted that water extracts of top growth (especially leaves) produce more allelopathy for seedlings than those from roots and crowns of alfalfa (*Medicago sativa* L.) (Miller, 1996), and that shoot extract from the reproductive stage was more inhibitory than from the vegetative stage under laboratory conditions (Chung and Miller, 1995a; Hegde and Miller, 1992a). Chung and Miller (1995a) ranked autotoxic effects of water extracts of plant parts of alfalfa as leaf (greatest), seed, root, flower, and stem (least). Chou and Leu (1992) reported that extracts from flowers of *Delonix regia* (BOJ) RAF exhibited highest inhibition against three test plants, alfalfa, lettuce (*Lactuca sativa*), and Chinese cabbage (*Brassica chinensis*). Chon (2004a) reported that phytotoxic effects of a series of aqueous extracts from leaves, stems, roots and flowers of common thistle (*Cirsium pendulum* Fisch.) on alfalfa (*Medicago sativa*) seedlings showed highest inhibition in the extracts from flowers and leaves, and followed by stems, and roots (Fig. 1).

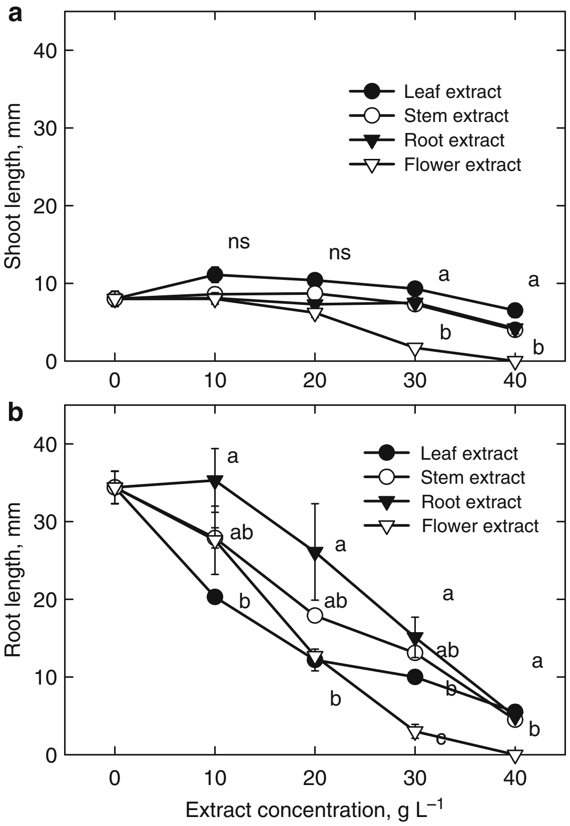

Fig. 1 Effects of *Cirsium japonicum* leaf, stem, root and flower extracts on shoot (**a**) and root lengths (**b**) of alfalfa as affected by different concentrations. Within an extract concentration, means followed by the same letter are not significantly different at $P < 0.05$. Each bar represents standard error of the mean. Adapted from Chon et al. (2003a)

3.2 Morphological Responses

Some plant genotypes are likely to escape the allelopathic chemical (s) by being hypersensitive. In this regard the root tip may actually be strongly affected by allelochemical (s) and have its growth rate nearly stopped. But if the seedling quickly enables to produce several secondary roots, the number of apices per soil volume increases at higher position in soil profile. A study demonstrated microscopically that alfalfa extract reduced both root growth and root hair density of alfalfa (Hegde and Miller, 1992b). Stimulation of lateral root growth to the detriment of the primary root also suggests disruption of hormonal balance (Dayan et al., 2000). Anatomical responses of tissue cells upon water-soluble substances or allelochemicals need to be elucidated. The morphology of seedlings grown in the presence of a phytotoxin may also yield important information. Stimulation of lateral root growth to the detriment of the primary root also suggests disruption of hormonal balance (Dayan et al., 2000). Benzoic and cinnamic acids-treated soybean plants showed fewer lateral roots and tended to grow more horizontally compared to the untreated plants. Their lateral roots were stunted and less flexible (Baziramakenga et al., 1994).

Not many microscopic approaches at ultrastructural level have been conducted on allelopathic effects of extracts or allelochemicals. Chon et al. (2002)

suggested that the root systems, especially root tips of alfalfa, were stunted and swollen by the aqueous alfalfa leaf extracts at 30 g L^{-1} and coumarin at 10^{-3} M. Duke et al. (1987) discovered artemisinin, a constituent of annual wormwood (*Artemisia annua*), marginally increased the mitotic index of lettuce root tips at 33 μM. At the ultrastructural level by means of electron microscopic study, however, chromosomes were less condensed during mitosis in artemisinin-treated than control meristematic cells. Liu and Lovett (1993) demonstrated that barley allelochemicals, hordenine and gramine affected damage of cell walls, increase in both size and number of vacuoles, autophagy, and disorganization of organelles. More recently, a study on allelopathic interference of benzoic acid against mustard (*Brassica juncea* L.) seedling growth showed irregular shaped cells arranged in a disorganized manner and disruption of cell organelles at cellular level (Kaur et al., 2005). Their result indicates that damage to the mustard root at cellular level was clearly evidenced by the changes in cell morphology and internal organization.

3.3 Residue Incorporation in Soil

Generally, residue inhibition of seedling growth was enhanced if crop residue was incorporated before planting, drastically reduced if residue remained on the surface (Cochran et al., 1980; Elliott et al., 1981). Crop residue toxicity to winter wheat seedlings was likely caused by either an allelopathic compound or N immobilization due to increased microbial populations. The allelopathic compound was either a water-soluble compound leached from residue or a compound produced during microbial decomposition of plant residue (Cochran et al., 1980; Elliott et al., 1981). Another study (Kadioglu, 2000) showed that the inhibitory effect of ground hearleaf cocklebur (*Xanthium strumarium* L.) on *Amaranthus retroflexus*, *Amaranthus sterilis*, and *Conium maculatum*. Chon and Kim (2005) reported that the effect of residue incorporation with *Xanthium occidentale* plant samples into soil on seedling growth of barnyard grass was examined in the greenhouse, and results showed that the leaf residues at 100 g kg^{-1} inhibited shoot and root dry weights of test plants by 70–90% (Fig. 2).

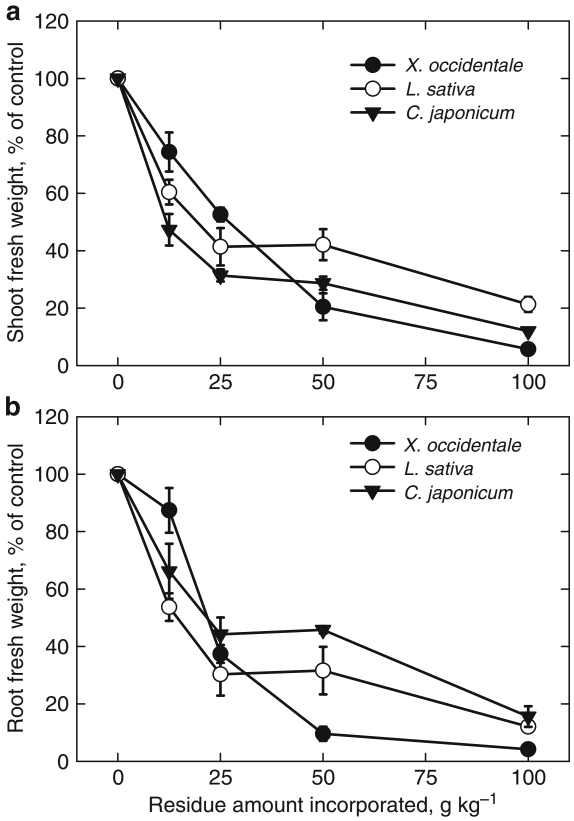

Fig. 2 Effects of incorporation with dried leaf material of *Xanthium occidentale*, *Lactuca sativa*, and *Cirsium japonicum* on shoot (**a**) and root (**b**) fresh weight of barnyard grass 15 days after seeding or treatment. Material was incorporated by mixing with soils. After incorporation, barnyardgrass was seeded and immediately subirrigated. Shoot and root fresh fresh weights were measured 15 days later. Adapted from Chon and Kim (2005)

3.4 Field Experiments

A field research described that the early stage of grassland succession in Korea would be composed of *Plantago asiatica*, *Artemisia princeps* var. *orientalis*, *Oenothera odorata*, and *Zoysia japonica* was inhibited greatly by the *Artemisia* extracts. These contrasting views could result from differences between laboratory work and field observations (Park, 1966). Yun and Kil (1992) reported that field soil collected under the *Artemisia* plants significantly reduced or somewhat promoted growth of the test plants. These results are in agreement or disagreement with following results: soil collected from some plant fields exhibited phytotoxicity by reducing growth (Al-Naib and Rice, 1971;

AlSaadawi and AlRubeaa, 1985; Inam et al., 1989) and soil from below some test plants did not inhibit germination and growth of the test species (Fadayomi and Oyebade, 1984; Goel and Sareen, 1986).

4 Discovery of Causative Allelochemicals

4.1 Compounds Involved

Most important approach on allelopathy is to successfully isolate, identify, and quantify causative allelochemicals that present in plants or soils. It is essential that potential allelopathic compounds can also relate to the levels originally in the whole extracts. Natural products identified as allelopathic agents have been classed into the following (a) toxic gases, (b) organic acids from Krebs cycle and aldehydes, (c) aromatic acids, (d) simple unsaturated lactones, (e) courmarins, (f) quinones, (g) flavonoids, (h) tannins, (i) alkaloids, and (j) terpenoids and steroids, etc. Although many of these compounds are secondary products of plant metabolism, several are degradation products that occur in the presence of microbial enzymes. Several biosynthetic pathways lead to production of the various categories of allelopathic agents. The inhibitors usually arise through either the acetate or through the shikimic acid pathway. Several types of inhibitors, which originated from amino acids, come through the acetate pathway. Most of compounds that cause allelopathy were derived from amino acids, via the shikimic acid pathway (Rice, 1984).

The source of the active agents may be living plants, litter, detritus, leachates, soil bateria and fungi, mycorhizal fungi, root exudates, the atmosphere, water, air-borne particles, or pathogenic organisms. Many organisms may be involved simultaneously in a particular interaction. Compounds isolated from plants or their leachates often do not reproduce the observed allelopathic effects without the associated factors. In many instances, these plant-derived compounds are modified by oxidation, reduction, photochemical activation (Fisher et al., 1994), detoxification, or biochemical activation by bacteria and fungi. Bioactive molecules may leave the system by being adsorved onto inorganic particles or organic matter in the soil, or leached from the system.

4.2 Identification and Quantification

Causative allelochemicals reported from Compositae plant species have not been identifieed sufficient. Identifying and quantifying the causative allelochemicals in plan and associated environments are the most important approaches for allelopathy study. Phenolic acids in the literature on allelopathy are often mentioned as putative allelochemicals and are perhaps the most commonly investigated compounds among potential allelochemicals. They are found in a wide range of soils or plants, and their phytotoxic potential against various plants has been demonstrated under controlled conditions. Phytotoxicity-based extraction and fractionation were employed to separate allelochemicals contained in each plant extracts. Chon et al. (2003a) reported that by means of high-performance liquid chromatography (HPLC) analysis, the responsible causative allelopathic substances present in *L. sativa, X. occidentale*, and *C. japonicum* were isolated from various fractions and identified as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, and chlorogenic acid.

Table 2 Quantitative determination using HPLC on concentration of some phenolic compounds present in leaves of *Xanthium occidentale* (Chon et al., 2003a)

	Fractions				
Compound	Hexane	EtOAc	BuOH	Water	Total
			- mg 100 g^{-1} -		
Coumarin	0.14	2.07	2.02	0.19	4.41
trans-cinnamic acid	8.39	20.16	4.20	0.2	32.95
p-coumaric acid	nd	0.24	nd	nd	nd
Chlorogenic acid	2.24	0.24	3.59	33.33	39.40
Total	10.77	22.67	9.81	33.54	76.76

[a] nd: none detected.

4.3 Fractionation and Bioassay of Each Solvent Fractions

Methanol extracts from ground plant samples are used for the following bioassay and fractionation. Generally, for instance, crude methanol extracts are diluted with distilled water and hexane to collect hexane fraction using a separating funnel. After hexane collection, the distilled water fractions are added with ethylacetate to obtain ethylacetate fraction in the same way. The same procedure is used in preparing other solevent fractions. The fractions are taken to dryness on a rotary evaporator at 40–50°C, and transferred into vacuum freeze dryer to obtain dry matters and used for quantification and bioassay. The dried samples concentrated from fractions are again dissolved in MeOH to use for bioassay (Chon et al., 2003a). Each of these fraction solutions are pippeted in a 9-cm plastic Petri dish lined with one Whatman No. 1 filter paper and evaporated to dryness for 24 h at 24°C. For the control, 4 mL of methanol is applied to Petri dishes. After evaporation, distilled water is added onto the filter paper and then seeds of test plant are placed on the paper and grown for 6 days. Chon et al. (2003a) reported that these major phenolic compounds present in Compositae species were total phenol compounds of all fractions in *C. japonicum*, *L. sativa*, and *X. occidentale* by 60.3, 18.5, and 84.4 mg 100 g^{-1}, respectively. They reported also, through the bioassay procedure, *X. occidentale* which had the highest total concentration, showed the most inhibitory effect on test plants in Compositae plant species (Table 2).

4.4 Phytotoxicity of Major Causative Allelopathic Compounds

Chon et al. (2003a) reported that among 10 phenolic compounds assayed for their phytotoxicity on root growth of alfalfa, coumarin, *trans*-cinnamic acid and *o*-coumaric acid were most inhibitory. Especially, coumarin at 10^{-3} M significantly inhibited root growth of alfalfa. Methanol extracts from BuOH, EtOAc, hexane, and water fractions were also assayed to confirm their phytotoxic effects. The results showed that methanol extracts of *X. occidentale* were most inhibitory on root growth of alfalfa, and that methanol extracts from BuOH and EtOAc fractions of *X. occidentale* reduced alfalfa root growth more than did those from hexane and water fractions. Methanol extracts from hexane and EtOAc fractions of *L. sativa* and *C. japonicum* reduced alfalfa root growth more than did those of BuOH and water fractions. Especially, methanol extracts from hexane and EtOAc

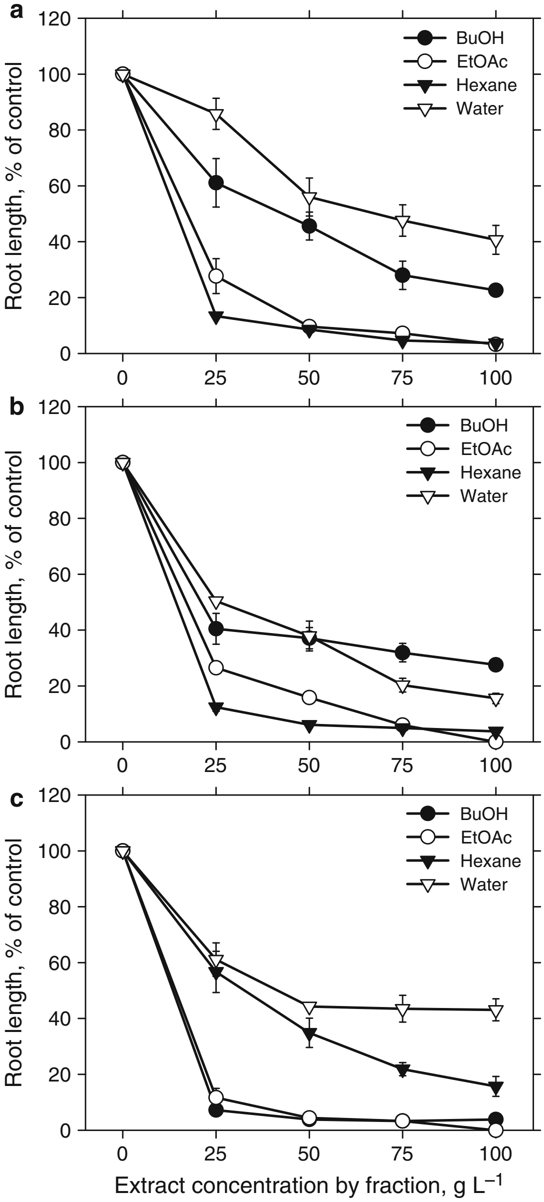

Fig. 3 Effects of various fractions from methanol extracts of *Cirsium japonicum* (**a**), *Lactuca sativa* (**b**), and *Xanthium occidentale* (**c**) on alfalfa root length 6 days after seeding. Adapted from Chon et al. (2002)

fractions at 50 g L^{-1} reduced root growth by each 85%, while treatment at same concentration of BuOH and water fractions reduced root growth by 40 and 15%, respectively (Fig. 3).

Acknowledgements This research was conducted with support from a part of the 2005 MAF-ARPC research fund (105088-33-1-HD110). Appreciation is expressed to Dr. Young-Min Kim at Biotechnology Industrialization Center, Dongshin University, Naju, Korea, for his technical assistance.

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Part VI
Soil Health

Assessing the Productivity Function of Soils

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Contents

Abstract The development and survival or disappearance of civilizations has been based on the performance of soils to provide food, fibre, and further essential goods for humans. Amongst soil functions, the capacity to produce plant biomass (productivity function) remains essential. This function is closely associated with the main global issues of the 21st century like food security, demands of energy and water, carbon balance and climate change. A standardised methodology for assessing the productivity function of the global soil resource consistently over different spatial scales will be demanded by a growing international community of land users and stakeholders for achieving high soil productivity in the context of sustainable multifunctional use of soils. We analysed available methods for assessing the soil productivity function. The aim was to find potentials, deficiencies and gaps in knowledge of current approaches towards a global reference framework. Our main findings were (i) that the soil moisture and thermal regime, which are climate-influenced, are the main constraints to the soil productivity potential on a global scale, and (ii) that most taxonomic soil classification systems including the World Reference Basis for Soil Resources provide little information on soil functionality in particular the productivity function. We found (iii) a multitude of approaches developed at the national and local scale in the last century for assessing mainly specific aspects of potential soil and land productivity. Their soil data inputs differ, evaluation ratings are not transferable and thus not applicable in international and global studies. At an international level or global scale, methods like agro-ecological zoning or ecosystem and crop modelling provide assessments of land

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_33,

from Mueller et al., Agron. Sustain. Dev. 30 (2010) 601–614. DOI: 10.1051/agro:2009057

productivity but contain little soil information. Those methods are not intended for field scale application to detect main soil constraints and thereby to derive soil management and conservation recommendations in situ. We found also that (iv) soil structure is a crucial criterion of agricultural soil quality and methods of visual soil assessment like the Peerlkamp scheme, the French method "Le profil cultural" and the New Zealand Visual Soil Assessment are powerful tools for recognising dynamic agricultural soil quality and controlling soil management processes at field scale. We concluded that these approaches have potential to be integrated into an internationally applicable assessment framework of the soil's productivity function, working from field scale to the global level. This framework needs to serve as a reference base for ranking soil productivity potentials on a global scale and as an operational tool for controlling further soil degradation and desertification. Methods like the multi-indicator-based Muencheberg Soil Quality Rating meet most criteria of such a framework. This method has potential to act as a global overall assessment method of the soil productivity function for cropping land and pastoral grassland but needs further evolution by testing and amending its indicator thresholds.

Keywords Soil functions • Soil productivity • Soil quality • Soil structure • Soil classification • Sustainable agriculture • Land rating

1 Introduction - The Demand for Information on the Productivity Function of Soils

Soils cover most lands of the earth, but regarding their service for humans they are a limited and largely non-renewable resource (Blum, 2006). On the globe about 3.2 billion hectares are used as arable land, which is about a quarter of the total land area (Scherr, 1999; Davis and Masten, 2003). Total agricultural land covers about 40–50% of the global land area (Smith et al., 2007).

The development and survival of civilizations has been based on the performance of soils on this land to provide food and further essential goods for humans (Hillel, 2009). Global issues of the 21st century like food security, demands of energy and water, climate change and biodiversity are associated with the sustainable use of soils (Lal, 2008, 2009; Jones et al., 2009; Lichtfouse et al., 2009). Feeding about 10 billion people is one of the greatest challenges of our century. Borlaug (2007) stated: "The battle to alleviate poverty and improve human health and productivity will require dynamic agricultural development". There are serious concerns that increases of global cereal yield trends are not fast enough to meet expected demands (Cassman et al., 2003). However, agricultural development cannot be intensified regardless of the bearing capacity of soils, ecosystems and socio-economical environment. It has to be imbedded within balanced strategies to develop multi-functional landscapes on our planet (Wiggering et al., 2006; Helming et al., 2008). Handling of soils by societies must be in a sustainable way in order to maintain the function of all global ecosystems (Rao and Rogers, 2006; Ceotto, 2008, Hillel, 2009). This includes the use of soils by agriculture for high productivity (Lal, 2009; Walter and Stützel, 2009). Global carbon, water and nutrient cycles are also affected by agriculture (Bondeau et al., 2007).

Soils have to provide several ecological and social functions (Blum, 1993; Tóth G et al., 2007; Lal, 2008; Jones et al., 2009). Based on a definition of Blum (1993), one of the six key soil functions is "food and other biomass production". The soil protection strategy of the European Commission (EC, 2006; Tóth G et al., 2007) addresses "biomass production" as a main soil function which must be maintained sustainably. We call this the "productivity function". The productivity function is related to the most common definition of soil quality as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). Based on this definition, the objective comes close to the assessment of "agricultural soil quality".

Although the productivity function of soils is of crucial importance, it is sometimes ill-defined or its description may be very different. In the German soil protection Act (BBodSchG, 1998) the productivity function is about "utility for agriculture and forestry". Amongst those utility functions (agriculture, resources, settlement and traffic), soils used by agriculture and forestry have a unique position.

Firstly, agricultural soils have to be used sustainably to maintain their productivity potential long-term. Secondly, natural soil functions (habitat, nutrient cycling, biofiltering) are not only the domain of soils in natural protected areas. Agricultural soils have to fulfil their natural functions too, e.g. provide or support ecosystem services (Foley et al., 2005). Assessing the productivity function is not restricted to specific land use concepts with regard to management intensity. It embraces the capacity of soils for low- input and organic farming approaches. Also, soils in more natural ecosystems may provide some productivity function. This chapter focuses on the productivity function of soil on agricultural land. We shall analyse available methods and tools for assessing the state of soils concerning their ability to provide the productivity function. We consider which evaluation tools are available to quantify soil productivity and which tools are needed to meet further demands under changing climate and soil management. We start from the hypothesis that a growing community of land users and stakeholders has to achieve a high productivity without any significant detrimental long-term impact on soils and the environment. This requires an increasing awareness of a demand to assess the productivity of their soils using internationally standardised frameworks and simple diagnostic tools.

Our focus shall be on answering the following questions:

- Which properties of soils most affect their productivity?
- Which information on soil productivity potentials do existing soil classification systems provide?
- What methods of assessing the productivity function of soils are available?
- How useful are these methods in assessing different aspects of agricultural soil quality?

Conclusions are made for the development of a framework and evaluation tools of agricultural soil quality consistently over different scales as a basis for monitoring and sustainable management of soils.

2 Soils and Their Constraints to Plant Growth

Soils are components of terrestrial ecosystems. The productivity of these systems is controlled by natural factors and by human activity. Most important external natural factors are solar radiation, influencing temperature and evapotranspiration, and/or precipitation (Lieth, 1975). Soils may provide for plant growth if climate, as the main soil forming factor, is in an appropriate range (Murray et al., 1983, Lavalle et al., 2009). Thus, on a global scale, natural constraints to soil productivity can be classified into three major groups. The first group includes the thermal and moisture regimes of soils. Plants require appropriate soil temperatures and moisture for their growth (Murray et al., 1983; Lavalle et al., 2009). For most soils, thermal and moisture regimes are directly dependent on climatic conditions. They define the frame for limitations like drought, wetness, or a too short vegetation period, limiting the productivity (Fischer et al., 2002).

Worldwide, soil moisture is the main limiting factor in most agricultural systems (Hillel and Rosenzweig, 2002; Debaeke and Aboudrare, 2004; Ciais et al., 2005; Verhulst et al., 2009; Farooq et al., 2009). Drylands cover more than 50% of the global land surface (Asner and Heidebrecht, 2005). Available soil water is a prerequisite for plant growth. In all climates suitable for agriculture, the water storage capacity of soils is a crucial property for soil functionality including the productivity function (AG Boden, 2005; Shaxson, 2006; Jones et al., 2009). It is closely correlated with crop yields (Harrach, 1982; Wong and Asseng, 2006).

The second group of restrictions includes other internal soil deficiencies mainly due to an improper substratum limiting rooting and nutrition of plants. These include shallow soils, stoniness, hard pans, anaerobic horizons, or soils with adverse chemistry such as salinity, sodicity, acidity, nutrient depletion or contamination which may cause severe restrictions to plant growth or the utilisation of biomass (Murray et al., 1983; Louwagie et al., 2009).

The third group includes topography, sometimes considered as an external soil property, preventing soil erosion and providing accessibility by humans and machinery (Fischer et al., 2002; Duran Zuazo, 2008).

There seems to be an interaction between natural constraints to soil productivity and societal factors. Historically, many countries with poor soils tended to be poorly developed. This has led to accelerated soil degradation. Currently, in developing countries, about two thirds of soils have severe constraints to agriculture. Their low fertility (38%), sandy or stony soils (23%), poor soil drainage (20%) and steep slopes (10%) are the main limits to productivity (Scherr, 1999).

3 Information on Taxonomic Soil Classification Systems for Soil Productivity Potentials

Soil classification systems are based on a combination of different criteria. Attributes used for classification may reflect both pedogenesis and pedofunction (Schroeder and Lamp, 1976; Beinroth and Stahr, 2005). Whilst morphological and functional criteria dominated soil classification until the 19th century, pedogenic criteria prevail at higher levels in national soil classification systems since the 20th century (Ahrens et al., 2002; Beinroth and Stahr, 2005). Functional information like the type of substrate is also part of most current soil classifications. In some cases pedogenic and functional criteria are combined, and genetic soil types provide information about soil productivity potentials. For example, Chernozems, which have developed mainly from loessial material and have a mollic epipedon, rich in humus, have a high crop yield potential, whilst Leptosols are shallow soils of low productivity. Podzols are leached sandy soils lacking nutrients and water storage capacity. These examples show that if the soil type or reference soil group is associated with typical substrate and climate conditions, some functional properties may be determinable.

Apart from these extremes, functional information derivable from higher level soil classifications is relatively low. Some soil types or reference soil groups such as Cambisols, Fluvisols or Regosols may have developed from different soil substrates in different climatic environments. In those cases, more relevant information about possible soil productivity at a local or regional scale is provided if the classification includes further soil attributes like texture, organic matter, degree of trophy and pH. Soil texture is correlated with other important functional attributes like water and nutrient storage capacity and thus has become a dominant criterion of all existing functional classification systems since soil began to be managed (Storie, 1933; Rothkegel, 1950; Feller et al., 2003, Feller et al., 2003; Beinroth and Stahr, 2005; Begon et al., 2006).

As the USDA soil classification (Keys to Soil Taxonomy, 2006) includes climate information in terms of soil moisture and temperature regime classes, correlations of soils with their productivity at a hierarchy level of great groups (3rd level) are relatively high. In contrast, the FAO soil map of the world and the latest reference base for soil resources (WRB, 2006) lack information about temperature and moisture regimes and thus information on soil productivity potentials. For a rough assessment of soil productivity potentials in Africa, Eswaran et al. (1997), had to translate the FAO soil map of Africa into the USDA soil taxonomy by supplementing climate information.

At the lowest levels of the soil classification hierarchy, functional information on particular soils is greatest. Soils classified at series level in USDA Soil Taxonomy, in the UK soil classification, or local soil types on forest sites in some federal states of Germany, contain detailed information on soil morphological and functional properties, which can be linked with soil productivity data (Mausel et al., 1975; Kopp and Schwanecke, 2003). However, the specific data and correlations cannot be transferred to other regions.

Soil taxonomic classifications sometimes include information on soil structure, which often reflects anthropogenic impacts within human timescales on soil. This information provision can be relatively high with some soils like Histosols in the AG Boden (2005), and Keys to Soil Taxonomy (2006), but it is low with most mineral soils.

4 Soil Structure as a Criterion of Agricultural Soil Quality

Soil structure is a complex category and a key to soil biological, chemical and physical processes (Jackson et al., 2003; Karlen, 2004; Bronick and Lal 2005; Kay et al., 2006; Roger-Estrade et al., 2009). The spatial arrangement of aggregates and porosity is a main aspect of soil structure. Structure is related to soil function, e.g. to the productivity function or to water and solute transport. Unfavourable structure can result in lower crop yields and greater leaching losses (Kavdir and Smucker, 2005). Current structure features and function result from soil substratum, genetic and management factors. Soil structure is vulnerable to change by compaction and erosion and its preservation is key to sustaining soil function. Crop rotation and tillage strategies should aim to produce optimum soil structure for high and sustainable crop yields (Hulugalle et al., 2007). A good soil structure for plant growth may play a particularly important role in organic farming while poor soil structure cannot be compensated by an extra input of agrochemicals in those systems (Munkholm et al., 2003).

Visible soil structure revealed by digging up the soil shows the abundance and arrangement of soil aggregates and roots which may indicate properties of soils that are dependent on soil management (Shepherd, 2000; McKenzie, 2001; Lin et al., 2005; Mueller et al., 2009). It reflects important aspects of the dynamic indicators of soil quality, indicators that can be categorised and used to monitor and control the status of soil. Farmers and gardeners do this in an individual, experienced-based visual-tactile manner. Visual-tactile recognizable soil features like colour, texture, moisture conditions, earthworm casts may serve to evaluate and classify the quality of soil (Shaxson, 2006).

As indigenous people have done before, soil science and soil advisory services utilise the same common field diagnostic criteria within defined frameworks and check their validity over larger scales. Over the past decades, the interest in soil structure evaluation as a diagnostic tool for assessments of dynamic, e.g management-induced, soil quality has been recognised and has evolved (Shepherd, 2000; McKenzie, 2001; Lin et al., 2005; Shaxson, 2006). Methods of visual soil structure examination enable semi- quantitative information for use in extension and monitoring (Shepherd, 2000; McKenzie, 2001) or even modeling (Roger-Estrade et al., 2004, 2009). One of their advantages is a quick, reliable assessment of good, acceptable or poor states of soil structure. Soil structural features meet the farmer‘s perception on soil quality (Shepherd, 2000; Batey and Mc Kenzie 2006) and are correlated with measured data of physical soil quality (Lin et al., 2005) and crop yield (Mueller et al., 2009). However, clearly defined rules and scoring methods are necessary to minimise subjective errors.

Several methods have been developed over the past five decades. One of the oldest but most accepted methods is that of Peerlkamp (1967). The traditional French method “Le profil cultural” (Roger-Estrade et al., 2004) belongs to a group of more sophisticated methods providing detailed information on the total soil profile. A quantitative comparison of some methods and their correlations with measured physical parameters after standardizing data revealed that most methods provided similar results (Mueller et al., 2009). Types and sizes of aggregates and abundance of biological macropores were the most reliable criteria as related to measurement data and crop yields. Differences in soil management could be recognised by visual structure criteria (Mueller et al., 2009). Unfavourable visual structure was associated with increased dry bulk density, higher soil strength and lower infiltration rate but correlations were site-specific. Effects of compaction may be detected by visual examination of the soil (Batey and Mc Kenzie 2006).

Visual methods based on, or supplemented by illustrations, have clear advantages for the reliable assignment of a rating score based on visual diagnostic criteria. The latest development of the Peerlkamp method provided by Ball et al. (2007) is well illustrated (Fig. 1). Also, the New Zealand Visual Soil Assessment (VSA, Shepherd, 2000, 2009) as an illustrated multi-criteria method, enables reliable assessments of the soil structure status. These are feasible tools for structure monitoring and management recommendations. However, they may explain only part of crop yield variability, as the influence of inherent soil properties and climate on crop yield is dominant, particularly over larger regions.

5 Methods of Assessing the Overall Productivity Function of Soil

5.1 *Soil and Land Evaluation in a Historical Context*

In a global context, the utilisation of the soil productivity function in agriculture requires not only soils but also an appropriate climate and human activity. Methods for the evaluation of the potential for the productivity of soil have recently been called “land” evaluation methods. “Land evaluation” has been defined as “the process of assessment of land performance when used for specific purposes (FAO, 1976). Historically, land evaluation has developed from soil science. As soil is the most important component of the land resource,

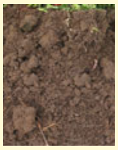
Structure quality	Ease of break up (moist soil)	Size and appearance of aggregates	Visible porosity	Roots	Appearance after break-up: various soils	Appearance after break-up: same soil different tillage	Distinguishing feature
Sq1 Friable (tends to fall off the spade)	Aggregates readily crumble with fingers	Mostly < 6 mm after crumbling	Highly porous	Roots throughout the soil			 Fine aggregates
Sq2 Intact (most is retained on the spade)	Aggregates easy to break with one hand	A mixture of porous, rounded aggregates from 2mm - 7 cm. No clods present	Most aggregates are porous	Roots throughout the soil			 High aggregate porosity
Sq3 Firm	Most aggregates break with one hand	A mixture of porous aggregates from 2mm -10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present	Macropores and cracks present. Some porosity within aggregates shown as pores or roots.	Most roots are around aggregates			 Low aggregate porosity
Sq4 Compact	Requires considerable effort to break aggregates with one hand	Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm	Few macropores and cracks	All roots are clustered in macropores and around aggregates			 Distinct macropores
Sq5 Very compact	Difficult	Mostly large > 10 cm, very few < 7 cm, angular and non-porous	Very low; macropores may be present; may contain anaerobic zones	Few, if any, restricted to cracks			 Grey-blue colour

Fig. 1 Revised Peerlkamp scale as an example of soil structure evaluation (Ball et al., 2007). The evaluation focuses on aggregates, porosity and roots. Photographs enable a reliable allocation of scores to real visible features of the topsoil. Intermediate scores and layers of differing scores are possible

soil evaluation is crucial for land evaluation (Rossiter, 1996). In many cases, there is no clear differentiation between soil and land evaluation (Van Diepen et al. 1991; Van de Steeg, 2003). Climate as a main precondition for the production of plant biomass varies over larger spatial scales than soil. Approaches to evaluate the productivity potential of soils from a more regional perspective in similar climates (fields, agricultural regions, smaller countries) tend to prefer the term "soil" for their object of assessment and rating. Approaches coming from a more global perspective (globe, continents, larger countries) tend to emphasise the role of climate and humans in biomass production and favour the term "land". The latter became dominant over the past 40 years, whilst evaluations of the productivity potential of "soil" have a long history, beginning with farming and animal husbandry. Ahrens et al. (2002) stated "…pedology and soil science in general have their rudimentary beginnings in attempts to group or classify soils on the basis of productivity. Early agrarian civilizations must have had some way to communicate differences and similarities among soils." At the beginning of the 19th century the German agronomist A. D. Thaer created a 100 point rating system for the productivity potential of soils based on texture, lime and humus content (Feller et al., 2003). It is one example of a predecessor for some of our current evaluation schemes of agricultural soil quality (Gavrilyuk, 1974; Feller et al., 2003).

5.2 Methods of Soil and Land Rating

5.2.1 Traditional National Soil Ratings

At national level, specific methods for the evaluation and classification of the productivity potential of soils and land have been developed. In Europe they have existed for about 60–100 years. In many countries they are defined by acts of government, have been done by soil surveys and have a high coverage in terms of mapped areas. Examples of those well known soil and land productivity rating systems at national levels are the Storie Index Rating (Storie, 1933), the German and Austrian Soil Rating (German term "Bodenschaetzung"), (Rothkegel, 1950; Pehamberger, 1992; AG Boden, 2005) and the system of soil rating of the former Soviet Union (Gavrilyuk, 1974). These methods try to cover the overall agricultural land with 100% coverage in some countries and are still applied for different purposes, ranging from land taxation to soil protection planning (Hartmann et al., 1999; Preetz, 2003; Rust, 2006). Ratings of these systems have a 100 point scheme in many cases. Data are ordinally scaled. Some methods have been updated and adapted to altered conditions. A main reason was to provide better correlations with current crop yields. The Austrian Soil Rating was amended by climate factors (Bodenaufnahmesysteme in Österreich 2001), whilst other systems like the German Soil Rating have remained unchanged for about 80 years.

5.2.2 More Recent Land Evaluation Systems at National Levels

Over the past 20 years, specific soil and land evaluation systems have been developed or are under construction. Examples of these systems are the US LESA system (Pease and Coughlin, 1996) and the Canadian Land Suitability Rating System for Agricultural Crops (LSRS, Agronomic Interpretations Working Group, 1995). The LESA system consists of a soil evaluation component (Storie Rating) and other factors that contribute to the suitability of land for agriculture, like location, surrounding use and infrastructure. The LSRS system is mainly based on soil attributes and climate factors (Agronomic Interpretations Working Group, 1995). Other countries with substantial agricultural production and fast growing demands like China and Brazil intend to implement quantitative evaluation systems of soil and land productivity (Peng et al., 2002; Bacic et al., 2003; Van de Steeg, 2003; Zhang et al., 2004). Also in Russia there are efforts to establish contemporary soil and land information and evaluation systems (Karmanov et al., 2002; Yakovlev et al., 2006). In the Ukraine, Medvedev et al. (2002) developed an evaluation system of the suitability of land for growing cereals based on soil information and climate data. In Hungary, a modern land evaluation system is being established, containing on-line soil evaluation, which is based on the real-time calculation of D-e-Meter soil fertility index using GIS to produce soil maps at a scale of 1:10 000 (Tóth T. et al., 2007).

All these soil and land evaluation systems are specific in approach, data and scale and their outputs are not or only rarely comparable. Approaches that have been developed for larger countries cover a broader variability of soils and climate and seem to have a better potential for evaluation of agricultural soil quality in trans-national studies.

5.2.3 Soil Capability and Suitability Classifications

Besides productivity ratings, in many countries, classifications of agricultural land limitations (steep lands, dry lands, stony lands), or final allocations to categories like "prime farmland" have been mapped. Examples of those national soil and land capability classifications are the US capability classification (Klingebiel and Montgomery, 1961; Helms, 1992), the UK system developed by the Macaulay Land Use Research Institute (Bibby et al., 1991), the New Zealand land use capability system (Lynn et al., 2009) and the soil fertility classes for agriculture in Australia (Hall, 2008).

Those capability classes are nominal, categorical data, useful for land use planning but not for more detailed productivity assessments within these categories. Data of modern national or federal state soil and land information systems provide tailored medium scale capability classifications.

Soil suitability classifications express soil productivity potentials in terms of the possibility of growing specific crops. In the nineteenth century in German states, soil suitability classification systems using classes ranging from "Prime wheat soil" to "Rye soil" or "Oats soil" were common, and were based on work of Thaer and others (Meyers Lexikon, 1925). As requirements of plants regarding the functional status of soil may differ, all recent soil productivity relevant classifications must have a certain stratification or orientation on crops or groups of crops. Cereals are a basic source of human food supply and while they reflect differences in agricultural soil quality, some systems (Rothkegel, 1950; Agronomic Interpretations Working Group, 1995; Mueller et al., 2007) refer to cereals or cereal-dominated rotations. In the UK, soil suitability classifications have been developed for specific purposes such as direct drilling or reduced tillage. Such systems emphasise the limitations of soil structure and drainage status (Cannell et al., 1978). The presence of climatic data within land use capability classification systems means that such systems can accommodate climate parameters projected into the future. Thus climate change scenarios can be used to identify future changes in land capability (Brown et al., 2008).

5.2.4 Global and Large Regional Soil and Land Evaluations and Classifications

The concept of agro-ecological zoning (AEZ) was developed by the International Institute for Applied Systems Analysis (IIASA) and the FAO (Fischer and Sun, 2001). This sophisticated methodology and model provide a framework for the characterization of climate, soil, and terrain conditions relevant to agricultural production. GIS-based suitability classes for estimating specific crops and their yields over the globe have been calculated and mapped from the sub-national to the global level (Fischer et al., 2002). The system processes soil information, including the FAO/UNESCO Digital Soil Map of the World, with climate information playing the most important role.

The Fertility Capability Classification (FCC Buol et al., 1975) is based on soil survey data and aims to make soil management recommendations and crop yield interpretations. It focuses on those properties and data of soils, topsoils in particular, that are important to fertility management (Sanchez et al., 1982). The system has been mainly applied to the tropics (Sanchez et al., 2003) and updated to a global soil functional capacity classification, providing overviews on single soil constraints to productivity like waterlogging, erosion risk, salinity and others. The basis of both the AEZ methodology and the FCC system are low resolution maps and a limited set of soil parameters and data.

Computer aided land evaluation and classification systems provide capability assessments. MicroLEIS (De la Rosa, 2005) is a system of agro-ecological land evaluation and interpretation of land resources and agricultural management. It has been extended to a decision support system, providing a multifunctional evaluation of soil quality using soil survey input data (De la Rosa et al., 2009).

Crop productivity estimators (Tang et al., 1992) can also be used as research tools and in planning studies. They combine both quantitative and qualitative data to estimate attainable crop yield for different soil units (Verdoodt and van Ranst, 2006). Examples of productivity models with focus on soil erosion are the Productivity Index (PI) model (Pierce et al., 1983), its modifications (Mulengera and Payton, 1999; Duan et al., 2009) and the Erosion Productivity Impact Calculator, EPIC (Williams et al., 1983; Flach, 1986).

The Muencheberg Soil Quality Rating (M-SQR, Mueller et al., 2007) has been developed as a potential international reference base for a functional assessment and classification of soils (Fig. 2). It focuses on cropland and grassland and is based on productivity-relevant indicator ratings which provide a functional coding of soils. Two types of indicator are identified. The first are basic and relate mainly to soil textural and structural properties relevant to plant growth. The second are hazard, relating to severe restrictions of soil function. The sum of weighted basic indicator ratings and multipliers derived from ratings of the most severe (active) hazard indicator yield an overall soil quality rating index. Indicator ratings are based on a field manual and utilize soil survey classifications (AG Boden, 2005; FAO, 2006), soil structure diagnosis tools, and local or regional climate data.

5.2.5 Models Predicting Biomass

There are a large and fast growing number of crop growth and ecosystem models that estimate the local productivity for specific crops, soils and weather data. Models are specific in purpose, vary in their spatial and local scale of resolution, in their focus on particular plants or land use systems, in their proportion and attributes of soil information data and other criteria. These crop growth models can be utilised for assessing the soil productivity for regions where yield data bases exist and the models were parameterised and validated.

On a global scale, modelling climate change relevant issues like possible shortfalls in food production (Tan and Shibasaki, 2003), drought risk

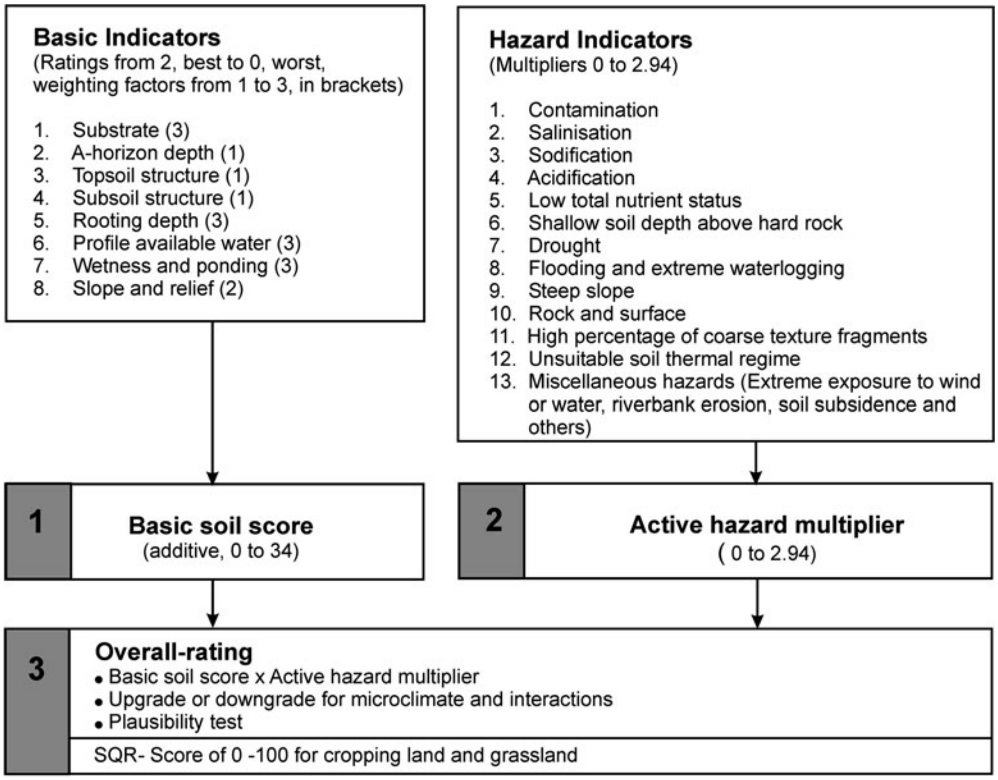


Fig. 2 Indicator system of the Muencheberg Soil Quality Rating (Mueller et al., 2007). Indicator ratings of soil states are based on rating tables given in a field manual which also contains, where relevant, hazard indicators and their thresholds. Best soils for cropping and grazing do not have values of hazard indicators which exceed the thresholds

(Alcamo et al., 2007), carbon balance (Bondeau et al., 2007) or GHG emissions (Stehfest et al., 2007) requires reliable calculations of the terrestrial biomass, crop growth and yield. Terrestrial biogeochemical models like the Global Assessment of Security (GLASS) model (Alcamo et al., 2007) containing the Global Agro-Ecological Zones methodology of Fischer et al. (2002) may provide this. Models of this group are valid on a global scale, but the spatial resolution is relatively low. They are sophisticated research tools, not designed for local scale calculations or even management decisions in agriculture.

On a daily temporal basis and local scale working crop production and ecosystem models like DAISY (Hansen et al., 1990), the CERES model family (Ritchie and Godwin, 1993; Xiong et al., 2008), WOFOST (Supit et al., 1994; Hijmans et al., 1994; Reidsma et al., 2009), CANDY (Franko et al., 1995), AGROTOOL (Poluektov et al., 2002), SIMWASER (Stenitzer and Murer, 2003), THESEUS (Wegehenkel et al., 2004), the AGROSIM model family (Mirschel and Wenkel, 2007), DAYCENT (Del Grosso et al., 2005), HERMES (Kersebaum, 2007, 2008) and many others provide productivity estimates of sites under varying conditions of weather, soil moisture or even soil management status.

Models of this group have in common that they are sophisticated and specific from methodology and design to their purpose and site situation. Their validation requires comprehensive knowledge and data (Bellocchi et al., 2009). They run well in the environment they are created for, but their transferability to other locations, scales or purposes is limited. Their data input demand, effort for soil data adaptation to other environments, and their calculation time is currently relatively high as compared with straightforward soil and land rating approaches of Section 5.2.4. However, because of their sophisticated process-based background and further advances in technology, biomass prediction models have great potentials to serve as reliable and fast decision tools.

Their flexibility in handling will remain limited in comparison with simple soil and land rating approaches.

5.2.6 Direct Recordings of Biomass and Crop Yield Data

Crop yield is a part of the net primary production (NPP) in managed ecosystems. Yield and NPP are often satellite driven, recorded and modelled (Smit et al., 2008; Prieto-Blanco et al., 2009; Kurtz et al., 2009). Also, permanent recording of spatial crop yield data as done in precision farming (Ritter et al., 2008; Schellberg et al., 2008; Lukas et al., 2009) may produce databases which have the potential to predict the productivity of land by statistical procedures of spatio-temporal auto-regressive forecasting, state-space approaches (Wendroth et al., 2003) or combinations of models and data (Reuter et al., 2005; Schellberg et al., 2008). The latter approaches developed for precision farming may provide excellent GIS-based modelling or even forecasting of land productivity in the field and at a regional scale but algorithms are rarely transferrable to other regions. Over larger regions and at a range of scales, the availability of soil survey information has to be taken into account. The combination of soil information systems with recorded crop yield data allows an identification of crop-yield relevant soil properties.

All these approaches represent major areas of soil scientific progress over the past 40 years (Mermut and Eswaran, 2001) but include two common risks of data gathering. First, the speed in developing algorithms and models often cannot keep pace with the rate of increase of available data. A second implication may be the loss of "ground adhesion", e.g. the difficulty of incorporating large amounts of data and sophisticated models into participatory approaches of decision support and in-situ decision procedures. Soil quality assessments for sustainable land use require straightforward tools, reliable but easy to implement into more complex decision models. Approaches based on simple soil functional classifications which are cross-validated with satellite and aerial data show great versatility for modelling policy scenarios (Baisden, 2006).

5.3 Comparison of Methods of Soil Evaluation Relevant to Soil Productivity

The comparability of soil productivity-related methods for assessing overall soil quality has been evaluated by different criteria including scale of validity, field method capability, reliability, relation to soil and climate data, plant suitability and others. Table 1 shows a list of criteria applied for the evaluation of the methods. For reasons of overview and readability of the table, only the rating values of a few distinct methods are provided. Values demonstrate that all existing methods have their merits and weakness regarding specific criteria. Figure 3 is an arbitrary similarity–dissimilarity plot by neighbourhood for evaluating systems of soil productivity potentials using a statistical procedure of multi-dimensional scaling (Procedure MDS, SPSS Inc., 1993). This plot is a computed map based on extending Table 1 by including more available methods and weightings of some criteria like performance over scales and correlations with crop yields. Wide separations indicate dissimilarities of methods. This procedure shows clear separation between traditional soil ratings (Storie Index Rating, German Soil Rating and dynamic visual assessments of soil quality (VSA)). The rating system of the former Soviet Union (Gavrilyuk, 1974) is similar to the Storie Index Rating. Crop models and the AEZ methodology are similar both in purpose and in results. They are located far from the centre as these procedures are not field methods of soil assessment and are mainly based on climate information.

Soil data sets (examples: minimum data set of Wienhold et al. (2004), or Cornell soil health test (Schindelbeck et al., 2008), also occupy isolated positions as, although they contain detailed soil information, they do not contain climate information and are based on laboratory analyses.

The soil management assessment framework of Andrews et al. (2004) would also be located in their vicinity. The Canadian Land Suitability Rating System (LSRS), and the Muencheberg Soil Quality Rating, (M-SQR) which include more crop yield relevant parameters (climate, soil structure) are in-between and closer to the centre. While rating procedures are

Table 1 Evaluation criteria and scheme of some existing methods for assessing overall agricultural soil quality (evaluation numbers 0 = none/false/worse; 1 = low/few/slow; 2 = medium; 3 = high/many/much/fast/good; 3 is always the best rating)

Criterion ↓	Storie index (1)	German BS (2)	AEZ (3)	VSA (4)	M-SQR (5)
Purpose of method					
Overall soil rating	3	3	0–1	0	3
Capability rating potential	3	0	1–2	0–1	3
Crop suitability rating	0	0	3	0–1	0–1
Tool for soil monitoring	0	0	1	3	2–3
Tool for soil management/extension	0	0	0	3	2–3
Tool for land use planning	2–3	2	3	1	3
Performance in spatial scales					
Field to regional level	3	3	0	3	3
Large regional to nation level	3	3	3	2–3	3
Trans-National	2	1	3	1–2	3
Indicator criteria					
Number of inherent SQ(6) indicators (a)	2	1	2	0	3
Number of dynamic SQ indicators	0	0	0	2	1
Climate inclusion	0	0	3	0	2
Interactions between indicators considered	0	0–1	2–3	0	0–1
Potential for assessing soil functions other than productivity	1–2	1	1	1	2–3
Further key criteria					
Simplicity in the field	3	3	0	2	2
Applicable without soil test kits	2–3	3	3	3	2–3
Speed of field rating (b)	2–3	3	0	3	2–3
Changes with soil depth included?	2	2	1	1	3
Correlation of scores with crop yields					
Field to regional level	2	2	0–1	1–2	2
Large regional to nation level	1–2	1	3	1–2	2
Trans-National	1	0–1	3	0	2

Abbreviations and references: (1) Storie index (Storie, 1933), (2) German BS (German Soil Rating, Rothkegel, 1950), (3) AEZ (Agro-ecological zoning, Fischer et al., 2002), (4) VSA (Visual Soil Assessment, Shepherd, 2000), (5) M-SQR (Muencheberg Soil Quality Rating, Fig. 2, Mueller et al., 2007) (6) SQ (Soil Quality).

[a] Number of indicators/criteria 1, few <5, 2 medium (5–15), 3 high >15.

[b] Time required for field rating (minutes per pedon/unit): 3 fast < 20, 2 medium 20–40, 1 slow >40, 0 no field method.

different, inputs are similar. M-SQR indicator ratings are expert based and validated with crop yield data from Germany, Russia and China.

6 Targets and Steps to Assessing the Soil Productivity Function in the 21st Century

All approaches for assessing mainly regional-specific and particular aspects of the soil potential for productivity have their eligibility and merits. However, in the resource-limited global world of the 21st century we need more precise instruments for monitoring and controlling the functionality of the soil resource by clearly defined but not only locally valid criteria. A global soil functional assessment and classification framework will enable creation of reliable indicators of farmland quality, consistently over spatial scales, for example a reliable agri-environmental indicator “High quality farmland” which is currently not available. Based on our analysis such a global assessment framework of the soil productivity function has to meet the following requirements:

- a monitoring, controlling and modelling tool of the functional status of the soil resource for crop productivity;
- precise in operation, based on indicators and thresholds of the most functionally relevant

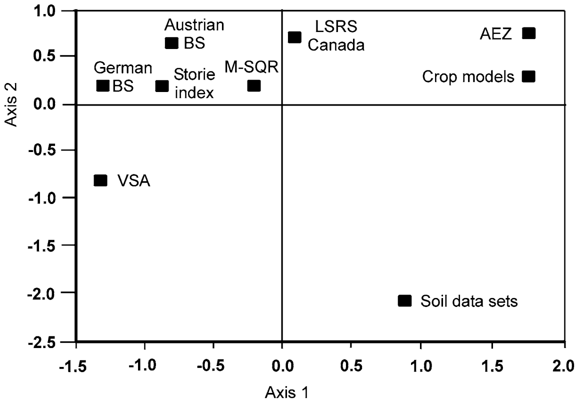


Fig. 3 Similarity plot of some soil productivity-relevant evaluation systems. Similarity is expressed by local neighbourhood. Axes are based on computed complex factors and have thus arbitrary meaning. Abbreviations and references: German BS = German Soil Rating (Rothkegel, 1950), Austrian BS = Austrian Soil Rating (Bodenaufnahmesysteme, 2001), Storie index (Storie, 1933), M-SQR (Muencheberg Soil Quality Rating, Fig. 2, Mueller et al., 2007), VSA (Visual Soil Assessment, Shepherd, 2000), LSRS Canada (Land Suitability Rating System, Agronomic Interpretations Working Group, 1995), AEZ (Agro-ecological zoning, Fischer et al., 2002)

parameters identified as soil moisture and temperature regimes, and textural and structural soil attributes;

- consistently applicable over different scales, from a field method to global overviews based on the soil map of the world;
- potential for suitability and capability classifications;
- straightforward for the use in extension and enabling participatory assessments;
- relevant to crop performance, with potential as a crop yield estimator and thus acceptable to farmers and other stakeholders;
- compatible with existing FAO soil classifications and capable of being integrated into new land evaluation frameworks of the 21st century (FAO, 2007).

Both the Canadian Land Suitability Rating System and the Muencheberg Soil Quality Rating meet the majority of these criteria. They contain information on climate and soil properties relevant to crop yield, and soil structure in particular. They have the potential for consistent ratings of the soil productivity function on a global scale but they need to be tested and evolved for this purpose in major agricultural regions. The selection and quantification of indicators and definition of thresholds and testing of the accuracy and sensitivity of the overall rating outputs under different environments will be a task of high priority. The latest results of Huber et al. (2008) about identified indicators and thresholds for main threats and degradation risks of soils in the EU will also need to be integrated.

Recent calls and approaches for the standardisation of soil quality attributes and their analyses (Nortcliff, 2002; FAO, 2007; Schindelbeck et al., 2008) will be very important for comparing productivity relevant soil states over the globe. The selection of attributes, data sets and indicators is the basic problem, and needs also to be relevant on a global perspective. Further locally proven and tested approaches and their indicator sets and thresholds (Kundler, 1989; Wienhold et al., 2004; Zhang et al., 2004; Barrios et al., 2006; Ochola et al., 2006; Govaerts et al., 2006; Sparling et al., 2008) referring to typical regions or countries have to be tested on inclusion into the frameworks.

Key indicators are single highly relevant attributes reflecting complex systems. Besides soil structure, soil organic carbon is such a key indicator of soil quality, associated with many soil functions other than productivity. It is also beneficial to agricultural productivity (Kundler, 1989; Rogasik et al., 2001; Lal, 2006; Martin-Rueda et al., 2007; Pan et al., 2009; Jones et al., 2009) at a limited level of inputs of farming but specific targets or thresholds are difficult to specify (Sparling et al., 2003). Despite this difficulty, from a broader perspective of soil functionality, organic carbon must be evolved as a globally key indicator of agricultural soil quality.

7 Conclusions

(i) There is a lack of a standardised methodology to assess soil productivity potentials for a growing global community of stakeholders achieving a sustainable use of the soil resource. Existing soil and land evaluation and classification systems operate on a regional or national basis. The soil types or reference groups of many existing soil classifications including the latest World Reference Base for Soil Resources are largely based on pedogenic criteria and provide insufficient information on soil functionality. A common internationally applicable method providing field soil productivity ratings is required but does not exist.

(ii) We advocate a straightforward indicator-based soil functional evaluation and classification system supplementing the WRB soil classifications. This could provide a useful tool for monitoring and controlling the soil status for sustainable land use at an internationally comparable scale. It could also serve as a soil productivity estimator providing a fast appraisal of attainable crop yields over different scales.

(iii) This framework has to meet the following criteria: precise in operation, based on indicators and thresholds of soil, consistently applicable over different scales, potential for suitability and capability classifications, adequately crop yield relevant, and capable of being integrated into new land evaluation frameworks of the 21st century.

(iv) Evolving this framework based on favoured methods for this purpose, the Muencheberg Soil Quality Rating (M-SQR) and the Canadian Land Suitability Rating System (LSRS), will be a starting point for assessing sustainable agricultural productivity without compromising soil quality.

Acknowledgements Authors thank Dr. Eric Lichtfouse and two anonymous reviewers for their helpful suggestions and comments.

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Long-Term Effects of Organic Amendments on Soil Fertility

Mariangela Diacono and Francesco Montemurro

Contents

Abstract Common agricultural practices such as excessive use of agro-chemicals, deep tillage and luxury irrigation have degraded soils, polluted water resources and contaminated the atmosphere. There is increasing concern about interrelated environmental problems such as soil degradation, desertification, erosion, and accelerated greenhouse effects and climate change. The decline in organic matter content of many soils is becoming a major process of soil degradation, particularly in European semi-arid Mediterranean regions. Degraded soils are not fertile and thus cannot maintain sustainable production. At the same time, the production of urban and industrial organic waste materials is widespread. Therefore, strategies for recycling such organic waste in agriculture must be developed. Here, we review long-term experiments (3–60 years) on the effects of organic amendments used both for organic matter replenishment and to avoid the application of high levels of chemical fertilizers. The major points of our analysis are: (1) many effects, e.g. carbon sequestration in the soil and possible build-up of toxic elements, evolve slowly, so it is necessary to refer to long-term trials. (2) Repeated application of exogenous organic matter to cropland led to an improvement in soil biological functions. For instance, microbial biomass carbon increased by up to 100% using high-rate compost treatments, and enzymatic activity increased by 30% with sludge addition. (3) Long-lasting application of organic amendments increased organic carbon by up to 90% versus unfertilized soil, and up to 100% versus chemical fertilizer treatments. (4) Regular addition of organic residues, particularly the composted ones, increased soil physical fertility, mainly by improving aggregate stability and decreasing soil bulk density. (5) The best

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_34,

from Diacono and Montemurro, Agron. Sustain. Dev. 30 (2010) 401–422. DOI: 10.1051/agro:2009040

agronomic performance of compost is often obtained with the highest rates and frequency of applications. Furthermore, applying these strategies, there were additional beneficial effects such as the slow release of nitrogen fertilizer. (6) Crop yield increased by up to 250% by long-term applications of high rates of municipal solid waste compost. Stabilized organic amendments do not reduce the crop yield quality, but improve it. (7) Organic amendments play a positive role in climate change mitigation by soil carbon sequestration, the size of which is dependent on their type, the rates and the frequency of application. (8) There is no tangible evidence demonstrating negative impacts of heavy metals applied to soil, particularly when high-quality compost was used for long periods. (9) Repeated application of composted materials enhances soil organic nitrogen content by up to 90%, storing it for mineralization in future cropping seasons, often without inducing nitrate leaching to groundwater.

Keywords Soil fertility • Soil carbon and organic matter • Organic amendments • Long-term experiments

1 Introduction

Indiscriminate use of agro-chemicals, excessive and deep tillage, and luxury irrigation have degraded soils, particularly in semi-arid Mediterranean areas, as well as polluted surface and groundwaters, and contaminated air (Lal, 2008). Soil is an essential nonrenewable resource with potentially rapid degradation rates and extremely slow formation and regeneration processes (Van-Camp et al., 2004). Soil degradation is as old as agriculture itself, its impact on human food production and the environment becoming more serious than ever before, because of its extent and intensity (Durán Zuazo and Rodríguez Pleguezuelo, 2008). There is a strong link between soil degradation and desertification on the one hand, and risks of accelerated greenhouse effects and climate change on the other (Komatsuzaki and Ohta, 2007). Durán Zuazo and Rodríguez Pleguezuelo (2008), confirmed that reduced precipitation or increased temperature accelerates land degradation through the loss of plant cover, biomass turnover, nutrient cycling and soil organic carbon storage, accompanied by higher greenhouse emissions. The reason for the earth's increased temperature along with change in rainfall amount and distribution, must be sought in the use of fossil fuel, that has drastically disturbed the global carbon cycle with its attendant impact on climate change (Lal, 2008). According to Komatsuzaki and Ohta (2007), there is also evidence that continuous cropping and inadequate replacement of nutrients, removed in harvested materials or lost through erosion, leaching, and gaseous emissions, degrade soil physical, chemical and biological properties, intensifying global warming. Moreover, the production of urban and industrial organic wastes is increasing worldwide, and strategies for disposal in such a way that these do not further degrade soil, contaminate water or pollute air must be developed and optimized (Düring and Gäth, 2002; Lal, 2008).

Soil fertility can be defined as the capacity of soil to provide physical, chemical and biological needs for the growth of plants for productivity, reproduction and quality, relevant to plant and soil type, land use and climatic conditions (Abbott and Murphy, 2007). It is becoming understandable that the proper agricultural use of soil resources requires equal consideration for biological, chemical and physical components of soil fertility, thus attaining a sustainable agricultural system.

The term "sustainable agriculture" is used in this review with the meaning given by Tilman et al. (2002), as referring to practices that meet current and future society needs for food and feed, ecosystem services and human health, maximizing the net benefit for people. Namely, sustainability implies both high yields, that can be maintained, and acceptable environmental impact of agricultural management.

It is relevant to note that organic farming is the only sustainable form of agriculture legally defined. According to the current legislation (EC Council Regulation N. 834, 2007), soil fertility management relies on a complex long-term integrated approach rather than the more short-term one of conventional agriculture. One of the possible main tools for the maintenance and the improvement of soil fertility in organic farming is to adopt crop rotations, including a mixture of leguminous fertility-building crops and plants with different rooting depths (Watson et al., 2002). Furthermore, organic wastes such as animal manures, by-products of several kinds and composted residues can be used as amendments to increase soil fertility, since they are important sources of nutrients for growing crops and means for enhancing the overall soil quality (Davies and Lennartsson, 2005).

1.1 Soil Management Strategies for a Sustainable Agriculture

Soil organic matter plays an important role in long-term soil conservation and/or restoration by sustaining its fertility, and hence in sustainable agricultural production, due to the improvement of physical, chemical and biological properties of soils (Sequi, 1989). The organic matter content is the result of the inputs by plant, animal and microbial residues, and the rate of decomposition through mineralization of both added and existing organic matter. More specifically, the generic term "organic matter" refers to the sum of all organic substances present in the soil. This sum comes from residues at various stages of decomposition, substances synthesized through microbial-chemical reactions and biomass of soil microorganisms as well as other fauna, along with their metabolic products (Lal, 2007). Decomposition of organic matter is chiefly carried out by heterotrophic microorganisms. This process is under the influence of temperature, moisture and ambient soil conditions and leads to the release and cycling of plant nutrients, especially nitrogen (N), sulfur and phosphorus (Murphy et al., 2007).

The different fractions of organic matter undergo the "humification" process, which is the changing from recognizable parts and pieces of plants or animals into an amorphous, rotted dark mass. The products of humification are the humic substances that in soil are dark brown and fully decomposed, i.e. humified (Fig. 1). The humic substances are one of the most chemically active compounds in soils, with cation and anion exchange capacities far exceeding those of clays. They are long-lasting critical components of natural soil systems, persisting for hundreds or even thousands of years (Mayhew, 2004). In fact, the turnover rate of organic materials varies considerably, from less than 1 year, as for microbial biomass, to more than 1 thousand years of stable humus (Van-Camp et al., 2004). The organic matter is being progressively depleted, particularly in the Mediterranean area, where the warm climate and the intensity of cultivation increase the rate of decomposition (Montemurro et al., 2007). By contrast, the build-up of organic matter in soils is a process much slower and more complex than its decline (Van-Camp et al., 2004). Since organic matter contents are difficult to measure directly, a great number of methods measure the soil organic carbon level, multiplying it by conversion factors ranging from 1.7 to 2.0 to obtain organic matter values (Baldock and Nelson, 2000).

Fig. 1 The diagnostic horizons of a Vertisol (Sparacia experimental farm - Department of Agronomy, University of Palermo) showing the presence of well-transformed, dark-colored humified organic matter in the topsoil

Several investigations have demonstrated that soil organic matter is a very reactive and ubiquitous soil quality indicator that influences the productivity and physical well-being of soils (Lal, 2006; Komatsuzaki and Ohta, 2007). As a consequence, agricultural management practices that enhance soil organic matter content are used for preserving farming output and environmental quality; thereby they can be considered as sustainable activities (Lal, 2004).

Conservative soil tillage systems, e.g. no-till, which leaves more residues on the surface because the soil is not turned over, can maintain or improve the organic carbon content and the related soil fertility properties (Ismail et al., 1994; Johnson et al., 2005). Crop rotations usually increase organic matter and prompt changes in N sources, affecting their availability for plants and, as a consequence, the N efficiency is greater when a crop rotation is adopted (Montemurro and Maiorana, 2008).

The inclusion in a rotation of cover crops or green manures can also enhance the efficient use of nutrients by plants, mainly owing to the increase in soil microbial population and activity (Watson et al., 2002). Cover crops are generally grown to provide soil cover during the winter months, thus preventing soil erosion by wind and rainwater strength, which reduces organic matter content in the long run. Moreover, Komatsuzaki (2004) indicated that cover crop utilization is a technique that limits nutrient leaching, scavenging the soil residual N and making it available for subsequent cultivation.

In addition, the summer green manures are field crops or forage ones, such as leguminous and non-leguminous plants, usually incorporated into the soil soon after flowering, to improve soil fertility. In particular, leguminous green manures can fix large quantities of atmospheric N_2. They also provide useful amounts of organic matter, as well as non-leguminous crops which, nevertheless, cannot fix atmospheric N_2 (Davies and Lennartsson, 2005). During 32 years of winter wheat cropping, Procházková et al. (2003) found higher average yields with green manuring compared with straw incorporation into the soil, probably due to a slower decomposition and following release of nutrients by the latter.

There is increasing interest in the alternative fertility building strategies previously described, because conventional inputs, such as synthetic fertilizers, should be excluded or reduced in sustainable agricultural management. Within this context, soil fertility could also be improved with organic waste application such as by-products of farming, or municipal activities including animal manures, food processing wastes and municipal biosolids, wastes from some industries, such as sewage sludges, wastewaters, husks and vinasse. Both groups of wastes present generally notable contents of organic matter and substantial quantities of nutrients and their use in agriculture can contribute to closing the natural ecological cycles (Montemurro et al., 2004; Montemurro and Maiorana, 2008). Increased recycling of organic residues as fertilizers and soil amendments on cropland avoids both utilization of non-renewable resources, e.g., fossil fuel and peat, and excess of energy expenses, i.e., production of chemical fertilizers and pesticides, treatment and landfill disposal of such organic wastes (Mondini and Sequi, 2008). Biodegradable wastes can also be considered valuable resources to promote soil fertility. However, this benefit occurred only if they were applied according to good practices, taking into account the needs of the soil, its use and the climatic conditions (Van-Camp et al., 2004). Moreover, it is necessary to adopt waste management strategies, such as controlled biodegradation processes, able to both minimize their potentially negative environmental impact and increase agricultural utilization (Montemurro et al., 2009).

Despite the fact that manure characteristics are influenced by many factors such as species and age of the animal, ration fed, and collection and storage method, it can be generally assumed that their ratio of nutrients are different from those removed by common crops (Edmeades, 2003). As a consequence, soil manure application, often in excess of crop requirements, can cause a significant build-up of phosphorus (P), N and salt. Nowadays, the industrialization of livestock enterprises has led to other problems linked to the land distribution of solid and liquid animal manures, enhancing the interest in pretreatment technologies, which can convert manure into a more valuable and easily usable resource called “compost” (Figs. 2a and 2b).

Compost is a stabilized and sanitized product of composting, which is the biodegradation process of a mixture of organic substrates carried out by a microbial community composed of various populations, both in aerobic conditions and solid state (Insam and de Bertoldi, 2007). During the composting process, the simple carbonaceous and nitrogenous compounds are transformed through the activity of microorganisms into more stable complex organic forms, which chemically resemble soil humic substances (Epstein, 1997).

The soil application of co-composted manure has several advantages over fresh manure, such as reduced numbers of viable weed seeds, reduced volume and particle size, which facilitates land distribution, a better balanced nutrient composition, stabilized organic matter and a slower release of nutrients. This topic has recently been reviewed by Moral et al. (2009).

From all the above, it can be summarized that the environmental impact of conventional farming practices and global concerns about soil degradation have increased the interest in sustainable agricultural strategies such as land application of waste materials. This is a way to avoid disposal costs and recycle nutrients into soil, unlike commercial fertilizers (Miller and

Fig. 2 Aerobic biodegradation can enhance the quality of wastes which will be applied to soils. Figure 2a shows a composting windrow stirred mechanically by a turning machine, while Figure 2b shows an experimental field with compost application

Miller, 2000; Van-Camp et al., 2004). However, it is necessary to point out that the utilization of various organic residues in agriculture depends on several factors, including the characteristics of the waste, such as nutrient and toxic element content, availability, the transportation costs and the environmental regulations, as reviewed in detail by Westerman and Bicudo (2005).

1.2 Purpose of this Review Article

The Rothamsted experiments, lasting for more than 100 years, are the oldest and most continuous agronomic trials in the world, that measure the effects on crop yields of inorganic fertilizers in comparison with farmyard manure and other organic materials. Since their results are well summarized in the "Guide to the classical and other long-term experiments, datasets and sample archive", they will not be discussed further in this review (Rothamsted Research, 2006).

This paper, focusing on recently published data, gives emphasis to long-term field trials, particularly regarding raw and composted agro-industrial and municipal waste application. Although there is a large amount of literature relating to the influence of raw and composted organic materials on soil fertility, only a few published studies have focused on studies about long-term effects of these amendments on the soil-plant system, for a sustainable crop production. The present work attempts to address this issue by using the results from long-lasting fertility trials. Long-term research on organic amendment use on cropland is particularly relevant because many effects, e.g. organic matter enrichment and possible soil toxic element accumulation, evolve slowly and are difficult to predict.

We have collected published literature, investigating a broad array of organic residues, composts and experimental conditions, especially analyzing their impact on soils and crops in relation to modern sustainable agriculture. Experimental data of field trials were selected lasting for at least 3 years up to 60 years, stressing longer-term research. Therefore, experimental designs considered in this review ranged from those usually known as mid- to long-term ones. Summaries of the essential data for the longest duration trials are given in Table 1.

The organic amendments covered in this paper are defined in Section 2 along with their effects on the soil-plant system, whereas the environmental impact and sustainability are outlined in Section 3.

2 Effects of Organic Amendments on the Soil-Plant System

Miller and Miller (2000) highlighted that organic material application to cropland could affect soil properties, but the effects generally may not be apparent over a short time period. More specifically, Tittarelli et al. (2007) pointed out that the simplest method of examining the agronomic value of stabilized

Table 1 Summary of the main data of long-term trials (selected data from experiments of ⩾10 years)

Site	Organic materials	Application rate	Crop	Trial period	Reference
Punjab, India	Rice straw compost	8 t ha^{-1}	Rice–wheat rotation	10 years	Sodhi et al. (2009)
Obere Lobau near Vienna, Austria	Biowaste compost	9, 16 and 23 t ha^{-1}	Cereals and potatoes	10 years	Erhart et al. (2005); Hartl and Erhart (2005); Erhart et al. (2008)
Turin, Italy	(1) Cattle slurry; (2) Composted farmyard manure	(1) 100 t ha^{-1}; (2) 40 t ha^{-1}	Maize for silage	11 years	Monaco et al. (2008)
Linz, Austria	Composts of: urban organic waste; green waste; cattle manure or sewage sludge	175 kg N ha^{-1}	Maize, summer-wheat and winter-barley rotation	12 years	Ros et al. (2006a)
Bennett, CO, USA	Anaerobically digested biosolids	2.2, 4.5, 6.7, 8.9 and 11.2 t ha^{-1}	Two-year wheat–fallow rotation	12 years	Barbarick and Ippolito (2007)
Ravenna, Italy	Municipal-industrial wastewater sludge: (1) Anaerobically digested (liquid slurry); (2) belt filtered (dewatered sludge); (3) composted with wheat straw	(1)–(3) 5 and 10 t ha^{-1}	Winter wheat–maize–sugarbeet rotation	12 years	Mantovi et al. (2005)
Toledo, Spain	(1) Barley straw and crop waste; (2) two-year-old cattle manure	(1) 3 and 2.5 t ha^{-1}; (2) 30 t ha^{-1}	Barley, wheat and sorghum	16 years	Dorado et al. (2003)
Murcia, Spain	Municipal solid waste	65, 130, 195 and 260 t ha^{-1}	none	17 years	Bastida et al. (2008)
Orange, VA, USA	Aerobically digested sewage sludge	42, 84, 126,168 and 210 t ha^{-1}	Barley, radish and romaine lettuce	19 years	Sukkariyah et al. (2005)
Tanashi City, Tokyo, Japan	(1) Dried sewage sludge; (2) rice husk compost; (3) sawdust compost	(1) 259.8 t ha^{-1}; (2) 82.5 t ha^{-1}; (3) 77.2 t ha^{-1}	Maize, barley and rye	19 years	Kunito et al. (2001)
Tokyo, Japan	(1) Sewage sludge composted with rice husk; (2) sewage sludge composted with sawdust	(1)–(2) 240 kg N ha^{-1}	Maize and barley	23 years	Zaman et al. (2004)
Alberta, Canada	Solid beef cattle manure	30, 60 and 90 t ha^{-1} in dryland soils and 60, 120 and 180 t ha^{-1} in irrigated soils	Barley, canola, triticale and maize	25 years	Whalen and Chang (2002)

(continued)

Table 1 (continued)

Site	Organic materials	Application rate	Crop	Trial period	Reference
Yamaguchi, Japan	Rice straw–cow dung compost	15 t ha^{-1}	Double cropping (paddy rice and barley)	25 years	Shindo et al. (2006)
Czech Republic	(1) Straw harvest; (2) straw harvest + green manuring; (3) straw incorporation; (4) straw incorporation + green manuring; (5) straw burning; (6) farmyard manure	(6) 10 t ha^{-1}	Winter wheat continuous cropping	32 years	Procházková et al. (2003)
Punjab, India	Farmyard manure	20 t ha^{-1}	Rice–wheat and maize–wheat systems	34 years	Kukal et al. (2009)
Gumpenstein, Austria	(1) Cattle slurry + straw; (2) animal manure (solid); (3) animal manure (liquid); (4) cattle slurry (semi-liquid)	(1) 120 kg N ha^{-1} + 0.2 kg m^{-2}; (2)–(4) 240 kg N ha^{-1}	Cereals; rape; pea; flax	38 years	Antil et al. (2005)
Fidenza, Italy	(1) Farmyard manure; (2) aerobically digested sewage sludge	(1)–(2) 5 t ha^{-1} digested sewage maize and wheat	Two-year rotation consisting mainly of	(1) 40 years; (2) 12 years	Saviozzi et al. (1999)
Legnaro, Italy	(1) Farmyard manure; (2) liquid manure	(1) 20 and 60 t ha^{-1}; (2) 120 t ha^{-1}	Maize	40 years	Nardi et al. (2004)
Bologna, Italy	Cattle manure, cattle slurry, wheat or corn residues	6 t ha^{-1} after wheat and 7.5 t ha^{-1} after maize crops	Two-year maize-winter wheat rotation	34 years	Triberti et al. (2008)
Central Sweden	Farmyard manure	4 t carbon ha^{-1}	Maize	47 years	Elfstrand et al. (2007)
Martonvásár and Keszthely Hungary	Farmyard manure	35, 40, 80 and 104 t ha^{-1}	Martonvásár: wheat–wheat–maize–maize; Keszthely:wheat–wheat–sugar beet–maize–maize	Martonvásár: 54 years; Keszthely: 41 years	Sleutel et al. (2006)
Towada, Japan	Rice straw compost	11 t ha^{-1}	Potato, maize and soybean in rotation	60 years	Takeda et al. (2005)

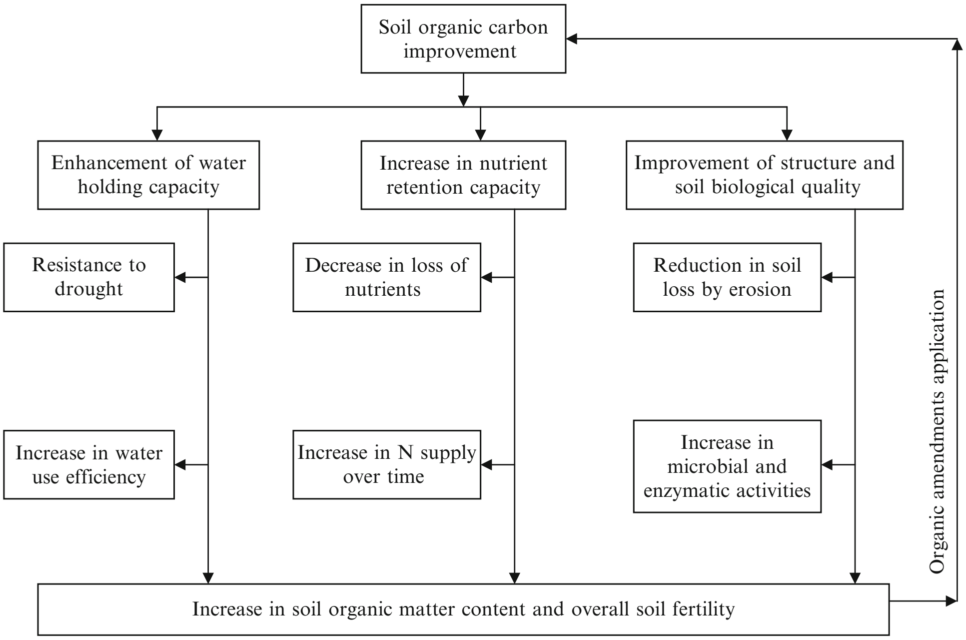

Fig. 3 Effects of increasing soil organic matter content and overall soil fertility by soil organic carbon improvement (adapted from Lal, 2006)

organic materials is the calculation both of organic matter supply and plant nutrients. The slow release of these nutrients is responsible for the increase in crop yields in the subsequent years, thus determining the difficulty of quickly evaluating the true agronomic value of these organic materials as amendments. However, there is a considerable variability between experimental techniques, climate, soil type and organic material characteristics, and therefore attention must be paid to generalizing the effects of composts and raw waste application on the soil-plant system.

2.1 *Effects on Soil Biological, Chemical and Physical Fertility*

Van-Camp et al. (2004) found that organic amendments influence soil characteristics by the interdependent modification of biological, chemical and physical properties (Fig. 3). Also, fertility improvement through an effective management of these properties has the capability of optimizing crop production. In this subsection, we selected papers whose primary purpose was to examine the effect of different organic amendments on overall soil fertility. However, it is necessary to take into account that sometimes it was difficult to compare the results, because of the different assessment methods used in the studies.

2.1.1 Biological Fertility

Microbiological and biochemical soil properties are very reactive to small changes occurring in management practices. Therefore, it is possible to use them in a basic analysis for evaluating the effects of the application of different sources and amount of organic matter on soil characteristics during experimental trials. Microorganisms, e.g. bacteria, fungi, actinomycetes and microalgae, play a key role in organic matter decomposition, nutrient cycling and other chemical transformations in soil (Murphy et al., 2007). Since organic carbon (C) is utilized for energy by decomposer microorganisms, its fate is to be either assimilated into their tissues, released as metabolic products, or respired as carbon dioxide (CO_2). The macronutrients N, P and sulfur (S), present in the organic chemical structures, are converted into inorganic forms. Subsequently, they are either immobilized and used in the synthesis of new microbial tissues or mineralized and released into the soil mineral nutrient pool (Baldock and Nelson, 2000). For assimilation by microorganisms of decomposing organic residues, the N has to be assimilated in an amount determined by the C/N ratio of the microbial biomass. More specifically, the amount of N required by the microorganisms is 20 times smaller than that of C. If there are both a low concentration of easily decomposable C compounds and a larger N quantity in respect to that required by the microbial biomass, there will be net N mineralization with release of inorganic N. On the contrary, Corbeels et al. (1999) found that if the amount of N present in the residues is smaller than that required by the microbial biomass, further inorganic N will need to be immobilized from the soil to complete the decomposition process.

It is difficult to distinguish between the direct and the indirect effects of an amendment on the behavior of soil microorganisms. In soils amended with compost or other raw organic materials, even in association with mineral fertilizer N, autochthonous microbiological activity and growth can be stimulated. However, different authors (Ros et al., 2006a; Kaur et al., 2008) suggest that a direct effect from microorganisms introduced with the compost is detectable. Several long-lasting experiments have demonstrated that soil biological properties, such as microbial biomass C, basal respiration and some enzymatic activities, are significantly improved by compost treatments. This is particularly evident in the upper layers of the soil because of the added labile fraction of organic matter, which is the most degradable one (Zaman et al., 2004; Ros et al., 2006a, b; Tejada et al., 2006, 2009). Since generally the composts are slowly decomposed in the soil, the continuous release of nutrients can sustain the microbial biomass population for longer periods of time, compared with mineral fertilizers (Murphy et al., 2007). In fact, an interesting residual effect of composts on the microbial activity has often been observed in many experimental seasons after their application, which also results in a longer availability of plant nutrients. Ginting et al. (2003), for example, found 4 years after the last application of compost and manure that the residual effects resulted in 20 to 40% higher soil microbial biomass C compared with the N fertilizer treatment. Research on the effect of different doses of raw municipal solid waste in Mediterranean semiarid conditions, ranging between 65 and 260 t ha^{-1}, demonstrated 17 years after a single application of this organic amendment an average increase of 70% in organic matter content. The water-soluble C fractions also increased by up to 195 t ha^{-1} application rate, above which they leveled off (Bastida et al., 2008). The authors also found that the enzymatic activities of urease, β-glucosidase, alkaline phosphatase and o-diphenyloxidase associated with humic substances increased significantly in all the amended plots, thus improving soil biochemical quality.

Sixteen years after 1 and 3 t ha^{-1} year^{-1} sludge application, in a field situated close to Lund in the southern part of Sweden, an increasing trend for substrate induced respiration was found, ranging from 20 to 22%, due to the organic material inputs, and enhanced by from 11 up to 33% acid phosphatase activity involved in the mineralization of organically-bound P (Mats and Lennart, 1999). This behavior follows the increasing rates of sludge addition.

As a general rule, the quantity and quality of organic material added to soils are the major factors in controlling the abundance of different microbial groups and the activity of microorganisms involved in nutrient cycling. Enzyme activity and microbial biomass analysis indicated that microbial properties were stimulated, e.g. microbial biomass C increased to about 100%, more by high rates and composted than by low rates and fresh paper mill residuals (78 t ha^{-1} and 22 t ha^{-1}, respectively) (Leon et al., 2006).

As regards the effects of the quality of amendment, Monaco et al. (2008) pointed out that microbial activity, measured by the potential soil respiration parameter, gave a reliable and useful indication of the amount of easily decomposable organic C, and this parameter was lower when the C was partially humified before soil input. In fact, after 11 years of repeated applications of different organic materials, soil respiration was in the order of $640.9 < 682.1 < 755.1$ mg C-CO_2 kg^{-1} for farmyard manure, cattle slurry and straw, respectively. The effect of green manure amendments was studied in a 47-year field experiment (Elfstrand et al., 2007). The authors found a higher abundance of bacteria and fungi in the green manure treatment, i.e. 34.3 and 1.8 nmol g soil^{-1}, respectively, in respect to the unfertilized one (20.3 and 0.9 nmol g soil^{-1}), measured before maize sowing. Furthermore, a higher fungal/bacterial ratio was noticed, equal to 0.054 compared with farmyard manure

(0.036) and sawdust (0.046) application. This behavior might be attributed to differences in the quality of the organic matter added.

The reviewed results suggest that exogenous organic matter applications to cropland lead to an improvement in soil biological functions, depending on the quantity and type of materials applied.

2.1.2 Chemical Fertility

A considerable number of studies, concerning long-term fertility trials, pointed out that soil organic material applications increased the organic carbon stock and, therefore, increased the cation exchange capacity. This effect was due to the high negative charge of organic matter. This is important for retaining nutrients and making them available to plants (García-Gil, 2004; Ros et al., 2006b; Weber et al., 2007; Kaur et al., 2008). Figure 4 shows the organic carbon increase, as a consequence of several organic amendments' long-lasting application, ranging from 24 to 92%.

Habteselassie et al. (2006a) found that, over a 5-year period, the C pool was enhanced by 115% in dairy-waste compost-treated soil. Moreover, the dairy-waste compost increased organic carbon by 143 and 54% as compared with ammonium sulfate and liquid dairy-waste treatments, respectively, applied at the same available N level (200 kg Nha^{-1}). This C stored in the soil organic matter accounts for approximately 11% of the total amount of C applied.

After 3 years of municipal solid waste compost and olive pomace compost application, the total organic carbon significantly increased by 24.0 and 43.2% for cocksfoot and alfalfa plots, respectively, in respect to the unfertilized control, indicating that these amendments positively affected the organic matter (Montemurro et al., 2006). Other authors also showed that municipal-industrial wastes stimulate plant growth, indirectly and with a long-term effect, by improving organic matter (Mantovi et al., 2005; Cherif et al., 2009).

Under 7–36-year fertility experiments in five different rice-based cropping systems, the application of organic amendments at 5–10 t ha^{-1}year^{-1}, through farmyard manure or compost combined with balanced mineral NPK, increased organic carbon by 10.7% (Mandal et al., 2007).

In a rice–wheat system, farmyard manure application at 20 t ha^{-1} showed, after a period of 32 years, higher organic carbon concentration of 17% compared with NPK fertilizers in the 0–15 cm soil layer (Kukal et al., 2009). Nevertheless, the results of repeated applications of either digested sewage sludge over 12 years or farmyard manure for 40 years indicated that such organic amendments were inadequate for restoration of organic matter lost as a consequence of cultivation (Saviozzi et al., 1999). In fact, the amount of organic carbon in the undisturbed site was 120 and 156% higher than that in farmyard and sludge cultivated soils, respectively. On the other hand, contrary to common belief, over a 25-year period of intensive rice-wheat cropping, a depletion of organic

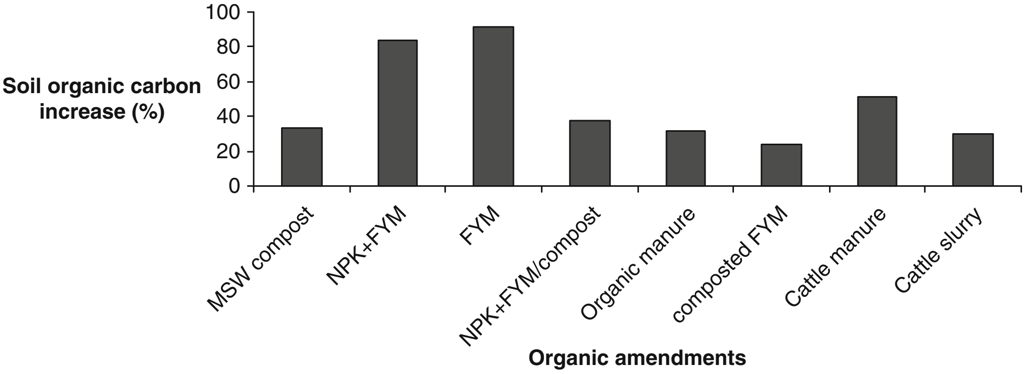

Fig. 4 Soil organic carbon increases after different long-term organic amendment applications. Note: data taken from: Montemurro et al. (2006) (means of crops); Kaur et al. (2008) (at 0–15 cm depth); Kukal et al. (2009) (means of crops; at 0–15 cm depth); Mandal et al. (2007) (means of 5 trials' data); Meng et al. (2005); Monaco et al. (2008); Triberti et al. (2008). SOC = soil organic carbon; MSW = municipal solid waste; FYM = farmyard manure

carbon did not occur, but rather an improved organic carbon concentration of 38% (Benbi and Brar, 2009).

In terms of sustainability, only farmyard manure fertilization maintained the total organic carbon level of 40 t C ha^{-1}, measured in the top soil layers at the start of a 40-year experiment, while the average total organic C depletion was 23% with liquid manure and mixed fertilization treatments, 43% with mineral fertilizers alone and 51% in the control (Nardi et al., 2004). Furthermore, the presence of weakly acidic chemical functional groups on organic molecules makes organic matter an effective buffer, as supported by the findings of García-Gil (2004). These authors observed a long- (9 years) and short-term improvement in the soil humic acid buffering capacity in municipal solid waste compost-amended soils, derived from a residual effect of a single application and cumulative effects from repeated ones. These distributions will favor the general soil fertility status and crop production.

There are reports in the literature of long-term compost and manure application both increasing (Eghball, 2002; García-Gil et al., 2004; Butler and Muir, 2006) and decreasing (Meng et al., 2005; Bastida et al., 2008; Bi et al., 2008) the pH of soils, depending on their initial pH and organic residues. Butler and Muir (2006) observed that soil pH increased on average by 0.5 units as the dairy manure compost rate doubled in magnitude from 11.2 to 179.2 t ha^{-1}.

As previously explained, with long-term compost use the residual effects on crop production and soil properties can last for several years, since only a fraction of the N and other nutrients becomes available for plants in the first year after spreading (Hartl et al., 2003; Eghball et al., 2004). As an estimation of the the available N from compost treatment in the first year of application, Tittarelli et al. (2007) mentioned a release of only 30–35% of the total N content. The N release from compost will mostly occur in the first two years after application, suggesting that a distribution frequency of once in every second year may be better than other application strategies, especially with higher rates (Zhang et al., 2006).

More specifically, it is well known that many microorganisms convert organic N into inorganic N forms by mineralization. A large number of authors confirmed that N mineralization from compost is very limited in the short term. However, there is a significant residual effect from the cumulative applications which becomes visible later after 4–5 years, resulting in deferred higher N availability and yields (Eghball, 2002; Blackshaw et al., 2005; Barbarick and Ippolito, 2007; Leroy et al., 2007). Regular addition of organic material to soil for more than 10 years, through compost or manures, enhanced both soil C and N stocks and resulted in build-up of N, indicating a physical protection of this nutrient within macroaggregates (Whalen and Chang, 2002; Meng et al., 2005; Mallory and Griffin, 2007; Sodhi et al., 2009). According to Hartl and Erhart (2005), the organic N content increases by about 10% compared with the control, in the upper 30 cm of soil, after 10 years of compost treatments. This result was complemented by significant increases in organic C, of 22%, indicating that the organic N was tied up in organic matter. After 4 years of vegetable compost applications, significantly higher soil total N concentration was observed on compost plots compared with plots without it (Nevens and Reheul, 2003).

The C/N ratio of organic material can be used as a good indicator of nutrient supply. Tejada et al. (2009) showed an optimum balanced C/N ratio (10–12) for soils amended with composts originating from leguminous residues, due to organic matter mineralization overcoming immobilization. These findings were not in accordance with those of Weber et al. (2007), who found the C/N ratio clearly increased, from 10.7 up to 22.2, in the third year after municipal solid waste compost application. This behavior can be explained by a depletion of N reserve, probably because of plant N uptake. However, there are often other explanations for such an increase in the C/N ratio. In fact, it is well known that when a compost that has a high C/N ratio is added to soil, the microbial population competes with plants for soil N, thus immobilizing it (Amlinger et al., 2003).

Soil available potassium (K) content increased on average by 26%, as compared with control, in 5-year compost treatments derived from organic household wastes and yard trimmings (Hartl et al., 2003). These treatments are a rich source of K, probably due to the large proportion of woody plant material and kitchen refuse in the raw material.

As regards the P from organic amendments, He et al. (2001) reported that compost applications can increase plant-available P in the soil. The biosolids-municipal solid waste co-compost, applied once in 4 years, has also been found to effectively supply P to soil at 0–15 cm depth. The soil extractable P concentration increased on average from 7.2 to 86 mg kg^{-1} soil with enhanced application rates from 0 to 200 t ha^{-1} (Zhang et al., 2006). Furthermore, Eghball (2002) suggested that 4-year beef cattle manure and composted manure application based on N needs of corn could eventually result in soil accumulation of P, since the manure or compost N/P ratio is usually smaller than the corn N/P uptake ratio.

Our overall literature analysis demonstrates that several organic amendments' long-lasting applications enhanced soil available potassium, extractable phosphorous and organic carbon content, and resulted in deferred N availability.

2.1.3 Physical Fertility

Aggregate stability is a keystone factor in questions of soil physical fertility and can be enhanced by means of an appropriate management of organic amendments, which can maintain an appropriate soil structure. This agronomic procedure could improve pore space suitable for gas exchange, water retention, root growth and microbial activity (Van-Camp et al., 2004). Soils rich in organic matter are less prone to erosion processes than soils with low content, such as those which predominate in arid and semi-arid areas (Durán Zuazo and Rodríguez Pleguezuelo, 2008).

The topic of soil structural stability has been reviewed recently by Abiven et al. (2008). Their literature analysis validated the conceptual model proposed by Monnier (1965). This author considers different patterns of temporal effects on aggregate stability, depending on the nature of the organic inputs. Easily decomposable products have an intense and transient effect on aggregate stability, while more recalcitrant ones, such as lignin and cellulose, have a lower but longer-lasting effect.

Results of Albiach et al. (2001) pointed out that organic matter and carbohydrates appeared to be the parameters most closely related to structural stability of soil aggregates obtained with five applications of a municipal solid waste compost. Another 5-year field trial confirmed these findings, suggesting that municipal solid waste compost, applied every 2 years, can be used to increase soil aggregate stability by 29.3% in respect to the control, thus improving soil resistance to water erosion (Annabi et al., 2006).

The organic matter stabilizes soil structure by at least two different mechanisms: by increasing the inter-particle cohesion within aggregates and by enhancing their hydrophobicity, thus decreasing their breakdown, e.g. by slaking. More specifically, the increase in soil microbial activity, especially due to the addition of composted residues, could be responsible for the increase in soil structural stability (Van-Camp et al., 2004). The relationships between soil biological activity and the functioning of soil are very complex. According to Abiven et al. (2008), several biological binding agents have been recognized as accountable for aggregation and aggregate stability. Polysaccharides synthesized by microorganisms, particularly at the beginning of the organic matter decomposition, tend to adsorb the mineral particles and increase their inter-cohesion. Conversely, products rich in humic compounds, such as manures or composts, would also be expected to increase aggregate hydrophobicity of clays. This has mainly been proved in long-term trials. In fact, after 16 years of either farmyard manure or crop waste applications, substantial improvements in soil physical properties have been noticed, markedly aggregate stability and water retention, due to the increased concentration of humic colloids in soil (Dorado et al., 2003). In particular, the authors found that the structural instability index decreased by 2.5 units with respect to control plots.

Tejada et al. (2009) observed that three different composts, consisting of leguminous plants, non-leguminous plants and the combination of both plant residues, applied at rates of 7.2 and 14.4 t organic matter ha^{-1} during a period of 4 years, had a positive effect on soil physical properties. More specifically, at the end of the experimental period and at the highest rate, the soil structural stability was the highest in the non-leguminous plant compost treatment (28.3%), followed by the combined one (22.4%). This result was due to greater amounts of humic acids provided to the soil, 63.6 and 59.5 g kg^{-1}, respectively, which are then directly involved in clay–organic complex formation.

Another recent study showed that, after 10 cycles of rice–wheat cropping, the amount of water-stable aggregates was significantly higher in plots amended with rice straw compost at 8 t ha^{-1} to both rice and wheat, as compared with inorganic fertilizers (Sodhi et al., 2009). The authors also suggested that this higher amount of water-stable aggregates can be ascribed to the regular addition of organic matter to soil, resulting in enhanced microbial activity and production of microbial decomposition products, which helps with binding of aggregates. Leon et al. (2006) found that the application of medium and high rates, equal to 38.1 and 78.4 t ha^{-1}, of composted paper mill residuals over 4 years, caused an increase in amounts of water-stable aggregates on average of 25% compared with the control. There was no significant difference ($P \leqslant 0.05$) between the two compost rates tested, suggesting that the lowest amount of amendment was enough to maximize water-stable aggregation.

These positive results of long-term compost application agreed with the findings reported by Tejada et al. (2008). The authors observed that composted leguminous plants, alone or mixed with beet vinasse, an agro-industrial by-product, had a positive impact on soil structural stability, which increased by 5.9 and 10.5% compared with the unfertilized treatment, respectively. Conversely, fresh beet vinasse application decreased soil structural stability by 16.5% in respect to the control, probably because high quantities of destabilizing monovalent cations were introduced into the soil by this organic material.

Whalen and Chang (2002) noted that 25 years of annual beef cattle manure applications, at rates of more than 30 t ha^{-1} year^{-1} to dry land soils and at more than 60 t ha^{-1} year^{-1} to irrigated soils, can shift the aggregate size distribution from larger (>12.1 mm) to smaller (< 2.0 mm) dry-sieved aggregates, because of dispersive agents in the manure. Consequently, many years of continuous manure applications could raise the risk of wind erosion because of a greater proportion of the soil small aggregates. Moreover, different authors have shown typical decreases in soil bulk density, on average of 15%, after long-term compost, farmyard manure or digested sewage sludge agricultural use, suggesting that the addition of composted and fresh organic matter facilitated, as a consequence, the soil porosity connected to soil bulk density (Saviozzi et al. 1999; Meng et al., 2005; Tejada et al., 2008; 2009). Porosity is a measure of the size and system of voids in the soil matrix, affecting both aeration and water movement. On the other hand, Eghball (2002) reported that soil bulk density was unaffected by 4-year manure or compost application.

Leon et al. (2006) found a strong correlation ($r = 0.65$, $P \leqslant 0.001$) of total C with soil moisture content after application of paper mill residual by-products, corroborating that high C level in soils increases the water-holding capacity because of the effect of organic matter on soil aggregation. This increase provides more available water to plants, also helping with resistance to drought.

On the basis of the information presented in this subsection, it can be summarized that repeated applications of organic amendments can increase soil physical fertility, mainly by improving aggregate stability. In general, a rise in the above investigated overall soil fertility influences crop yield response, as framed in the following subsection. Selected studies are summarized in Table 2, focusing on the effects of organic amendments on yields.

2.2 Effects of Organic Amendments on Plant Nutrition and Yielding Responses

The N dynamics in compost-amended soils could be affected by different site-specific factors, e.g. compost matrices, composting conditions, climate, soil properties and management practices. It can be generally assumed that the prompt availability of N to plants is low, as already explained in this review, since the majority of total compost N is bound to the organic N-pool (Amlinger et al., 2003). In fact, the greatest total N content in compost is not readily available, but it can be mineralized and subsequently taken up by plants or immobilized, denitrified and/or leached.

It is important to take into account that the slow release of nutrients from compost or green manures should be adequately controlled to match temporal crop demand with nutrient supply. The increase in the N-use efficiency decreases loss to leaching when there is wide drainage, e.g. during the fall-winter period, or volatilization (Tilman et al., 2002). The plant-available N in the application year was expected to be higher for fresh manure than for stable composted manure. In fact, in a 4-year experiment, it ranged from 26 to 67 and from 12 to 18 kg ha^{-1} for fresh and stabilized manure, respectively (Blackshaw et al., 2005). Furthermore, Zhang et al. (2006) reported that the amount of N used by crops from municipal solid waste compost for barley, wheat and canola, at two different sites, was 11, 3, 1 and 2% for the first and subsequent 3 years. These results indicate the complexity of estimating N release from different composts and its relationship with plant N uptake. Barbarick and Ippolito (2007) showed that

Table 2 Summary of the effects of organic amendments on crop yields (selected data)

Crop and trial period	Amendments and application rate	Sub-treatment or comment	Highest yield (t ha^{-1})	Yield increase $^{(*)}$ (% t^{-1} of total C applied)	Reference
Wheat (5 years)	MSW composts C1 (40 t ha^{-1}) and C2 (80 t ha^{-1}); FYM (40 t ha^{-1}); MSW + Cf; FYM + Cf	Higher HM content in MSW compost treatments	C2$^{(**)}$: 60.2 t ha^{-1}; C2Cf: 61.9 t ha^{-1}	17	Cherif et al. (2009)
Wheat–maize–sugarbeet rotation (12 years)	Municipal-industrial wastewater sludge: (I) anaerobically digested, (II) dewatered and (III) composted (two rates: 5 and 10 t ha^{-1} year^{-1})	Mineral fertilizer dressings: four increasing rates of urea, plus superphosphate	I (at 10 t ha^{-1}year^{-1}) for sugarbeet: 60.5 t ha^{-1} II (at 5 t ha^{-1} year^{-1}) for wheat: 6.23 t ha^{-1};	10; 11	Mantovi et al. (2005)
Cereals and potatoes (10 years)	Biowaste compost: C1 (9 t ha^{-1} year^{-1}), C2 (16 t ha^{-1} year^{-1}) and C3 (23 t ha^{-1} year^{-1}) plus 5 treatments with combined fertilization	Mineral fertilizer: three increasing rates (N1; N2; N3)	N3 + C1: 5.2 t ha$^{-1(**)}$; N2 + C1: 4.9 t ha^{-1}	15	Erhart (2005)
Maize (7 years)	VFG compost: C1 (22.5 t ha^{-1}) and C2 (45 t ha^{-1}); cattle slurry	Mineral fertilizer	C2 plus cattle slurry: 22.4 t ha^{-1}	5 (from compost C applied)	Leroy (2007)
Tall fescue (7 years)	Three food waste compost with: (1) yard trimmings; (2) yard trimmings + mixed paper waste; 3) Wood waste + sawdust (each at 155 t ha^{-1}rate)	Two composting methods: (I) aerated static pile; (II) aerated turned windrow	(1) 79 t ha^{-1}	0.2$^{(***)}$	Sullivan et al. (2003)
Silage cornfield (5 years)	(1) Low dairy-waste compost rate (to provide 100 kg ha^{-1} of available N); (2) high dairy-waste compost rate (200 kg N ha^{-1}); (3) low liquid dairy-waste rate (100 kg N ha^{-1}); (4) high liquid dairy-waste rate (200 kg N ha^{-1})	Other treatments: ammonium sulfate at 100 kg N ha^{-1} and 200 kg N ha^{-1}	(1) 24.6 t ha^{-1}	9.7 (in the last year of the experiment)	Habteselassie et al. (2006a)

Note: MSW = municipal solid waste; HMs = heavy metals; Cx = compost (x = 1, 2, etc.); Cf = chemical fertilizer; (*) in respect to the control and regarding the yield indicated as (**); VFG = vegetable-fruit-garden waste; (***) in respect to the lowest yield, found in wood waste + sawdust, and for the second method.

six applications once every second year of anaerobically digested biosolids, in a 2-year wheat–fallow rotation, provided about 9 kg N t^{-1} biosolids. Sullivan et al. (2003) noted that 7 years of one-time food waste compost application consistently increased tall fescue N uptake by a total of 294 to 527 kg ha^{-1}. Therefore, the increase in grass uptake due to amendment was 15 to 20% of compost N applied. However, compost N immobilization/mobilization is predominantly linked to the degradability and balance of soil C pools, as will be better explained in Subsection 3.3 of this review.

Moreover, Eghball et al. (2004) found that the lowest rate of P (125 kg ha^{-1}) was applied for the annual P-based beef cattle manure whilst the greatest amount (594 kg P ha^{-1}) was applied for the biennial N-based cattle manure compost treatment. The latter resulted in P leaching to a soil depth of 45 to 60 cm. On the contrary, the P-based application was environmentally sound, since it provided nutrients for the crop, while maintaining the amended soil P at a level similar to the untreated control.

2.2.1 Yield Response

There are three possible scenarios relating crop yield or agronomic productivity to organic C content of soil: (i) increase in crop yield as a consequence of organic carbon pool enhancement; (ii) no or little decrease in crop yield with reduction in the organic carbon pool, and (iii) increase in crop yield with decrease in the organic carbon pool (Lal, 2006). These apparently conflicting responses depend on several factors such as the previous organic carbon pool, soil management, and use of chemical fertilizers and organic amendments.

As demonstrated by several long-term experiments on crop nutrition and yielding responses, the benefits of increased organic matter content will differ on the basis of the rate supplied. In a 5-year trial, Hartl et al. (2003) found that every second year spreading of 40 t ha^{-1} biowaste compost, from source-separated organic household waste and yard trimmings, resulted in slightly higher (9%) rye yields than other rates. This result suggested that beneficial use depends on choosing the best amount and frequency of compost application.

Frequently, the best rate is the highest, as supported by Butler and Muir (2006), who observed the greatest tall wheatgrass dry matter yield with the highest composted dairy manure rate of about 180 t ha^{-1}. More specifically, forage yield increased from 32 to 96% with 11.2 and 179.2 t compost ha^{-1}, respectively, in the first of two growing seasons. Sullivan et al. (2003) highlighted the long-lasting effect of a one-time high rate (155 t ha^{-1}) food waste compost application in providing slow-release N for crop growth, over a 7-year period. These results agreed with the findings of Habteselassie et al. (2006a), who found that soils with about 100 t ha^{-1} dairy-waste compost maintained N supply to the plants through continuous mineralization, as shown by available inorganic N pools, silage corn yield and plant N content analysis.

Wheat grain yield was enhanced on average by 246%, in respect to the control, with a high rate (80 t ha^{-1}) of municipal solid waste compost applied annually over 5 years, alone or combined with mineral fertilizer, reaching 60.2 and 61.9 t ha^{-1}, respectively (Cherif et al., 2009). Similarly, Erhart et al. (2005) investigated the agronomic performance of biowaste compost, on cereals and potatoes, showing that the highest application rate equal to 23 t $ha^{-1}year^{-1}$ increased crop yields by 10% compared with the unfertilized control, on the average of 10 years. These findings are in contrast with those of Mantovi et al. (2005). In repeated sewage sludge applications for 12 years on a winter wheat–maize–sugarbeet rotation, the composted sludge gave a significantly lower yield, equal to 51.8 t ha^{-1}, for sugarbeet with higher dose spreading compared with non-composted biosolid treatment yield (60 t ha^{-1}, on average). Moreover, residual effects of amendments on both soil properties and crop production can last for several years, as referred to in the preceding subsection of this review. For instance, in a 7-year trial Eghball et al. (2004) confirmed that available P concentration in the soil surface can contribute to corn crop P uptake for more than 4 years after the last biennial N-based compost application, being 241% higher than the control.

The examined literature pointed out that the use of composts or of other organic amendments in combination with mineral fertilizers enhanced crop yield in many cropping systems over more than 10 years, compared with compost and amendments alone (Ros et al., 2006a; Bi et al., 2008). The subject has recently been discussed by Montemurro (2009), who found that the multiple application of municipal solid waste compost associated with mineral fertilizer increased wheat yield by 8% compared with mineral N alone and induced a more productive stability and N uptake throughout the years. Furthermore, maize grain yield was the highest where farmyard manure at 10 t ha^{-1} was applied along with recommended NPK fertilizer for 34 years, under a maize–wheat cropping system (Kaur et al., 2008). In a study by Sleutel et al. (2006), 41 years of application of 35 t farmyard manure ha^{-1}, plus an equivalent amount of NPK in mineral fertilizer, every 5 years in a wheat–wheat–sugarbeet–maize–maize crop rotation, increased grain yield by 124 and 55% for maize and wheat in respect to the control. Moreover, in a 7-year study, Leroy et al. (2007) investigated vegetable-fruit-garden-waste compost combined with cattle slurry applied at both 22.5 t ha^{-1} yearly and 45 t ha^{-1} every other year. Both the application strategies resulted in 25 to 43% higher maize yields in respect to the two organic amendments provided alone. On the contrary, Edmeades (2003), after reviewing 14 long-term soil fertility trials, concluded that, despite the positive effects of organic manures on soil biological and physical properties, no advantage to crop yields with respect to the application of the same amounts of nutrients as mineral fertilizers was found. However, it is necessary to note that in organic farming systems the use of mineral fertilizers is not a possible alternative. After all, the analyzed soil management practices used in sustainable farming systems have potential for producing comparable yields to conventional farming ones. Poudel et al. (2002) reported that the average tomato fruit and corn grain yields, for a 5-year trial period, were 71.0 and 11.6 t ha^{-1}, respectively, both not significantly different among organic, low-input and conventional farming systems.

From all the above discussion, there is clear evidence that the best agronomic performance of compost, particularly if combined with mineral fertilizers, is often obtained with both high rates and frequency of applications, leading to residual effects as a slow-release nitrogen fertilizer.

2.2.2 Quality Response

From the yield quality viewpoint, the reviewed long-term research showed, for example, that source-separated organic waste compost as well as mixtures of sugarbeet vinasse composted with other agro-industrial solid wastes did not adversely affect the quality of products (Madejón et al., 2001; Erhart et al., 2005). In particular, winter rye protein concentration was similar in compost and mineral fertilized treatments, and the nitrate concentration of potato tubers in compost treatments was not significantly higher than in the unfertilized control (Erhart et al., 2005). The crop quality in some cases was even improved by compost fertilization. In one above-mentioned study, the partial substitution of mineral with organic N not only did not reduce the quality of wheat, with respect to mineral fertilizer, but also increased the protein content by 6% in comparison with the unfertilized control (Montemurro, 2009). In another study the wheat grain quality, expressed by its apparent specific weight as means of 12 cropping years, was worsened by excessive N supply, so high rates (10 t ha^{-1} year^{-1}) of liquid and dewatered sludge were particularly detrimental, while compost was safer (Mantovi et al., 2005). In fact, a downward trend was observed as $74.7 > 73.5 > 72.8$ kg hL^{-1} for composted, dewatered and liquid sludge, respectively.

Therefore, considering that stabilized organic amendment application does not reduce the crop yield quality, as reviewed here, but can even enhance it, their use can appear more profitable.

3 Environmental Impact and Sustainability of Organic Amendments

As recently highlighted by Lichtfouse et al. (2009), while conventional agriculture is driven almost solely by productivity and profit, sustainable agricultural systems aim at developing new farming practices that are also safe and do not degrade the environment. By taking this into account, and from the previous discussions, it is possible to suggest that appropriate organic matter management is a fully sustainable pattern, because it fulfils three requisites: sustainability of resources, human health and economic sustainability. Conversely, organic amendments that can improve organic matter and the linked soil fertility can also be a source of environmental pollution, especially when they are improperly used. In fact, when the application rates of manure are calculated on the N crop requirement, the amount of P added often exceeds the plant P requirement, resulting in soil P accumulation (Miller and Miller, 2000). According to Komatsuzaki and Ohta (2007), soil management strategies for sustainable agriculture should focus not only on increasing organic matter in the soil, but also on the uptake or stocking of soil residual nutrients in such a way to prevent excess nutrient leaching into the groundwater.

The cropland application of immature compost can produce environmental and agronomic problems. In fact, if the organic material has not been sufficiently stabilized, its application increases ammonia volatilization, decreases the soil oxygen concentration, produces some phytotoxic compounds and immobilizes soil mineral N. Therefore, organic products of high quality must be produced and their stability must be accurately assessed.

With regard to toxic elements, e.g. heavy metals, their accumulation in soils is the most often cited potential risk, particularly for biosolid waste compost use. On the other hand, there is increasing positive evidence of the impact that composts and wastes can have on soil C sequestration, as we will discuss in the following pages.

3.1 Organic Matter Evolution and Soil Carbon Sequestration

In recent years, global concern over increased atmospheric CO_2 and methane (CH_4) emissions has raised interest about the potential role of soils as a source or sink of C and in studying organic matter dynamics and related C sequestration capacity. In fact, organic matter varies in both decomposition rate and turnover time. and the soil C pool can be a source or sink for the atmospheric pool, depending on land use and management (Van-Camp et al., 2004). When organic materials, such as composted wastes, are added to soil, at least a share of their organic C is decomposed producing CO_2, which is a good indicator of the decomposition rate, while another part is sequestered in the soil.

For the purpose of this review, the term "carbon sequestration" is used according to Lal (2004, 2007), implying the transfer of atmospheric CO_2 into the soil C pool through: (i) humification of crop residues and other wastes added to the soil, (ii) formation, in arid and semi-arid regions, of secondary carbonates or leaching of bicarbonates into the groundwater, so CO_2 thus adsorbed is not immediately re-emitted, (iii) formation of organo-mineral complexes which encapsulate C and protect it against microbial activities, and (iv) translocation of organic carbon into the sub-soil, that can move it away from the zone of disturbance by plowing and other agronomic practices, minimizing the risks of being removed by erosional processes.

Article 3.4 of the Kyoto Protocol emphasizes the agricultural role in CO_2 sequestration in tilled soils and advocates sustainable cropping techniques for this purpose. These practices primarily include the reduction of soil disturbance and the optimization of water-use efficiency. However, soil incorporation of organic materials and N fertilization can also influence C dynamics (Triberti et al., 2008). Any attempt to enrich the organic carbon reservoir through sequestration of atmospheric C will help to manage global warming.

According to Feller and Bernoux (2008) findings, it is essential to consider that when an organic waste contains a high percentage of fossil carbon, the C in soils originating from this fraction should not be referred to as sequestered C, i.e. originating from the atmosphere, but as "stored C".

As explained by Mondini and Sequi (2008), it is also important to consider that the reduction in the soil of the C pool contributes both to an atmospheric enrichment in CO_2 concentration and an involution in soil fertility. This reduction also induced the onset of degradative processes such as erosion, salinization, desertification, compaction, nutrient deficiency, etc. However, whereas the exact magnitude of the historic loss of organic carbon may be debatable, Lal (2004) suggested that the troubling process of organic carbon depletion can be reversed.

The effects of organic amendments on organic carbon increase should be studied through long-term field experiments, as already seen in Subsection 2.1.2. of this review, because of the long time needed to attain a new equilibrium after environmental change in the organic matter.

Triberti et al. (2008) reported that 29 years after the start of a trial comparing cattle manure, cattle slurry and crop residues with mineral fertilization, the cattle manure gave the quickest organic carbon stock build-up, 0.26 t organic carbon ha^{-1} $year^{-1}$. Each t dry matter ha^{-1} of applied cattle manure, containing 33.1% of organic C, increased the organic carbon stock by 27 kg C ha^{-1} in the 0–0.4 m soil layer. This increase corresponded to the highest sequestration efficiency, equal to 8.1% of the C added, due to its low degradability, as compared with 3.8 and 3.7% in the instances of cattle slurry and cereal crop residues. On the whole, the recycling of cattle farming by-products for CO_2 sequestration purposes poses environmental risks, such as pollution by nitrate N (NO_3–N), that can limit its sustainability in fertile cropland of developed countries.

About 25 and 36% of applied manure and compost C remained in the soil after 4 years of application, indicating greater C sequestration with composted than non-composted manure (Eghball, 2002). According to Sodhi et al. (2009), a 10-year application of rice straw compost, either alone or in combination with inorganic fertilizers, results in C sequestration in macroaggregates. In fact, with the application of 8 t compost ha^{-1}, the C concentration in the 1–2 mm size fraction increased by 180 to 191%, respectively, over unfertilized control. From trials still in progress for more than 10 years, there is evidence that the organic carbon sequestration rate increased more due to farmyard manure and composted farmyard manure in comparison with mineral fertilizers or other organic materials (Monaco et al., 2008; Kukal et al., 2009).

Mandal et al. (2007) observed that the total quantity of soil C sequestered over a long period was linearly related to the cumulative crop residue C inputs, and the rate of the conversion to organic carbon was higher in the presence of added organic materials, i.e. 6.9% of each additional t C input ha^{-1}, than in their absence (4.2%). Indeed, it has been calculated that the rate of organic carbon sequestration is on average 0.3 to 0.5 t C ha^{-1} year^{-1} under intensive agricultural practices (Lal, 2007). As reported by Mondini and Sequi (2008) organic matter is the largest C stock of the continental biosphere, with 1550 billion tons. On the time scale of several decades, in arable soils which receive high organic materials, relatively less organic matter is stabilized either by association with the silt plus clay mineral fractions, or by its inherent biochemical recalcitrance (Sleutel et al., 2006).

Shindo et al. (2006) reported that continuous compost application in a field subjected to 25 years of double cropping could increase both the amounts of fulvic and humic acids, and the total humus content. From the organic carbon sequestered quality standpoint, Nardi et al. (2004) found that, over 40 years, farmyard manure fertilization improved by 116% the production of humus with a high degree of polycondensation, a high-quality fraction usually linked to soil fertility. Conversely, the absence of organic fertilizer inputs determined the opposite, with a higher percentage of non-complex and lightweight humus.

Besides, the soil C sequestration should not be restricted to a mere quantification of C storage or CO_2 balance. All greenhouse gas fluxes must be computed at the plot level in C–CO_2 or CO_2 equivalents, incorporating as many emission sources and sinks as possible across the entire soil-plant system (Feller and Bernoux, 2008). For example, results of Ginting et al. (2003) showed that fluxes of CH_4 and nitrous oxide (N_2O) were nearly zero after 4 years of manure and compost applications. This evidence indicates that the residual effects had no negative influence either on soil C and N storage, or global warming. However, there is a lack of information and a strong need to further understand the greenhouse gas fluxes as related to organic material input dynamics in the soil.

The reviewed results suggest that the process of organic carbon depletion can be reversed by long-term organic amendment application. Soil carbon sequestration should be considered as a “win–win” strategy for increasing soil fertility and preventing soil erosion processes (Sánchez-Monedero et al., 2008).

3.2 Heavy Metal Concentration in the Agro-Ecosystem

Despite the numerous benefits, the agricultural utilization of raw and composted wastes could also induce adverse impacts on the environment. This is particularly due to the types of toxic elements contained in these organic materials that might enter the food chain, since they may be taken up by plants from soil.

The concentration of heavy metals in compost is generally higher than the normal concentration in soil, so the possibility exists of metal accumulation when the compost is repeatedly applied (Zhang et al., 2006). On the other hand, little research is available from studies lasting decades and longer about the availability of metals applied as constituents of composted organic matter. Over a 10-year trial period, the most abundant metals in the uppermost soil horizon were copper (Cu), zinc (Zn) and lead (Pb). Cadmium (Cd) was the least plentiful, corresponding to the mean metal concentrations in the municipal solid waste compost applied (Businelli et al., 2009). This is supported by the opposite findings of Bergkvist et al. (2003) who found, during a period of 41 years, 92% of applied Cd was recovered in the topsoil in sludge treatment, indicating measurable losses by both downward movement and crop uptake.

Six-year consecutive applications of a swine compost resulted in significantly higher concentrations of Cu and Zn at 10–20 cm depth of the compost-amended soil, relative to the control, with an increase from 102.8 to 127.4 mg kg^{-1} for Cu and from 111.9 to 165.7 mg kg^{-1} for Zn (Zhao et al., 2006). On the other hand, Bartl et al. (2002) found that $32t$ ha^{-1} of biowaste compost did not influence the total contents of Cd, manganese (Mn), molybdenum (Mo) or nickel (Ni) in soil, in 5 years. The total soil contents of Zn and Pb were significantly higher in soils with compost treatment than in the unfertilized soils.

From experiments longer than 10 years, it is possible to suggest that sludge and composted sludge showed a high accumulation of Zn, Cu and chromium (Cr), probably due to the notably higher concentration of these metals in the raw materials (Saviozzi et al., 1999; Kunito et al., 2001). The soil enzyme activities, dehydrogenase, urease and β-D-glucosidase were also found to be adversely affected by the metals derived from the addition of sewage sludge (Kunito et al., 2001).

Notwithstanding, the concentration of pollutants in composts may be reduced through the correct separation of organic wastes at source, which offers the opportunity of high-quality input material for aerobic biodegradation (Montemurro et al., 2009). For the long-term protection of the environment, it is also necessary to develop and implement other preliminary treatments for potentially polluting wastes. Erhart et al. (2008) showed that, after 10 years of application, the use of high-quality biowaste compost gives no variation in either total heavy metal concentrations or available fractions. In particular, with total applications of 95, 175 and 255 t biowaste compost ha^{-1}, no heavy metal significant increase was measurable, except for Zn in the treatment with the highest application rate.

However, the environmental hazard is strictly linked to the mobility of metals, and to their concentration in the soil solution rather than to the total soil concentration. According to Businelli et al. (2009), metal mobilization is not an immediate process, but it involves various equilibria that control their adsorption and desorption. These behaviors depend on soil characteristics and climatic conditions and they involve biochemical processes responsible for the microbial degradation of autochthonous and compost-derived organic matter. This highlights the importance of long-term experimentation when studying the environmental fate of heavy metals, particularly in compost-amended soils on large scales (Businelli et al., 2009).

Within this context, according to Tittarelli et al. (2007), the main factors that affect the environmental behavior of heavy metals are: (i) cation exchange capacity, as an index of the soil capacity to adsorb and hold metal cations; (ii) humic substances, that can interact with heavy metals, forming complexes with different solubility, and consequently mobility, and (iii) the water and thermic regime of the soil, which affect the organic matter decomposition.

It can also be generally assumed that extractability and uptake of heavy metals decline as the soil pH becomes more alkaline, especially after repeated compost application. By contrast, low pH in the soil, caused by more than 60 years of rice straw compost applications, may have enhanced the concentration of metals in the water-soluble fraction (Takeda et al., 2005).

The aerobic composting processes increase the complexation of heavy metals in organic waste residues. In this condition the metals are strongly bound to the compost matrix and organic matter, limiting their solubility and potential bioavailability in soil (Smith, 2009). However, 10 years after municipal solid waste compost application, a share of the heavy metals was further re-mobilized in the soil profile, also leading to a decrease in the percentage distribution of organically-bound heavy metals with time. According to Businelli et al. (2009), this metal mobilization was primarily influenced by organic matter dynamics. On the contrary, Sukkariyah et al. (2005) reported that in aerobically digested sewage sludge-amended soils, the extractability of the heavy metals steadily declined over 17 years, despite a significant decline in organic matter concentration in the amended plots. This outcome showed that the mineralization of exogenous applied organic matter did not increase heavy metal availability due to loss in metal binding capacity associated with organic matter.

Monitoring concentrations of various heavy metals, their fractionation and the percentage contribution of each fraction to the total concentration are important analytical tools. In fact, in soil receiving long-term application of compost or other organic materials with relatively high concentrations of metals, these determinations can produce important information at the present time and help to determine what might happen in the future (Zhao et al., 2006; Businelli et al., 2009).

In any case, moderate compost doses applied in current agriculture will not cause any risk of toxicity for plants or animals, and therefore it is reasonable that application rates always have to be chosen on the basis of limited heavy metal loadings. However, no adverse effect on plant growth or excessive amounts of plant metal uptake were noted 17 to 19 years after biosolid application, despite the high application rate, equal to 210 t ha^{-1}, containing concentrations of Cu and Zn that exceeded the pollutant concentration limits (Sukkariyah et al., 2005).

In general, heavy metal uptake by crops increases in leafy plants and it is higher in cereal leaves than in grain. Lettuce, for example, had higher assimilative capacity for Zn and Cd uptake than other non-leafy crops (Sukkariyah et al., 2005). Mantovi et al. (2005) reported that biosolid applications significantly increased the content of Zn and Cu in wheat grain and of only Cu in both sugarbeet roots and maize grain. Cadmium is one of the most significant potential contaminants of food supplies on arable lands and may limit sewage sludge suitability for soil amendment, because this organic material presents a large amount of this metal (Singh and Pandeya, 1998). Being relatively soluble in soils, it is readily taken up by crops and it is quite toxic to humans (Miller and Miller, 2000). This behavior is particularly evident at low soil pH. Cd solubility in equilibrium extracts of $Ca(NO_3)_2$ increased, during the 41-year trial above mentioned, by a factor of 20 in the sludge treatment compared with the control (Bergkvist et al., 2003). This was reflected in the Cd concentration of the straw fraction in barley, which was almost doubled in the sewage sludge treatment, compared with the control. The grain fraction, on the other hand, showed no significant increase in Cd concentration.

It can be concluded that there is no tangible evidence demonstrating negative impacts of heavy metals applied to soil, particularly when high-quality compost was used for a long period. Composting processes also inherently reduce metal availability compared with other organic waste stabilization methods (Smith, 2009).

3.3 N Pool Fate

Proper use of organic amendments requires the capacity to predict the release in the soil of inorganic nutrients from organic forms. Sikora and Szmidt (2001) suggested that a better understanding of coupled mineralization-nitrification is essential to manage the soil N pool with an environmentally sound application of amendments.

The C/N ratio, which is an important tool of amendment evaluation, cannot explain all differences in N mineralization, since organic materials with similar C/N ratios may mineralize different amounts of N. This behavior is probably due to other differences in their chemical compositions. Repeated long-term applications of organic amendments not only generally increase the size of the soil organic N pool, but also cause remarkable changes in soil characteristics, that influence N dynamics and can lead to a residual effect.

Habteselassie et al. (2006a) found an 89% increase in total soil N content after 5 years when dairy-waste compost at 200 kg N ha^{-1} was applied. Conversely, Zaman et al. (2004) observed, after 23 years of 240 kg Nha^{-1} sewage sludge plus sawdust compost application, an increase in total N content of approximately 14%, as compared with a chemical fertilizer supply. The increase in total soil N after compost, biosolids or compost-plus-N was closely related to the build-up of organic matter in the soil over time and may be attributed to the direct effect of organic inputs (Mantovi et al., 2005; Ros et al., 2006a). After 7 years of application, the soil N concentration in the topsoil accounted for 33% of compost N applied (Sullivan et al., 2003). Antil et al. (2005) showed that the highest total soil N content increase, by 70% in respect to NPK mineral fertilizer, was found after 38 years of animal-manure liquid applications, as compared with solid animal-manure, cattle slurry and half cattle slurry plus straw. Moreover, in the plots after 13 years of cropping, compared with those kept fallow for the same period, there was a clear impact of N removal due to both the harvest and N losses, following enhanced mineralization due to tilling.

The N uptake by crops depends on several factors such as the N content and C/N ratio of the amendment, soil characteristics, climatic conditions and, obviously, on the plant's N requirements. Research on an average of 10 years by Hartl and Erhart (2005) pointed out that the ratio of N output by harvested plant parts to total N input in the source-separated organic waste compost treatments ranged from 3 to 7% of the applied N. This result was lower compared with mineral fertilization (11 to 15%), even if it was not reflected by a higher proportion of NO_3–N in the soil profile at the end of the growing season.

Data collected on N recovery from different composts, related to N uptake or yield, showed that the N effect of compost application does not generally exceed 15–20% of total N supply in the first year, while the residual compost N pool is mineralized at rates of 3–8% in following years. Continuous compost amendments and crop rotations with high nutrient demand may increase N mineralization (Amlinger et al., 2003). Eghball (2000) found that of the organic N applied to provide corn N requirements, 151 kg available N ha^{-1} for an expected 9.4 t ha^{-1} grain yield, about 11% was mineralized from composted beef cattle manure and 21% from non-composted manure during the succeeding growing season. A lower N availability from compost reflects the presence of stable N compounds.

The organic matter mineralization process increases the amount of ammonium and nitrate in the soil. However, the NO_3–N is minimally adsorbed by the soil particles because it is very mobile and is susceptible to losses into ground- and surface waters by infiltrating water. Within this context, it is essential to remember that the synchronization of N supply with crop N demand, together with a proper application rate, is the best way to avoid the accumulation of soil mineral N, thus reducing the risk of NO_3–N leaching (Montemurro and Maiorana, 2008).

The long-term application effects of raw and composted organic materials on nitrate leaching have been evaluated by several researchers, but the results of the studies are often discordant. In fact, Mallory and Griffin (2007) in a 13-year experiment found that, despite similar NH_4–N inputs and rates of NH_4–N consumption for manure and fertilizer N treatments, NO_3–N accumulation was slower in the manure treatment. This is because the N from manure became available more slowly than fertilizer N. On the contrary, Basso and Ritchie (2005), in a 6-year maize–alfalfa rotation, observed the highest amount of NO_3–N leaching of 681 kg ha^{-1} in the manure treatment, followed by compost (390 kg ha^{-1}), inorganic N (348 kg ha^{-1}) and control (311 kg ha^{-1}). They suggest that, although manure applications can be valuable for organic matter increase, attention needs to be given to the possible environmental impact without benefiting from yield increase.

From several experiments longer than 8 years conducted under different soil and climate conditions, it might be concluded that compost fertilization resulted in equal or lower NO_3–N leaching losses to groundwater than corresponding mineral supply (Hartl et al., 2001; Hartl and Erhart, 2005; Erhart et al., 2007). These findings are observed even with higher amounts of amendments than used in practical farming. Nevens and Reheul (2003) reported that, at the end of a 4-year trial, the increase in residual soil NO_3–N was smaller with vegetable-fruit-garden waste compost and compost plus cattle slurry applications, in respect to mineral fertilizer treatment.

Regarding the effect of the application rates, over a 5-year field trial with permanent rye, the treatments receiving 20 to 40 t ha^{-1} of biowaste compost per single application showed smaller amplitudes in the soil NO_3–N levels in fall than the treatments receiving 60 t ha^{-1} (Hartl et al., 2003). Other studies have noted that compost produced more nitrate than needed for plant use throughout a 5-year period. This is particularly evident at a high rate of application. The continuing mineralization of the organic material, even after harvest, led to postharvest nitrate production and a consequent downward movement (Habteselassie et al., 2006a). Furthermore, measurements of gross N transformation rates are important to properly understand N cycling in agricultural soils, where both productive and consumptive processes occur. Habteselassie et al. (2006b) reported that, by applying dairy-waste compost and liquid dairy-waste annually for 6 years, the mean gross N mineralization rates were about 5.7 and 2.9 mg N kg^{-1} day^{-1}. Conversely, the gross nitrification rates were 10.2 and 1.6 mg N kg^{-1} day^{-1}, respectively, and the net mineralization rates were less than 35% of gross rates. The ratio of gross nitrification to mineralization for dairy-waste compost-treated plots were more than 100%, indicating a large increase in nitrifying capacity of the soil following repeated applications, which is undesirable due to the known high mobility of nitrate.

Zaman et al. (2004) found that the long-term application of sewage sludge, composted with rice husk or sawdust, has showed a positive effect on gross and net rates of N mineralization, and net nitrification rates, probably due to high levels of microbial and enzyme activities. The same authors also found that chemical fertilizer-treated soils showed negative net N mineralization rates, which is indicative of N immobilization. These assessments are contradicted by Poudel et al. (2002), who observed that organic systems managed with cover crops and composted animal manure showed 112% greater potentially mineralizable N pools than conventional systems. They also showed N mineralization rates to be lower by 100% throughout the growing season, as compared with conventional systems. This reduction corresponds to a reduced risk for NO_3–N leaching and groundwater pollution.

The valuation of the potentially mineralizable organic N pool for soils that have received organic amendments is important for estimating the capacity of these soils to provide N over time. For example, residual effects of 4-year compost and manure application resulted in 42 to 74% higher potentially mineralizable N compared with synthetic N fertilizer treatment (Ginting et al., 2003). Furthermore, when both organic amendments are added to soil and biological oxygen demand by decomposer microorganisms exceeds the supply, the anaerobic microenvironment necessary for denitrification is created and the intermediate product N_2O gas is formed. This gas is a strong “greenhouse gas” and can also be emitted into the atmosphere (Epstein, 1997). The long-term application of stabilized organic materials at 300 kg N ha^{-1} $year^{-1}$ significantly increased N_2O emissions from 150 g N_2O–N ha^{-1} $year^{-1}$ in the control treatment soil to 856 g N_2O–N ha^{-1} $year^{-1}$ in the organic ones (Meng et al., 2005). N_2O production was also significantly higher in the 0–15 and 15–20 cm layers of the compost-treated soils than in the respective layers of the chemical fertilizer-treated ones (Zaman et al., 2004). The higher N_2O emissions from some compost-treated soils highlight the need for quantifying N_2O emissions from agricultural land. This is particularly important where composts are used instead of chemical fertilizers (Sikora and Szmidt, 2001).

In summary, the repeated application of composted materials can enhance soil organic N content, storing it for mineralization in the following cropping seasons.

4 Conclusion

Only a few published studies have focused on the long-term effects of organic amendments on the soil-plant system for sustainable crop production. Therefore, our work attempted to address this issue by using the results from long-lasting fertility trials, providing a useful platform for future research.

The basic reflections of this review include:

(i) long-term trials are the best indicators of both organic material utilization and sustainability. In fact, many effects, e.g. release of nutrients, carbon sequestration and possible build-up of toxic elements in the soil, evolve slowly, thus needing time to be tested. These effects are present only when repeated applications occurred;
(ii) addition of exogenous organic matter to cropland can lead to an improvement in soil biological functions, even more than 15 years after spreading, depending on the quantity and quality of materials applied. For example, microbial biomass C increased up to 100% with high rate compost treatments, while enzymatic activity was enhanced by up to about 30% with sludge additions;
(iii) several organic amendments’ long-lasting application can enhance soil available potassium, extractable phosphorous and, particularly, organic carbon. Soil organic carbon increased by about 90%, in respect to unfertilized soil, and by more than 100% as compared with chemical fertilizers;
(iv) regular addition of organic amendments increased soil physical fertility, mainly by improving aggregate stability and decreasing soil bulk density;
(v) the best agronomic performance of compost is obtained with the highest rates and frequency of utilization. This is particularly evident if the amendment is combined with mineral fertilizers. Furthermore, there were residual effects because it is a slow-release nitrogen fertilizer;
(vi) crop yield was enhanced by about 250%, with high rates of municipal solid waste compost repeated applications. There is also some evidence that stabilized organic amendments do not reduce the crop yield quality, but even enhance it;

(vii) long-term organic amendment application can play a positive role in climate change mitigation by soil carbon sequestration, which in turn can reverse the process of soil degradation;

(viii) agricultural utilization of raw and composted wastes could induce adverse environmental impacts. However, there is no tangible evidence demonstrating negative impacts of heavy metals applied to soil, particularly when high-quality compost was used for a long period. Composting processes also reduce metal availability;

(ix) repeated application of composted materials can enhance soil organic N content, by up to 90%, storing it for mineralization in the following cropping seasons, often without raising the nitrate leaching to groundwater.

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Tillage Management Effects on Pesticide Fate in Soils

Lionel Alletto, Yves Coquet, Pierre Benoit, Djilali Heddadj, and Enrique Barriuso

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Abstract Reducing tillage intensity through the implementation of conservation practices is a way to reach a more sustainable agriculture. Reducing tillage is indeed an efficient way to control soil erosion and to decrease production costs. Nonetheless, the environmental impact of reduced tillage is not well known because conservation techniques may induce strong changes in soil physicochemical properties and biological activity. Knowledge on the fate of applied pesticides under conservation practices is particularly important from this point of view. We review here the advances in the understanding, quantification and prediction of the effects of tillage on pesticide fate in soils. We found the following major points: (1) for most dissipation processes such as retention, degradation and transfer, results of pesticide behaviour studies in soils are highly variable and sometimes contradictory. This variability is partially explained by the multiplicity of processes and contributive factors, by the variety of their interactions, and by their complex temporal and spatial dynamics. In addition, the lack of a thorough description of tillage systems and sampling strategy in most reports hampers any comprehensive interpretation of this variability. (2) Implementation of conservation tillage induces an increase in organic matter content at the soil surface and its gradual decrease with depth. This, in turn, leads to an increase in pesticide retention in the topsoil layer. (3) Increasing retention of pesticides in the topsoil layer under conservation tillage decreases the availability of the pesticides for biological degradation. This competition between retention and degradation leads to a higher persistence of pesticides in soils, though this persistence can be partially compensated for by a more intensive microbial activity under conservation tillage. (4) Despite strong

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_35,

from Alletto et al., Agron. Sustain. Dev. 30 (2010) 367–400. DOI: 10.1051/agro:2009018

changes in soil physical properties under conservation tillage, pesticide transfer is more influenced by initial soil conditions and climatic conditions than by tillage. Conservation tillage systems such as no-tillage improve macropore connectivity, which in turn increases pesticide leaching. We conclude that more knowledge is needed to fully understand the temporal and spatial dynamics of pesticide in soil, especially preferential flows, in order to improve the assessment of pesticide risks, and their relation to tillage management.

Keywords Conventional tillage • Conservation tillage • Herbicide • Retention • Degradation • Transport • Soil carbon

1 Introduction

Tillage can be defined as any mechanical operation on the soil and crop residues that aims at providing a suitable seedbed where crop seeds are sown. Soil preparation has always been an important component of traditional agriculture. Since 6000 BC, a wide variety of farming tools have been developed, from the simple digging stick to the paddle-shaped spade that could be pulled by humans or animals (Lal et al., 2007). In the 18th century, the mouldboard plough, a curved board that cuts and rotates the soil at a significant working depth, of more than 20 cm, was introduced in England. This tool was particularly efficient in incorporating organic residues, fertilisers and lime, in controlling weeds and helping the growing process. In the early part of the 19th century, the "initial" model of the mouldboard plough received several improvements to reach its "standardised" version by 1870, similar to current models (Hanson, 2006; Lal et al., 2007).

In the United States, between 1910 and 1930, the use of the mouldboard plough widely expanded, especially in the Great Plains of the Middle West. In Europe, its use started to be significant after 1945. These changes in tillage practices were accompanied by significant progresses in seed selection and by the development of the use of chemicals for plant protection, allowing a significant increase in crop yields. For example, wheat yields in France increased from 0.8-1 tons per hectare in 1945 to more than 8 tons in 1985. Because of its success, the mouldboard plough became a centrepiece of traditional agriculture.

In parallel with this growth of agricultural production, the widespread use of the mouldboard plough led to severe problems of soil and environmental degradation. One of the most well-known environmental problems was the drastic increase in wind erosion known as the "Dust Bowl" in 1930 in the US Great Plains (Lal et al., 2007; Masutti, 2004). Faced with this major problem, depriving some areas of any production capacity, the American reaction was swift. In 1935, the Soil Conservation Service – now called the Natural Resources Conservation Service – was created within the United States Department of Agriculture. Many important research programmes and communication efforts aimed at promoting the adoption of soil conservation techniques, grouped under the term "conservation tillage". These techniques are defined as any tillage and planting system that leaves at least 30% of the soil surface covered by crop residue after planting to reduce soil erosion by water, or at least 1.1 tons of crop residue/ha to reduce soil erosion by wind. A broad spectrum of farming methods can thus be classified as conservation techniques: from tillage techniques with a high working depth, such as sub-soiling, to reduced tillage of 0–15 cm or direct drilling techniques without any preliminary soil tillage, called "no-tillage". This last type of tillage technique remained less developed until 1940 with the discovery of hormonal herbicides such as 2,4-dichlorophenoxy acetic acid (2,4D) that allowed farmers to control weeds without ploughing. Fairly quickly, these North American techniques thrived in the South American countries, particularly in Brazil where water erosion was significant (Bernoux et al., 2006), and in Argentina and Chile. Tillage practices and their codes used in this review are reported in Table 1.

In Europe, because soil erosion was not as great as in the US, the interest of farmers in conservation tillage was limited. In the years 1970–1980, some attempts to develop these techniques were faced with decreases in crop yields, while, at the same time, farmers were encouraged to produce more to earn more. At the beginning of the 1990s, with the Rio conference (1992) which laid down the foundations for a sustainable agriculture, the concerns moved towards considering the vulnerability of water, soil and landscape resources. Moreover, the emergence of agricultural product surpluses led to lower prices. The control by farmers of their income more than ever implied lower production costs and a further increase in productivity, especially

Table 1 Tillage practice types used in this review article

Code	Tillage practice	% soil surface covered by crop residues[†]	Agricultural tool
CT	Conventional tillage	<30% (0–10%)	Mouldboard plough Disk plough Spading machine
CnT	Conservation tillage		
MT_{Dk}	Mulch tillage	>30% (30–50%)	Disk harrow
MT_{Ch}	Mulch tillage	>30% (30–50%)	Chisel plough, harrow…
SS	Sub-soiling	>30%(30–60%)	Sub-soiler, deep ripper, paratill
RT	Ridge tillage	>30%(40–60%)	Ridger
ST	Strip tillage	>30%(40–60%)	Strip-till
NT	No-tillage (or direct drilling)	>50% (depending on crop residues)	Seeding drill (with disc openers)

[†] (CTIC, 2006).

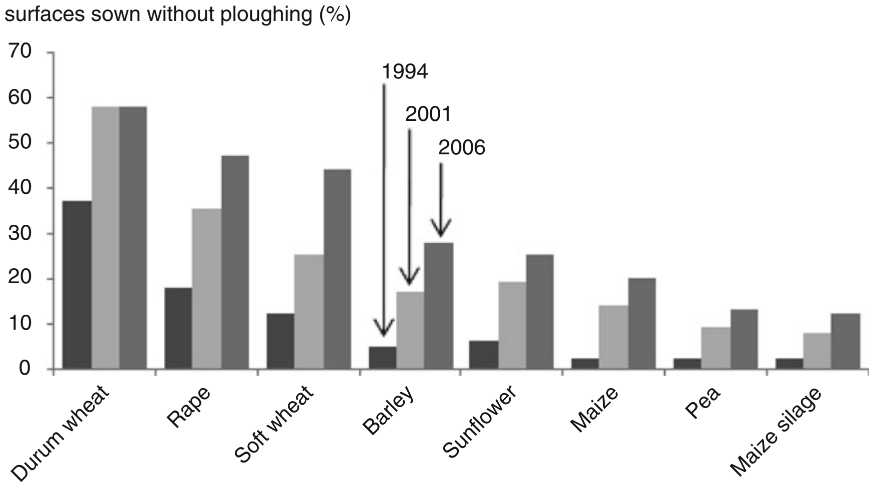

Fig. 1 Evolution of cultivated surfaces without ploughing in France between 1994 and 2006. Whatever the crop, the trend is an increase of surfaces cultivated without ploughing and winter crops are more often sown without ploughing than spring crops (Agreste, 2004, 2008)

as the size of farms expanded. Under this economic context, soil conservation tillage, deemed to be faster and less costly in time and energy than conventional tillage, finally started to become attractive.

Progressively, surfaces sown in France without ploughing increased for all crops (Fig. 1). Spring crops are, however, more frequently sown after ploughing than winter crops. Moreover, the development of conservation techniques varies according to the regions. For example, in the south-west part of France – Aquitaine and Midi-Pyrénées regions –, more than 75% of the wheat surfaces were not ploughed in 2006 against 50% in the whole country (Agreste, 2008).

One of the main roles of tillage is to provide an efficient control of weeds. Indeed, tillage influences weed populations by the combined effects of mechanical destruction of weed seedlings and by changing the vertical distribution of weed seeds in soil (Peigné et al., 2007). It also changes the soil climatic conditions which control weed dormancy, germination and growth. Reducing tillage intensity generally tends to increase the concentration of weeds in the topsoil (Moonen and Barberi, 2004; Torresen et al., 2003; Vasileiadis et al., 2007) and is very often associated with an increase in herbicide use (Fig. 2).

Although many studies have been conducted, mainly on the North American continent, the environmental fate of pesticides under conservation tillage presents many contradictions and remains, finally, poorly understood (Aubertot et al., 2005; Réal et al., 2007). Publication of articles concerning the effects of tillage practices on pesticide fate in soils, water and

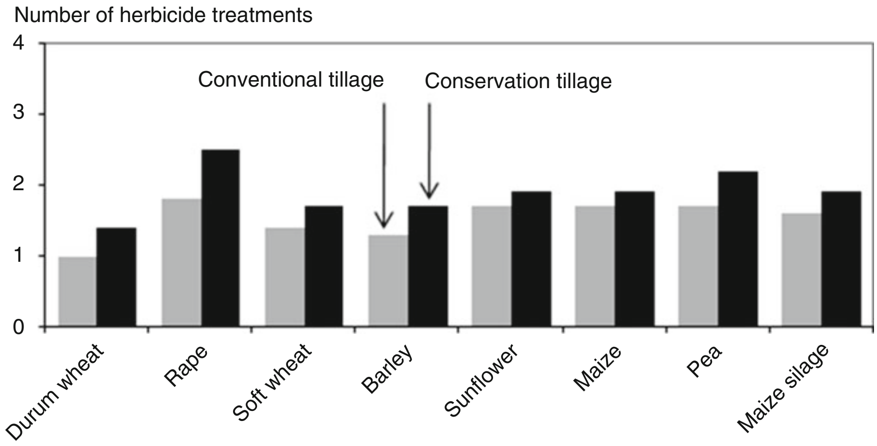

Fig. 2 Mean number of herbicide treatments applied in 2006 for several crops sown after ploughing (conventional tillage) or without ploughing (conservation tillage). Note the systematic increase of herbicide treatments with the implementation of conservation tillage (Agreste, 2004, 2008)

air started slowly during the 1970s and showed a rapid increase at the end of the 1980s followed by a peak rate of publication at the end of the 1990s (Fig. 3a). One-third of the published articles were found in one journal, and the first three journals were from the US, illustrating the great interest of US scientists in this subject (Fig. 3b).

Reducing tillage intensity leads to significant and complex changes in soil physical, chemical and biological properties, most often interrelated with each other, thus affecting the fate of the applied pesticides. A first review, conducted by Locke and Bryson (1997), dealt with herbicide interactions in soils under plant residue-managed farming systems. Following their work, we sought to assess the current state of knowledge of the effects of tillage on the processes involved in the fate of pesticides in soils cultivated with arable crops. Figure 4 summarises the main changes in soil properties due to tillage practices and their effects on the mechanisms involved in pesticide fate. As shown in this figure, understanding the effects of tillage on pesticide fate in soils implies a comprehensive evaluation of all the interactions between the different dissipation processes and of all the different soil factors affected by tillage operations. Some of these interactions are now well known, but most of them are still poorly understood. In this review, for each of these processes, we paid particular attention to the understanding of the mechanisms involved, their relative importance and the origin of the contradictory results found. The lists of the molecules, mainly herbicides, mentioned in this review are shown in Table 2.

2 Interception – Retention

Pesticide interception depends on several factors such as the type of application (pre- or post-emergence), the presence of a crop and, if any, plant growth stage and species, the presence of a mulch, etc. Because interception of pesticides by plant foliage and its effect on the environment have not been widely studied (Reddy and Locke, 1996), the subject developed in this part focuses mainly on the interception and retention by the mulch and by the soil. In some cases, due to the difficulty of formally separating interception and retention, these processes are discussed simultaneously.

2.1 Mulch Effect

The accumulation of organic residues on soil surface (mulch) in conservation tillage generally leads to an increase in the interception of applied pesticides, more particularly for apolar pesticides or pesticides with a low polarity (Locke, 1992; Novak et al., 1996; Reddy and Locke, 1998; Reddy et al., 1997a, b; Zablotowicz et al., 2000). This interception depends on the amount and type of crop residues and, for example, with a proportion $\geqslant 30\%$ of the soil surface covered, it was found to range from 40 to 70% of the applied dose for several preemergence herbicides (Banks and Robinson, 1982; Ghadiri et al., 1984; Isensee and Sadeghi, 1994; Sadeghi and Isensee, 1997). In terms of mass of residues, for a quantity >4.5 t ha^{-1}, the interception of

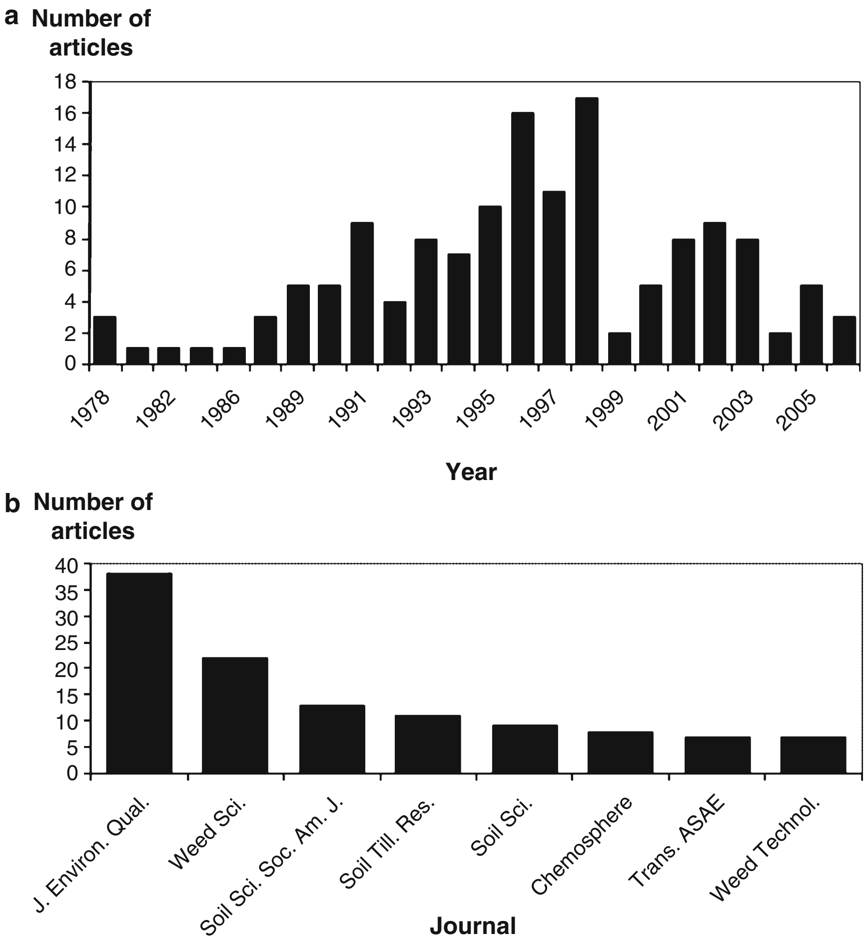

Fig. 3 Articles published on the effects of tillage practices on pesticide fate in soils, water and air. (**a**) Time series of articles found per year; and (**b**) major source journals found in (**a**). The plot includes the top 8 journals representing 115 articles out of the 144 references cited in the review

acetochlor, alachlor and metolachlor was higher than 90% of the applied doses (Banks and Robinson, 1986). Retention processes are closely associated with the interception of pesticides. Indeed, crop residues can have sorption capacities 10 to 60 times higher than soil (Boyd et al., 1990; Reddy et al., 1995b) and can significantly modify the (bio)availability and the migration of pesticides in soil. As a result, a loss of efficacy of some pesticides has been observed (Erbach and Lovely, 1975; Mills et al., 1989; Shelton et al., 1998) which can lead to an increase in the applied doses (Shelton et al., 1998; Worsham, 1991). By penetrating inside crop residues, most of the molecules are physically entrapped within cell wall structures, such as cellulose microfibrils embedded in a lignin-hemicellulose matrix (Dao, 1991) and, most often, lose their activity.

The nature and decomposition degree of crop residues both influence interception and retention of pesticides, but in contrasted ways depending on studies. For example, hairy vetch (*Vicia villosa* Roth) residues had a higher retention for chlorimuron than rye (*Secale cereale* L.) residues (Reddy et al., 1995) and a higher retention for fluometuron than wheat (*Triticum aestivum* L.) residues (Gaston et al., 2001) (Table 3). This greater sorption by vetch than rye or wheat residues may be related to differences in physical state, that offered a greater surface area for

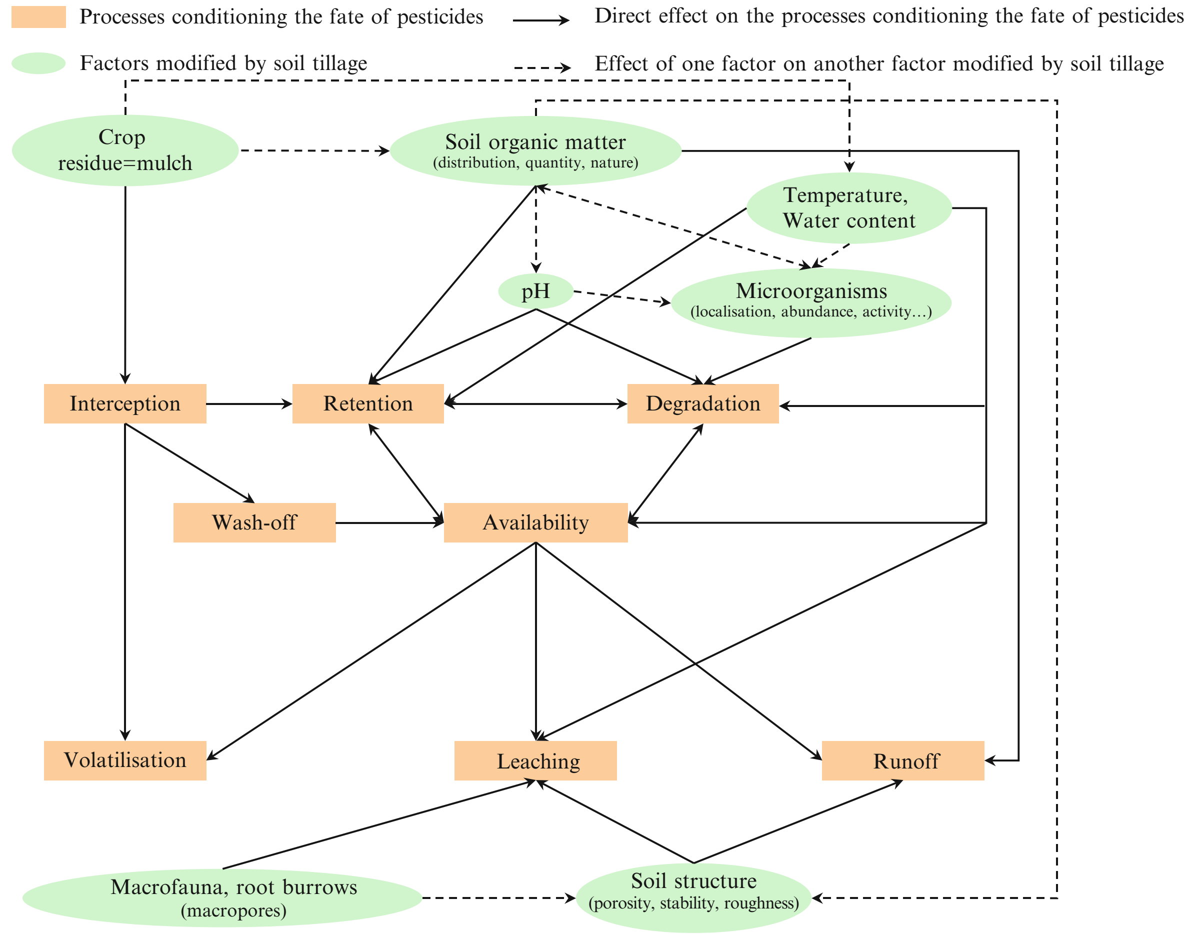

Fig. 4 Relationships between the processes conditioning the fate of pesticides in soils, water and air and the soil factors modified by tillage operations

herbicide sorption in the case of the vetch, and in composition of the residues that contained less cellulose and more amino acids (Gaston et al., 2001). Sigua et al. (1993) indicated that interception of atrazine was enhanced with fresh maize residues, due to a combination of a greater hydrophobicity and a higher sorption capacity of the fresh compared with the aged maize residues. In contrast, with metribuzin (Dao, 1991), chlorimuron (Reddy et al., 1995b) and cyanazine (Reddy et al., 1997a), interception was higher with aged residues. In these cases, the increase in sorption due to aging was attributed to both changes in the physical state and in the chemical composition of the crop residues. On one hand, physical alterations of the residues increase their external surface area for herbicide sorption compared with fresh residues. On the other hand, the chemical evolution of the residues during degradation leads to an increase in the lignin/cellulose ratio, resulting in an increase in the sorption of chlorimuron and metribuzin (Dao, 1991; Reddy et al., 1995b).

Interception is not only influenced by the amount of mulch. It is also conditioned by the formulation of the molecules. Compared with the commercial formulation, the use of microencapsulated or granular forms of alachlor led to a decrease in its interception and, finally, the herbicide reached the soil surface more rapidly (Johnson et al., 1989; Sadeghi et al., 1998).

Once intercepted by the mulch, the molecule can be washed off from the crop residues to reach the soil surface. This wash-off depends on the timing between pesticide treatment and the first rainfall and its intensity. On maize residues, Martin et al. (1978) reported a range of 30 to 60% wash-off by the first 5 mm of water for alachlor, atrazine, cyanazine and propachlor, which was equivalent to the wash-off

Table 2 Chemical and common names for pesticides mentioned in this review

Common name	Nature	Chemical name
Acetochlor	Herbicide	2’-ethyl-6’-methyl-*N*-(ethoxymethyl)-2-chloroacetylanilide
Acifluorfen	Herbicide	5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid
Alachlor	Herbicide	2-chloro-*N*-(2, 6-diethylphenyl)-*N*-(methoxymethyl)acetamide
Atrazine	Herbicide	(6-chloro-*N*-ethyl)-*N*’-(1-methylethyl)-1,3,5-triazine-2,4-diamine
Bentazone	Herbicide	3-isopropyl-1*H*-2,1,3-benzonthiadiazain-(4)3*H*-one 2,2-dioxide
Carbofuran	Insecticide	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Chlorimuron	Herbicide	Ethyl-2-[[[[4-chloro-6-methoxy-2-(pyrimidinyl)amino]carbonyl]amino]sulfonyl] benzoic acid
Clomazone	Herbicide	2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone
Clopyralid	Herbicide	3,6-dichloropicolinic acid
Chlorpyrifos	Insecticide	*O*, *O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate
Cyanazine	Herbicide	[2-4-chloro-6-(ethylamino) 1,3,5-triazin-2-yl]amino-2-methylpropanenitrile
Diazinon	Insecticide	*O*, *O*-dimethyl *O*-2-isopropyl-6-methylpyrimidin4-yl phosphorothioate
Dicamba	Herbicide	3,6-dichloro-2-methoxybenzoic acid
Diclofop	Herbicide	(±)-2-(4-(2,4-dichlorophenoxy)phenoxy)propanoic acid
Diclosulam	Herbicide	*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro-(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide
Diketonitrile	Herbicide	2-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-3-oxopropanenitrile
Dimethipin	Defoliant	2,3-dihydro-5,6-dimethyl-1,4-dithiin 1,1,4,4-tetraoxide
Fluometuron	Herbicide	*N*,*N*-dimethyl-*N*’-[3-(trifluoromethyl)-phenyl]-urea
Fonofos	Insecticide	*O*-ethyl *S*-phenyl ethylphosphonodithioate
Glyphosate	Herbicide	*N*-(phosphonomethyl)glycine
Imazapic	Herbicide	(RS)-2-(4,5-dihydro-4-isopropyl-4-methyl-5-oxoimidazol-2-yl)-5-methylnicotinic acid
Imazapyr	Herbicide	2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-pyridine carboxylic acid
Imazaquin	Herbicide	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid
Imazethapyr	Herbicide	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidiazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid
Isoproturon	Herbicide	3-(4-isopropylphenyl)-1,1-dimethylurea
Isoxaflutole	Herbicide	5-cyclopropyl-1,2-isoxazol-4-yl alpha alpha alpha -trifluoro-2-mesyl-p-tolyl ketone
Linuron	Herbicide	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
MCPA	Herbicide	4-chloro-2-methyphenoxy acetic acid
Metolachlor	Herbicide	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide
Metamitron	Herbicide	4-4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one
Metribuzin	Herbicide	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2, 4-triazine-5(4*H*)-one
Norflurazon	Herbicide	4-chloro-5(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2*H*)-pyridazinone
Oxyfluorfen	Herbicide	2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoro-methyl) benzene
Pendimethalin	Herbicide	*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine
Propachlor	Herbicide	2-chloro-*N*-isopropylacetanilide
Simazine	Herbicide	2-chloro-4,6-bis[ethylamino]-s-triazine
Sulfentrazone	Herbicide	*N*-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide
Terbufos	Insecticide	S-(((1,1-dimethylethyl)thio)methyl) *O*, *O*-diethyl phosphorodithioate
Terbuthylazine	Herbicide	*N*-2-tert-butyl-6-chloro-*N*-4-ethyl-1,3,5-triazine-2,4-diamine
Thidiazuron	Defoliant	*N*-phenyl-*N*-1,2,3-thidiazol-5-yl-urea
Tribufos	Defoliant	S,S,S-tributyl phosphorotrithioate
2,4 D	Herbicide	(2,4-dichlorophenoxy)acetic acid
2,4-Dichlorophenol	Herbicide precursor	2,4-Dichlorophenol

Table 3 Sorption properties of pesticides under different tillage practices

			Soil properties						
Pesticide	Tillage[1]	Depth cm	Clay	Organic carbon (OC) g kg^{-1}	pH	K_D L kg^{-1}	K_F L^{n_F} mg$^{(1-n_F)}$ kg^{-1}	K_{OC} L kg^{-1} OC	Reference
Acetochlor	NT	0–10	–	16.6	–	2.7	–	166	(Ferri et al., 2002)
	CT		–	13.3	–	1.7	–	126	
Acifluorfen	NT	0–10	–	10.2	5.6*	–	0.8	–	(Gaston and Locke, 2000)
	NT	10–10	–	5.6	5.3*	–	0.5	–	
	NT	20–30	–	4.4	5.7*	–	0.6	–	
	CT	0–10	–	8.7	5.8*	–	0.8	–	
	CT	10–20	–	6.4	5.8*	–	0.5	–	
	CT	20–30	–	4.9	5.8*	–	0.5	–	
Alachlor	NT	0–5	290	16.7	5.5*	5.4	5.6	323	(Locke, 1992)
	CT	0–5	260	10.2	5.5*	3.5	3.6	342	
Alachlor	NT	0–5	–	72.5	4.7*	–	6.0	–	(Clay et al., 1991)
	NT	5–10	–	29.0	5.0*	–	3.6	–	
	NT	10–15	–	29.5	6.2*	–	4.0	–	
	NT	15–20	–	32.5	6.4*	–	3.7	–	
	CT	0–5	–	30.0	5.5*	–	3.6	–	
	CT	5–10	–	26.0	6.0*	–	4.0	–	
	CT	10–15	–	29.0	5.2*	–	3.9	–	
	CT	15–20	–	28.0	5.7*	–	4.0	–	
Alachlore	NT + B[2]	0–15	–	8.4	–	0.9	–	110	(Xue et al., 1997)
	NT + V[2]		–	8.6	–	1.1	–	130	
	NT		–	6.7	–	1.0	–	143	
	CT + B		–	4.7	–	0.5	–	99	
	CT + V		–	5.7	–	0.5	–	95	
	CT		–	6.3	–	0.6	–	91	
Atrazine	NT	0–2.5	–	19.1	5.2	7.8	–	–	(Ghadiri et al., 1984)
	NT	2.5–5	–	15.1	5.0	7.1	–	–	
	NT	5–10	–	10.4	5.8	7.0	–	–	
	NT	10–15	–	10.4	6.2	7.3	–	–	
	NT	15–20	–	10.4	6.3	7.0	–	–	
	NT	20–25	–	8.1	6.5	6.2	–	–	
	NT	25–30	–	7.0	6.7	6.1	–	–	
	NT	30–35	–	6.4	6.8	6.1	–	–	
	NT	35–40	–	5.8	6.9	5.8	–	–	
	CT	0–2.5	–	12.8	5.6	7.4	–	–	
	CT	2.5–5	–	12.8	5.2	7.5	–	–	
	CT	5–10	–	11.6	5.7	7.6	–	–	
	CT	10–15	–	11.0	6.2	6.7	–	–	
	CT	15–20	–	10.4	6.3	6.5	–	–	
	CT	20–25	–	8.1	6.6	6.5	–	–	
	CT	25–30	–	7.0	6.7	6.0	–	–	
	CT	30–35	–	5.2	6.9	5.7	–	–	
	CT	35–40	–	4.7	7.0	5.5	–	–	

(continued)

Table 3 (continued)

			Soil properties						
Pesticide	Tillage[1]	Depth cm	Clay	Organic carbon (OC) g kg^{-1}	pH	K_D L kg^{-1}	K_F L^{n_F} mg$^{(1-n_F)}$ kg^{-1}	K_{OC} L kg^{-1} OC	Reference
Atrazine	NT	0–15	–	10.6	–	1.7	–	–	(Novak et al., 1996)
	CT		–	9.8	–	1.4	–	–	
Bentazon	NT	0–10	–	10.2	5.6*	0.03	0.04	–	(Gaston et al., 1996)
	NT	10–20	–	5.6	5.3*			–	
	NT	20–30	–	4.4	5.7*			–	
	CT	0–10	–	8.7	5.8*			–	
	CT	10–20	–	6.4	5.8*			–	
	CT	20–30	–	4.9	5.8*			–	
Chlorimuron	NT	0–7.5	280	30.6	6.6*	–	1.0	31	(Reddy et al., 1995a)
	CT		250	17.8	6.4*	–	0.6	31	
	NT		330	46.0	6.2*	–	6.5	142	
	CT		260	19.2	4.5*	–	2.1	108	
	NT		–	15.3	5.4*	–	2.1	141	
	CT		–	13.1	5.7*	–	1.6	125	
Clopyralid	NT (9)[3]	0–5	145	23.7	5.1	1.0	–	21	(Shang and Arshad, 1998)
	CT		145	22.7	5.8	0.3	–	2	
	NT (8)		532	47.0	4.9	1.6	–	51	
	CT		520	44.5	5.4	2.1	–	32	
Cyanazine	NT (10)	0–5	230	21.4	5.2	3.5	–	165	(Reddy et al., 1997b)
	CT		210	16.0	5.3	2.2	–	140	
Dicamba	NT (9)	0–5	145	23.7	5.1	0.2	–	13	(Shang and Arshad, 1998)
	CT		145	22.7	5.8	Undetected	–	Undetected	
	NT (8)		532	47.0	4.9	1.4	–	23	
	CT		520	44.5	5.4	1.6	–	23	
Diclosulam	NT	0–10	355	19.9	5.0*	1.9	1.8	98	(Lavorenti et al., 2003)
	CT	0–10	341	12.8	4.4*	2.0	1.9	159	
Fluometuron	NT+Adv[2]	0–3	–	13.9	5.2	–	2.0	–	(Gaston et al., 2001)
	NT+Adv	3–6	–	8.0	5.5	–	1.0	–	
	NT+V	0–3	–	15.6	5.5	–	1.8	–	
	NT+V	3–6	–	7.3	5.2	–	1.2	–	
	NT+B	0–3	–	11.2	6.1	–	1.8	–	
	NT+B	3–6	–	4.7	5.5	–	1.3	–	
	CT+Adv	0–3	–	7.4	5.7	–	0.8	–	
	CT+Adv	3–6	–	7.0	5.2	–	1.2	–	
	CT+V	0–3	–	7.4	5.5	–	0.9	–	
	CT+V	3–6	–	6.6	5.0	–	1.1	–	
	CT+B	0–3	–	7.8	5.8	–	1.0	–	
	CT+B	3–6	–	5.8	5.1	–	0.8	–	
Fluometuron	NT	0–4	150	20.0	5.1	2.2	–	–	(Brown et al., 1994)
	NT	4–8	150	10.0	5.5	1.7	–	–	
	NT	8–15	160	9.0	6.5	1.4	–	–	
	CT	0–4	130	13.0	5.5	1.7	–	–	
	CT	4–8	140	13.0	5.7	2.1	–	–	
	CT	8–15	150	11.0	6.3	1.4	–	–	
	NT+V	0–4	150	25.0	4.7	3.0	–	–	
	NT+V	4–8	150	11.0	4.9	1.0	–	–	
	NT+V	8–15	150	9.0	5.7	1.3	–	–	
	CT+V	0–4	140	15.0	5.0	2.1	–	–	
	CT+V	4–8	150	15.0	5.0	2.2	–	–	
	CT+V	8–15	150	11.0	5.6	1.6	–	–	

(continued)

Table 3 (continued)

			Soil properties						
Pesticide	Tillage[1]	Depth cm	Clay	Organic carbon (OC) g kg^{-1}	pH	K_D L kg^{-1}	K_F L^{n_F} mg$^{(1-n_F)}$ kg^{-1}	K_{OC} L kg^{-1} OC	Reference
Fluometuron	NT (11)	0–2	–	30.1	–	–	7.2	–	(Zablotowicz et al., 2000)
	NT	2–5	–	11.8	–	–	2.0	–	
	NT	5–10	–	5.9	–	–	1.4	–	
	NT	10–25	–	3.3	–	–	1.5	–	
	CT	0–2	–	20.3	–	–	1.9	–	
	CT	2–5	–	11.2	–	–	2.2	–	
	CT	5–10	–	6.9	–	–	1.0	–	
	CT	10–25	–	4.4	–	–	1.2	–	
Fluometuron	NT	0–8	130	24.5	5.1*	2.0	–	80	(Suba and Essington, 1999)
	CT	0–8	100	14.2	5.2*	1.3	–	92	
Fluometuron	NT	0–2	–	13.9	6.7	2.4	–	–	(Locke et al., 2005)
	NT	2–10	–	6.1	5.9	1.6	–	–	
	NT+Rg[2]	0–2	–	19.8	6.0	5.0	–	–	
	NT+Rg	2–10	–	6.9	5.7	1.4	–	–	
	CT	0–2	–	8.0	6.5	1.7	–	–	
	CT	2–10	–	6.0	5.8	1.6	–	–	
	CT+Rg	0–2	–	9.5	6.4	2.1	–	–	
	CT+Rg	2–10	–	7.3	5.7	2.0	–	–	
	RT	0–2	–	12.2	6.5	0.6	–	–	
	RT	2–10	–	6.7	6.7	0.4	–	–	
	RT+S	0–2	–	12.6	6.6	0.7	–	–	
	RT+S	2–10	–	7.1	6.6	0.5	–	–	
	CT	0–2	–	6.4	6.1	0.4	–	–	
	CT	2–10	–	8.4	6.4	0.4	–	–	
	CT+S	0–2	–	6.8	6.1	0.4	–	–	
	CT+S	2–10	–	7.9	6.6	0.5	–	–	
	RT	0–2	–	10.3	5.5	0.5	–	–	
	RT	2–10	–	9.5	5.5	0.5	–	–	
	RT+S	0–2	–	8.8	5.5	0.4	–	–	
	RT+S	2–10	–	8.5	5.9	0.5	–	–	
	CT	0–2	–	9.0	5.6	0.4	–	–	
	CT	2–10	–	8.6	5.4	0.4	–	–	
	CT+S	0–2	–	8.7	5.7	0.4	–	–	
	CT+S	2–10	–	8.6	5.5	0.4	–	–	
MCPA	NT (9)	0–5	145	23.7	5.1	2.0	–	63	(Shang and Arshad, 1998)
	CT		145	22.7	5.8	0.7	–	40	
	NT (8)		532	47.0	4.9	7.3	–	168	
	CT		520	44.5	5.4	10.4	–	182	
Metolachlor	NT	0–5	–	15.4	–	–	3.5	230	(Ding et al., 2002b)
	NT	10–15	–	3.5	–	–	1.3	360	
	CT	0–5	–	8.5	–	–	2.0	240	
	CT	10–15	–	5.4	–	–	1.4	256	
Norflurazon	NT	0–8	130	24.5	5.1*	12.1	–	496	(Suba and Essington, 1999)
	CT	0–8	100	14.2	5.2*	6.5	–	456	

(continued)

Table 3 (continued)

Pesticide	Tillage[1]	Depth cm	Soil properties		pH	K_D L kg^{-1}	K_F L^{n_F} mg$^{(1-n_F)}$ kg^{-1}	K_{OC} L kg^{-1} OC	Reference
			Clay	Organic carbon (OC) g kg^{-1}					
Norflurazon	NT	0–2	–	13.9	6.7	2.2	–	–	(Locke et al., 2005)
	NT	2–10	–	6.1	5.9	1.6	–	–	
	NT+Rg	0–2	–	19.8	6.0	3.6	–	–	
	NT+Rg	2–10	–	6.9	5.7	1.6	–	–	
	CT	0–2	–	8.0	6.5	1.6	–	–	
	CT	2–10	–	6.0	5.8	1.8	–	–	
	CT+Rg	0–2	–	9.5	6.4	2.3	–	–	
	CT+Rg	2–10	–	7.3	5.7	2.1	–	–	
Sulfentrazon	NT	0–7.5	280	30.6	6.6*	0.9	1.2	30	(Reddy and Locke, 1998)
	CT		250	17.8	6.4*	0.8	1.0	47	
	NT		330	46.0	6.2*	3.2	3.4	71	
	CT		260	19.2	4.5*	2.9	2.6	153	
	NT		230	21.4	5.2*	2.3	3.3	96	
	CT		210	16.0	5.3*	1.5	1.8	106	
2,4–Dichlorophenol	NT (9)	0–3	188	27.0	6.6*	4.2	–	155	(Düring, 2002)
	NT	3–10	177	16.7	6.6*	2.2	–	132	
	NT	10–25	168	10.3	6.4*	1.3	–	122	
	CT	0–3	132	11.3	6.1*	1.7	–	150	
	CT	3–10	155	11.6	6.1*	1.6	–	140	
	CT	10–25	143	11.3	6.2*	1.4	–	126	

[1] Codes of tillage practices are reported in Table 1.

[2] Adv: weed residues; B: wheat residues; Rg: ray-grass residues; S: rye residues; V: vetch residues.

[3] Numbers in parenthesis indicate the age in years of the tillage system.

* pH $CaCl_2$.

by the next 30 mm of water. At the end of their experiment, most of the applied chemicals had been washed off from the mulch. Timing between treatment and first rainfall also strongly influences the quantity of pesticides that could be washed from the mulch. It was found to be the highest after heavy rainfall occurring within two weeks following the treatment (Sadeghi and Isensee, 1996; 1997). Depending on rainfall quantity and intensity, between 70 and 96% of intercepted atrazine were released between the 1st and 3rd weeks after treatment (Ghadiri et al., 1984; Isensee and Sadeghi, 1994). However, this wash-off directly depends on the retention capacities of the pesticide on crop residues (Gaston et al., 2001). For example, metolachlor, once intercepted, seems to form stronger and less reversible bonds with crop residues than acetochlor or alachlor (Banks and Robinson, 1986). In some cases, a gradual release of the pesticide from the residues by wash-off may lead to an increase in its efficacy (Dao, 1991), but could also increase crop injuries (Barnes et al., 1989; Loux et al., 1989; Mills and Witt, 1991).

2.2 Organic Carbon Content Effect

One of the main changes related to the implementation of conservation tillage compared with conventional tillage is the redistribution of organic carbon in the soil (Balesdent et al., 1990; Tebrügge and During, 1999). Generally, organic carbon content increases in surface soil due to the presence and decomposition of the mulch and gradually decreases with depth (Lal et al., 1994; Pinheiro et al., 2004; Six et al., 1999). For most pesticides, organic carbon content and adsorption are positively correlated, resulting in a higher adsorption in surface soil under conservation tillage than under conventional tillage (Table 3). For molecules with low sorption capacity, such as bentazon (Gaston et al., 1996) or diclosulam (Lavorenti et al., 2003), the effect of tillage may not be significant. Very few studies aimed at analysing the effects of tillage on pesticide sorption kinetics. For acifluorfen (Gaston and Locke, 2000), chlorimuron (Reddy et al., 1995a) and cyanazine (Reddy et al., 1997b), no effect of tillage system was found on sorption kinetics and most of the sorption occurred during the first hour of contact. For some molecules, such as alachlor (Locke, 1992) and sulfentrazone (Reddy and Locke, 1998), sorption was faster under conservation tillage and was positively correlated with higher soil organic matter contents.

Tillage management also modifies pesticide desorption. For sulfentrazone and alachlor, whatever the tillage system, desorption was found to be hysteretic, but a greater proportion of the molecule, representing approximately 35% of the applied sulfentrazone, remained sorbed under no-tillage (against 20% under conventional tillage) (Locke, 1992; Reddy and Locke, 1998). For cyanazine (Reddy et al., 1997b), desorption was also more reversible under conventional tillage but, when ryegrass residues were added to the soil samples, desorption was similar under conventional and conservation tillage. As indicated by Locke (1992), desorption tends to decline with increasing contact time between soil and pesticide due to diffusion processes within the soil.

However, considering only the quantitative increase in organic carbon content in surface soil under conservation tillage is not sufficient to explain the increase in pesticide sorption found in some studies (Ding et al., 2002b; Novak et al., 1996; Zablotowicz et al., 2000). Although the analytical procedures used to extract soil organic matter can induce artefacts by modifying its molecular structure (Ding et al., 2002b; Salloum et al., 2001), it appears that the nature of soil organic matter and, hence, its reactivity, are also affected by tillage (Ding et al., 2002a; Sleutel et al., 2007; Tatzber et al., 2008) and by the nature of crop residues (Ding et al., 2006). In soil organic matter collected under a no-tillage system, Preston et al. (1994) found a higher number of reactive functional groups compared with that collected under a conventional tillage system. Ding et al. (2002a) found that humic acids and humin from conservation tillage contained more aliphatic carbon and less aromatic carbon than under conventional tillage. A larger amount of amino acids were also linked to these humic acids under no-tillage, whereas they were more linked to fulvic acids under conventional tillage (Szajdak et al., 2003). Moreover, reactive/recalcitrant peak ratios indicated that humic acids in the topsoil (0–5 cm-depth) were more biologically active under conservation tillage than under conventional tillage. With depth, the soil organic matter composition changes. Humification processes are more advanced in deeper soil layers and lead to an increase in aromaticity with the development of polycondensed rings (Ding et al., 2002a). The reactivity of organic matter fractions may also affect pesticide desorption. As an example, desorption of metolachlor from conventional tillage humic acids was less significant than from conservation tillage humic acids (Ding et al., 2002b). The hysteresis index (which is the ratio of Freundlich exponents for desorption and sorption) was lower for humic acids under conventional tillage, indicating that sorbed metolachlor molecules were more difficult to desorb. This may be caused by higher aromatic carbon contents in conventional tillage humic acids (Ding et al., 2002b; Xing, 2001).

The nature of crop residues also influences the composition of soil organic matter. For example, rye residues tend to form more aromatic and less aliphatic humic acids than a mix of vetch/rye residues (Ding et al., 2006), which could potentially affect pesticide behaviour and efficacy in soil (Ding et al., 2002a; Nanny and Maza, 2001). However, to our current knowledge, little work has been published on the effects of these changes in soil organic matter composition due to tillage on pesticide sorption. Ding et al., (2002b) highlighted a stronger sorption of metolachlor by humin than by humic acids, and humin content was found to be higher under no-tillage systems (Stearman et al., 1989). For humin, which is a highly condensed form of soil organic matter, several studies showed that sorption was more variable and that sorption isotherms were less linear (Xing and Pignatello, 1997; Yuan and Xing, 1999). In contrast, Stearman et al. (1989) observed a higher sorption of metribuzin and oxyfluorfen on fulvic and humic acids, which are abundant in conventional tillage, than on humin, which is abundant in conservation tillage. Dissolved organic carbon content is most often higher under conservation tillage and is mainly composed of small molecules, with a size similar to that of fulvic acids (Suba and Essington, 1999). In some studies, sorption of pesticides on dissolved organic carbon was found to be an efficient way to increase the mobility of some pesticides in soils, e.g. atrazine and 2,4D, leading to a significant transport of these chemicals through the soil profile (Chin et al., 1990; Gao et al., 1998; Lafrance et al., 1994; Li et al., 2005). In another study, sorption of fluometuron and norflurazon on the dissolved organic carbon formed under conservation tillage did not lead to a higher leaching of these molecules (Suba and Essington, 1999).

2.3 Soil pH Effect

Soil pH may be differently modified by tillage techniques: conservation tillage leads to an increase in pH (Reddy and Locke, 1998), no change (Comia et al., 1994; Rasmussen, 1991) or, more often, to a decrease in pH, especially in surface soil due to the accumulation of organic matter and fertilisers (Arshad et al., 1999; Doran, 1980; Levanon et al., 1994). For many pesticides, sorption is strongly influenced by soil pH and tends to increase when soil pH decreases (Barriuso and Calvet, 1992; Barriuso et al., 1992; Grey et al., 1997). In sandy soils, it has been found that, for similar organic carbon contents between tillage treatments, acidification in conservation techniques could significantly increase the sorption of several molecules according to their pKa (Shang and Arshad, 1998). For s-triazines (weak bases), the decrease in pH causes their protonation, promoting sorption on organic matter (Senesi and Testini, 1982) and decreasing their herbicide activity.

2.4 Other Effects

Tillage techniques modify other soil properties (Fig. 4) whose effects on retention mechanisms are still poorly understood. Due to the presence of a mulch, water content in conservation techniques is generally higher than in conventional techniques (Drury et al., 1999; Shelton et al., 1998). This increase in soil water content led, in some cases, to a decrease in pesticide retention (Dao and Lavy, 1991; Walker, 1971). In other cases, it led to an increase in adsorption that could be explained by a decrease in the hydrophobicity of the organic compounds and an access to sorption sites facilitated by the presence of water (Berglof et al., 2000; Ochsner et al., 2006). Soil temperature is also modified by the mulch under conservation tillage. The mulch intercepts light energy, thus reducing temperature at the soil surface in conservation techniques, from 1 to 5°C (Bragagnolo and Mielniczuk, 1990; Grant et al., 1990; Gupta et al., 1988). However, to our current knowledge, there is no published data about the consequences of soil water content and temperature modifications by tillage management on pesticide retention.

2.5 Sorption of Metabolites

Little information is available on tillage effects on metabolite retention. According to their molecular composition, metabolites can have lower sorption capacities, e.g. cyanazine metabolites (Reddy et al., 1997a), or higher sorption capacities, e.g. atrazine and bentazon metabolites (Clay and Koskinen, 1990; Gaston et al., 1996) than the parent compound. For cyanazine metabolites, as observed for the parent compound, sorption was higher under conservation tillage than under conventional tillage.

2.6 Summary and Recommendations

Regarding pesticide retention processes, most studies highlighted the role of the mulch in conservation tillage systems which intercepted the molecules. In a general way, tillage systems act on pesticide retention mainly through their effect on the distribution of organic matter in soil. Recent works on the characterisation of organic matter fractions and their reactivity towards pesticides show significant influences of the type of tillage and nature of crop residues. Using recent analytical technologies, these studies on the characterisation of organic compounds found in soils or crop residues are a significant advance in the qualitative evaluation of the effects of agricultural practices on pesticide retention. To progress in this direction, further research should not be focused on tillage management only, but rather on the whole cropping systems. As a matter of fact, other management options such as crop rotation, cover crops and manure application play important roles in the dynamics of soil organic matter. Regarding conservation tillage systems, research efforts should be made to clarify the indirect effects of the mulch via modifications of pH, water and temperature dynamics in the underlying soil.

3 Degradation

Understanding pesticide degradation in soils is a key step for assessing their persistence and their risks of transfer in the environment. Degradation studies can be carried out in the field, thus integrating a set of non-controlled phenomena such as fluctuations in temperature, soil water content or radiation (Table 4), or in the laboratory, where soil samples are kept under controlled conditions (Table 5). In all cases, degradation is conditioned by a combination of factors, i.e. microflora, organic matter, water content, temperature and pH, which are directly influenced by tillage (Fig. 4). Biotic degradation of pesticides is most often seen as quantitatively more important than abiotic degradation. In conservation tillage, the total microbial biomass is generally larger than in conventional tillage (Biederbeck et al., 1997; Dalal et al., 1991; Doran, 1980), but it remains strongly dependent on soil conditions. Fungi populations, which were found to be efficient in pesticide degradation (Barr and Aust, 1994), are generally larger in conservation tillage and their biomass increases as degree of tillage is reduced (Drijber et al., 2000; Kabir, 2005). These biological differences due to tillage have effects, sometimes contradictory, on the degradation of pesticides in the mulch and in the soil.

3.1 Pesticide Degradation in the Mulch

The interception by the mulch in conservation techniques can modify the persistence of the applied pesticides. These effects are sometimes negligible (Banks and Robinson, 1982), but more often they significantly affect the fate of pesticides in soil. Crop residues may have higher microbial activity and degradation capacity than the soil, thus reducing the concentrations of pesticide that reach the soil surface (Locke et al., 2005; Zablotowicz et al., 1998). Moreover, depending on the nature of the pesticide, interception by the mulch may generate photodegradation, thus reducing the persistence of the molecules (Selim et al., 2003). In other works, crop residues increased the residence time of pesticides because of the competition between retention and degradation processes, and a gradual release of the molecules by desorption was observed (Mazzoncini et al., 1998). By acting as a physical protector of the soil surface, crop residues may also limit the air flow between the soil and the atmosphere and within the soil, thus slowing down the activity of degrading microorganisms (Sorenson et al., 1991).

Depending on the nature of crop residues, the degradation of molecules can be affected by the presence of a mulch, but in contrasted ways. For example, in no-tillage, vetch residues accelerated the degradation of metolachlor by from 1.5 to 3 times, but had no effect on the degradation of atrazine (Teasdale et al., 2003). On the contrary, according to laboratory studies, vetch residues seemed to slow down fluometuron degradation compared with soil samples without vetch residues (Brown et al., 1994; Zablotowicz et al., 1998) or other types of residues such as wheat residues (Gaston et al., 2001), rye residues (Zablotowicz et al., 1998), or ray grass residues (Locke et al., 1995). This slowdown could be due to the abundance of nitrogen in legumes (Fabaceae). This nitrogen would be preferentially used by the microorganisms to the detriment of the N sources contained in pesticides. In the same way, Gan et al. (1996) observed a lower degradation rate of atrazine after an addition of nitrogen. However, experiments on undisturbed soil columns with simulated rainfall, that allowed nitrogen leaching and thus reduced the amount bioavailable in the soil column, relativised these results and no negative effect of vetch residues on fluometuron degradation was found (Gaston et al., 2003). This experiment provided an explanation of the difference between the results obtained in laboratory incubations where leaching of nitrogen is not possible (Gaston et al., 2001) and those obtained in the field where N can leach out through the soil (Brown et al., 1996).

3.2 Pesticide Degradation in Soil

Tillage practices were found to modify significantly pesticide degradation in soil, but in contrasted ways according to studies. Degradation of several pesticides was indeed found to be slower (Brown et al., 1994; Gaston and Locke, 2000; Otto et al., 1997; Ulbrich et al., 2005), equivalent (Gaynor et al., 1998; Locke et al., 1996; 2005; Monks and Banks, 1993; Reddy et al., 1995b; Renner et al., 1998), or faster (Gaston et al., 2001; 2003; Lavorenti et al., 2003; Levanon et al., 1994; Sadeghi and Isensee, 1997) under conservation tillage than under conventional tillage. Several reasons could explain these differences. To explain a lower degradation under conservation

Table 4 Field studies of pesticide degradation under different tillage practices

			Soil properties				
Pesticide	Tillage^1	Depth cm	Clay	Organic carbon g kg^{-1}	pH	DT$_{50}^2$ d	Reference
Alachlor	NT	0–30	–	–	–	<1	(Weed et al., 1998)
	CT	0–30	–	–	–	<1	
Alachlor encapsulated alachlor	NT	0–110	–	–	–	8–41	(Gish et al., 1994)
	CT	0–110	–	–	–	8–41	
	NT	0–110	–	–	–	4–20	
	CT	0–110	–	–	–	4–20	
Atrazine	NT	0–10	–	–	–	50	(Ghadiri et al., 1984)
	CT		–	–	–	42	
Atrazine	NT	0–50	–	–	–	71	(Gish et al., 1991)
	CT	0–70	–	–	–	73	
Atrazine encapsulated atrazine	NT	0–110	–	–	–	36	(Gish et al., 1994)
	CT	0–110	–	–	–	36	
	NT	0–110	–	–	–	110	
	CT	0–110	–	–	–	110	
Atrazine	NT	0–10	390	14.5	5.4	45, 36, 56^4	(Gaynor et al., 2000)
	NT+Rg^3	0–10				40, 33, 54	
	CT	0–10				46, 34, 54	
	CT+Rg	0–10				35, 59, 24	
Atrazine	NT	0–10	–	–	–	43, 56, 43, 35	(Gaynor et al., 1998)
	RT top		–	–	–	33, 75, 53, 36	
	RT furrow		–	–	–	31, 47, 53, 35	
	CT		–	–	–	33, 62, 58, 47	
Atrazine	NT	0–50	17–26	11.0	6–6.5	35, 25,12, 23	(Isensee and Sadeghi, 1994)
	CT		16–24	7.0	4.8–5	37, 21, 29, 18	
Atrazine	NT	0–150	55–166	0.1–9.7	5.3–6.1	149	(Heatwole et al., 1997)
	CT		59–179	1.2–4.4	5.5–6.7	215	
Atrazine	NT (7)^5	0–50	–	–	–	12	(Sadeghi and Isensee, 1996)
	CT		–	–	–	22	
	CT → NT		–	–	–	–, 21, 32	
	NT		–	–	–	–, 17, 23	
	(7) → CT						
Clomazon	RT	0–10	230	23.2	6.0	52, 91	(Curran et al., 1992)
	CT					58, 83	
Chlorimuron	NT	–	260	10.4	7.8	32, 14, 22	(Baughman et al., 1996)
	CT	–	260	10.4	7.8	44, 13, 18	
	NT	–	220	8.7	6.3	17, 82	
	CT	–	220	8.7	6.3	14, 22	
Cyanazine	NT	–0–50	–	–	–	13	(Gish et al., 1991)
	CT	0–70	–	–	–	13	
Fluometuron	NT, NT+Rg	0–2	–	–	–	7–15	(Locke et al., 2005)
	CT, CT+Rg		–	–	–		
Fluometuron	NT	0–8	150	8.7	5.2	30, 23	(Brown et al., 1996)
	NT+V^2		150	10.4	4.8	38, 19	
	CT		130	7.6	5.6	24, 26	
	CT+V		140	8.7	5.0	30, 25	
Imazapic	NT (3)	0–10	780	20.3	4.7	66	(Ulbrich et al., 2005)
	CT	0–10				45	
	NT	0–10	280	27.3	5.8	35	
	CT	0–10				32	
Imazapyr	NT (3)	0–10	780	20.3	4.7	53	(Ulbrich et al., 2005)
	CT	0–10				50	
	NT	0–10	280	27.3	5.8	43	
	CT	0–10				45	
Imazaquin	RT	0–10	230	23.2	6.0	39, 144	(Curran et al., 1992)
	CT					41, 117	

(continued)

Table 4 (continued)

			Soil properties				
Pesticide	Tillage[1]	Depth cm	Clay	Organic carbon g kg^{-1}	pH	DT$_{50}$[2] d	Reference
Imazaquin	NT	0–20	–	16.9, 19.8	5.9, 6.4	27, 33	(Mills and Witt, 1991)
	CT		–			53, 22	
Imazethapyr	RT	0–10	230	23.2	6.0	82, 53	(Curran et al., 1992)
	CT					122, 56	
Imazethapyr	NT	0–20	–	16.9, 19.8	5.9, 6.4	36, 40	(Mills and Witt, 1991)
	CT		–			40, 12	
Isoproturon	NT	0–30	170	6.1	–	12	(Otto et al., 1997)
	RT	0–30	170	5.8	–	8	
	CT	0–30	170	4.5	–	15	
Metolachlor	NT	0–30	170	6.1	–	9	(Otto et al., 1997)
	RT	0–30	170	5.8	–	26	
	CT	0–30	170	4.5	–	29	
Metolachlor	NT	0–150	55–166	0.1–9.7	5.3–6.1	45	(Heatwole et al., 1997)
	CT		59–179	1.2–4.4	5.5–6.7	34	
Metolachlor	NT	0–10	–	–	–	40, 65, 37, 30	(Gaynor et al., 1998)
	RT top		–	–	–	23, 87, 41, 35	
	RT furrow		–	–	–	28, 42, 40, 28	
	CT		–	–	–	32, 68, 43, 40	
Metolachlor	NT	0–10	390	14.5	5.4	46, 42, 72	(Gaynor et al., 2000)
	NT+Rg	0–10				42, 40, 69	
	CT	0–10				42, 45, 79	
	CT+Rg	0–10				44, 44, 97	
Metribuzin	NT	0–10	390	14.5	5.4	24, 27, 34	(Gaynor et al., 2000)
	NT+Rg	0–10				24, 26, 36	
	CT	0–10				23, 29, 32	
	CT+Rg	0–10				24, 29, 37	
Metribuzin	NT	0–5	–	13.3	5.7	12, 11	(Sorenson et al., 1991)
	CT	0–5	–			5, 17	
	NT	0–5	–	18.0	5.1	15, 15	
	CT	0–5	–			13, 11	
Terbuthylazine	NT	0–30	170	6.1		25	(Otto et al., 1997)
	RT	0–30	170	5.8		32	
	CT	0–30	170	4.5		22	

[1] Codes of tillage practices are reported in Table 1.
[2] DT$_{50}$: pesticide half-life.
[3] Rg: ray-grass residues; V: vetch residues.
[4] Commas are used to separate different years of study.
[5] Numbers in parenthesis indicate the age in years of the tillage system.

tillage, the most commonly mentioned phenomenon is the competition between retention and degradation. In conservation tillage, pesticide adsorption to the soil solid phase is generally increased and may lead to a decrease in the availability of the molecules for biological degradation (Zablotowicz et al., 2000). In some cases, microbiological activity in soil can be affected by a lower temperature (Sorenson et al., 1991) or higher soil acidity (Brown et al., 1994) under conservation tillage. Both of these consequences were found to increase sorption of pesticides and thus to reduce their bioavailability. For some authors, repeated fertiliser inputs (Gaynor et al., 1998; Ghadiri et al., 1984), the use of legumes as cover crops (Brown et al., 1994) and the absence of lime could lead to soil acidification and thus mask the effects of tillage practices, particularly for molecules of the s-triazine family whose sorption is highly sensitive to acidity. Other pesticides such as sulfonyl-ureas have their chemical stability reduced by acidification, leading to a faster degradation of these compounds under conservation techniques (Chapman and Cole, 1982). Global soil microbial activity can be correlated with mineralisation of the molecules (Lavorenti et al., 2003), but

Table 5 Laboratory studies of pesticide degradation under different tillage practices

			Soil properties									
Pesticide	Tillage^1	Depth cm	Clay	Organic carbon g kg^{-1}	pH	Water content g g^{-1}	Temperature °C	Incubation duration d	DT$_{50}^2$ d	CO$_2^2$ % applied dose	NER^2 % applied dose	Reference
Acifluorfen	NT	0–10	–	10.2	5.6*	0.35	25	49	108	5	9	(Gaston and Locke, 2000)
	NT	20–30	–	4.4	5.7*				165	4	5	
	CT	0–10	–	8.7	5.8*				74	6	12	
	CT	20–30	–	4.9	5.8*				169	5	3	
Alachlor	NT (7)^3	0–5	–	22.0	5.3*	0.35	25	54	6.5	13	54	(Locke et al., 1996)
	CT	0–5	–	11.6	5.1*				6.5	7	43	
Alachlor	NT	0–30	–	–	–	–	–	–	≈3	–	–	(Weed et al., 1998)
	NT	0–30	–	–	–	–	–	–	≈3	–	–	
Bentazon	NT	0–10	–	10.2	5.6*	0.30	25	22	50	2	15	(Gaston and Locke, 1996)
	NT	20–30	–	4.4	5.7*				87	2	8	
	CT	0–10	–	8.7	5.8*				39	3	20	
	CT	20–30	–	4.9	5.8*				77	2	9	
Bentazon (soil columns)	NT	0–10	–	–	–	–	–	–	17–23^4	0.1	15–17	(Gaston and Locke, 1996)
	NT	10–20	–	–	–	–	–	–	69	0.1	8–12	
	CT	0–10	–	–	–	–	–	–	12–14			
	CT	10–20	–	–	–	–	–	–	23–35			
Bentazon	NT (9)		–	15.3	5.4*			48	7	12	63	(Wagner et al., 1996)
	CT		–	13.1	5.7*				9	17	65	
	NT(16)		–	48.0	6.3*			48	9	18	60	
	CT		–	18.9	4.5*				15	14	53	
	NT(18)	0–7.5	–	30.6	6.8*	0.33	25	48	9	14	61	
	CT		–	17.8	6.4*				8	14	62	
	NT(4)		–	20.4	6.3*			48	11	15	59	
	CT		–	17.7	5.0*				11	14	59	
	NT(3)		–	10.2	5.6*			22	50	2	15	
	CT		–	8.7	5.8*				39	3	20	
Chlorimuron	NT		280	30.6	6.6*				–	10	22	(Reddy et al., 1995b)
	CT		250	17.8	6.4*				–	12	18	
	NT	0–7.5	330	46.0	6.2*	0.31	–	63	–	11	24	
	CT		260	19.2	4.5*				–	14	15	

(continued)

Table 5 (continued)

			Soil properties									
Pesticide	Tillage[1]	Depth cm	Clay	Organic carbon g kg^{-1}	pH	Water content g g^{-1}	Temperature °C	Incubation duration d	DT$_{50}^{2}$ d	CO$_2^2$ % applied dose	NER^2 % applied dose	Reference
	NT		–	15.3	5.4*				–	16	24	
	CT		–	13.1	5.7*				–	16	24	
Diclosulam	NT	0–10	355	19.9	5.0*	60	25	119	67	14	29	(Lavorenti et al., 2003)
	CT	0–10	341	12.8	4.4*	%WHC[5]			87	11	24	
Fluomeuron	NT(11)	0–2	–	30.1	–				20	–	24	(Zablotowicz et al., 2000)
	NT	2–5	–	11.8	–				19	–	25	
	NT	5–10	–	5.9	–				48	–	13	
	NT	10–25	–	3.3	–	0.33	28	25	50	–	9	
	CT	0–2	–	20.3	–				9	–	43	
	CT	2–5	–	11.2	–				11	–	31	
	CT	5–10	–	6.9	–				25	–	23	
	CT	10–25	–	4.4	–				94	–	7	
Fluometuron	NT + Adv[6]	0–3	–	7.2	5.2				6	–	–	(Gaston et al., 2001)
	NT+V[6]	0–3	–	9.1	5.5				44	–	–	
	NT+B[6]	0–3	–	9.2	6.1	33 kPa[7]	25	60	9	–	–	
	CT+Adv	0–3	–	3.4	5.7				44	–	–	
	CT+V	0–3	–	4.6	5.5				74	–	–	
	CT+B	0–3	–	4.9	5.8				23	–	–	
Fluometuron	NT+Adv	0–7.5	–	13.9	5.2				9	–	–	(Gaston et al., 2003)
	NT+V	0–7.5	–	15.6	5.5				9	–	–	
	NT+B	0–7.5	–	11.2	6.1	PS	25	109	9	–	–	
	CT+Adv	0–7.5	–	7.4	5.7				19	–	–	
	CT+V	0–7.5	–	7.4	5.5				12	–	–	
	CT+B	0–7.5	–	7.8	5.8				9	–	–	

Fluometuron	NT(11)	0–4	150	20.0	5.1				57	–	–	(Brown et al., 1994)
	NT	4–8	150	10.0	5.5				79	–	–	
	NT	8–15	160	9.0	6.5		30	84	49	–	–	
	CT	0–4	130	13.0	5.5				49	–	–	
	CT	4–8	140	13.0	5.7				55	–	–	
	CT	8–15	150	11.0	6.3	0.25			52	–	–	
	NT+V(11)	0–4	150	25.0	4.7				78	–	–	
	NT+V	4–8	150	11.0	4.9				90	–	–	
	NT+V	8–15	150	9.0	5.7				83	–	–	
	CT+V	0–4	140	15.0	5.0				71	–	–	
	CT+V	4–8	150	15.0	5.0				68	–	–	
	CT+V	8–15	150	11.0	5.6				52	–	–	
Imazaquin	SS(3)	–	660	16.9	6.4	0.25–0.30	30–25	6	12–16	–	–	(Seifert et al., 2001a)
	CT	–								–	–	
Sulfentrazone	NT	0–7.5	230	21.4	–	0.30	–	77	–	2	27	(Reddy and Locke, 1998)
	CT		210	16.0	–		–		–	2	23	

[1] Codes of tillage practices are reported in Table 1.

[2] DT_{50}: pesticide half-life; CO_2: mineralisation; NER: non-extractable residues.

[3] Numbers in parenthesis indicate the age in years of the tillage system.

[4] Hyphens are used to indicate the range of variation of values.

[5] Water content expressed in % of the water-holding capacity (WHC).

[6] Adv: weed residues; B: wheat residues; V: vetch residues.

[7] Water content expressed with the corresponding matric potential (kPa).

* pH $CaCl_2$.

not systematically (Reddy and Locke, 1998). The increase in soil microbial activity under conservation techniques did not always mean that specific microbial populations involved in the degradation of a molecule were more abundant (Gaston and Locke, 2000). In some cases, crop residues on the soil surface under conservation tillage seemed to disrupt microorganisms' activity (Locke and Harper, 1991b; Sorenson et al., 1991), and, compared with conventional tillage, lag phases in the activation of mineralisation may occur (Seifert et al., 2001b). In addition, a greater availability of carbon under conservation tillage compared with conventional tillage can defer the use of pesticides as a source of carbon and thus their degradation in soil (Locke and Harper, 1991b).

Mineralisation is considered as the last step of pesticide degradation, leading to its complete removal from the soil. The mineralisation results are highly contrasted according to pesticides, location sites and incubation conditions (Table 5) and do not allow any conclusion on an increase or a limitation of mineralisation in conservation tillage. In the same way, non-extractable residue fractions vary widely depending on pesticides, techniques and soils. However, the formation of non-extractable residues is often correlated with the degradation half-life of the molecules (Gaston and Locke, 2000; Lavorenti et al., 2003; Zablotowicz et al., 2000). Locke and Harper (1991b) have also shown that the difference in non-extractable residues of metribuzin between conventional tillage and conservation tillage was mainly due to the coarse fraction of organic matter.

Moreover, although little information is generally mentioned in the literature, the age of the tillage system seems to be a major source of differences in pesticide degradation (Wagner et al., 1996). Sadeghi and Isensee (1996) have evaluated the effect of reversing the tillage of 7-year-old no-tillage and conventional tillage field plots on atrazine degradation. Their results suggested that, after reversing a well-established tillage, time for the new tillage to fully develop its particular effects on atrazine degradation may be shorter for a new conventional tillage than for a new no-tillage. Another major source of degradation variability in field studies is the inter-annual variability of climatic conditions. It can hide or completely cancel the effects of tillage and it can lead to opposite conclusions depending on the year (Baughman et al., 1996; Mills and Witt, 1991; Sorenson et al., 1991).

In most cases, pesticide degradation forms one or several degradation products. In the same manner as for mother compounds, their degradation can be modified by tillage practices. For example, under a no-tillage system, an accumulation of polar metabolites formed by the degradation of metribuzin was observed, while they were degraded under conventional tillage (Locke and Harper, 1991a, b). On the contrary, the formation of alachlor metabolites was faster but their degradation was slower under conventional tillage than under conservation tillage (Locke et al., 1996). Similarly, the accumulation of a fluometuron metabolite, trifluoromethylphenylurea (TFMPU), occurred mainly under conventional tillage (Zablotowicz et al., 2000), and another of its metabolites, dimethylfluometuron, seemed to be formed mainly when the air flow conditions, due notably to tillage operations, were favourable (Locke et al., 2005).

Pesticide formulation also appeared to have important consequences on their persistence. For example, whatever the tillage system, starch encapsulation of atrazine and alachlor tended to increase their field persistence (Gish et al., 1994).

Spatial variability of local conditions seems to be an important source of pesticide degradation variation. In their study, Gaynor et al. (1987) observed that the ridge tops retained more herbicide than the furrows, resulting in differences in pesticide persistence. Recently, Alletto et al. (2008) have examined the degradation of the diketonitrile metabolite of isoxaflutole under two tillage systems. Under conventional tillage, the herbicide persistence was found to be highly variable vertically and laterally according to soil sample location within the tilled horizon. The main source of variation in this case was the tillage operation with the mouldboard plough. Under mulch tillage with disk harrowing, MT_{Dk} (Table 1), the effect of tillage was minimised but variations in diketonitrile persistence were associated with the vertical distribution of organic carbon. Both of these studies highlight the importance of the soil sampling strategy in order to provide accurate assessment of environmental impacts of agricultural practices. Last, the mulch can reduce water content and temperature variations (Bragagnolo and Mielniczuk, 1990; Unger, 1987), leading to a lower variability of degradation under conservation tillage compared with conventional tillage (Mills and Witt, 1991).

3.3 Summary and Recommendations

Results about tillage effects on pesticide degradation are highly contrasted. Studies of the effects of management practices on soil properties and microorganism activity have to deal with complex interactions between soil physics, physico-chemistry and microbiology which control the microbial activities involved in pesticide breakdown. Particularly the strong coupling between retention and degradation processes highly depends on soil physical and hydrodynamic properties, ensuring the access of microbes to water, substrates and the movement of solutes such as pesticides to sorption and degradation sites. Degradation studies should therefore pay great attention to the soil conditions (dynamics of temperature, water content, pH, N content, etc.), which have been shown to be largely modified by the presence of the mulch at the soil surface. In the same way, studies focusing on the understanding and prediction of pesticide degradation in soil should use dynamic experimental systems where soil solution is allowed to move and be renewed (e.g. soil columns) rather than static (batch) experimental systems. Improving knowledge and quantitative prediction of pesticide degradation in soils also implies an accurate estimation of the spatial distribution and temporal dynamics of active degradative populations. Research efforts should thus be oriented at (a) improving field study for a better understanding of the in situ dynamics of these microorganisms and their location ('hot-spots'), and (b) establishing a comprehensive link between this dynamics and agricultural operations. Particular attention should be paid to the soil sampling strategy in this regard.

4 Transfer of Pesticides

Depending on their mobility and their persistence, pesticides can migrate within and outside the soil and contaminate other compartments of the environment, such as water and air. The three main transfer processes are volatilisation, leaching and runoff. The relative importance of each of these processes depends on the application conditions, the pesticide properties, the climatic conditions and the soil properties partly governed by agricultural practices.

4.1 Volatilisation

Volatilisation is an important pathway for the loss of pesticide that is controlled by the pesticide properties (such as saturated vapour pressure, Henry constant, K_{OC}), the soil properties (temperature, water content, organic carbon content), the farming operations (mode of application, soil roughness, presence of a mulch) and the climatic conditions (wind, solar radiation, temperature) (Bedos et al., 2002). Although volatilisation is often mentioned in the literature to explain differences in the pesticide persistence due to tillage operations (Banks and Robinson, 1982; Curran et al., 1992; Gaynor et al., 2000), this phenomenon remains poorly studied. Whang et al. (1993) highlighted a higher volatilisation in conservation tillage than in conventional tillage that was attributed to the presence of a mulch. In their study, four days after treatment, transfer by volatilisation accounted for 48 and 18% of applied fonofos, 23 and 7% of applied chlorpyrifos and 0.9 and 0.7% of applied atrazine under no-tillage and conventional tillage, respectively. Wienhold and Gish (1994) also observed a larger volatilisation of alachlor and atrazine under conservation tillage, but only until the first rainfall occurred (5 days after treatment). Following this rainfall, volatilisation in conservation tillage was strongly slowed down, which could be explained by the migration of the pesticides from the mulch to the soil. After 35 days, the cumulative loss accounted for 9 and 14% of applied alachlor and 4 and 9% of applied atrazine under conservation tillage and conventional tillage, respectively. In addition, this study showed a significant effect of pesticide formulation on volatilisation. Starch-encapsulated alachlor was less volatilised under conservation tillage than the commercial formulation, and a similar effect was measured for atrazine under both conservation tillage and conventional tillage systems. Volatilisation directly depends on environmental conditions and is favoured first by high temperatures (Glotfelty, 1987; Weber et al., 2002) and also by wet soils that keep the molecules available in water solution. Weber et al. (2006) observed during a year of monitoring that volatilisation reached 22 and 32% of applied metolachlor in conservation tillage and conventional tillage, respectively. Due to the mulch, soil surface water content was higher under conservation tillage, which slowed down the soil warming and thus reduced the amount of herbicide lost.

4.2 Leaching

Pesticide properties play a decisive role in the modifications of the leaching risk by tillage. First, retention properties determine the mobility of the molecules (Singh N et al., 2002) and directly influence their transfer to groundwater. Masse et al. (1998) reported that leaching of atrazine and deethylatrazine was more significant under conservation tillage, whereas metolachlor leaching, that has a stronger sorption capacity, was not affected by tillage. Water solubility of pesticides also influences their leaching. As an example, alachlor and cyanazine leaching was related to the interaction between tillage and water solubility (Sadeghi and Isensee, 1997). Under no-tillage, the migration depth of alachlor in soil was lower than that of cyanazine, whereas the opposite was observed under conventional tillage. The highest solubility of alachlor compared with that of cyanazine allowed a migration through the soil matrix, thus increasing the possibilities of adsorption, while cyanazine rather circulated via macropores under conservation tillage. Water solubility of the molecules may be modified by soil pH, which could increase leaching risk (Li et al., 2003). For sulfentrazone, solubility in water was, for example, multiplied by a factor of 16 when soil pH rose from 6 to 7.5 (Reddy and Locke, 1998). Other studies have shown a positive correlation between the half-life of several molecules (alachlor, atrazine, cyanazine, metolachlor, metribuzin and simazine) and their concentrations measured in drains without any tillage effect on these transfers (Logan et al., 1994; Ritter et al., 1996).

Formulation, by modifying solubility in water, persistence and retention in soils of pesticides, also influences their transport. Micro-encapsulation of alachlor increased its solubility in water and its transport through the soil matrix (Sadeghi et al., 1998). Starch-encapsulation of atrazine limited its losses by leaching in both no-tillage and conventional tillage (Gish et al., 1994, 1995). Hall et al. (1998) also observed a good efficiency of starch-encapsulation of atrazine, allowing a reduction of the leaching losses by a factor of 2 to 4 compared with the commercial formulation, depending on the year and on the application rate. Likewise, coating of metolachlor with a polyurea polymer helped reduce losses by a factor of 2 compared with the commercial formulation (Hall et al., 1998). According to these results, an efficient control of leaching seems achievable via adequate pesticide formulations.

Pesticide leaching depends on soil physical properties, such as the hydraulic conductivity, which is directly influenced by the soil structure created by tillage. Many studies on pesticide leaching have been conducted under field conditions, on plots equipped with ceramic cups, lysimeters or drains (Table 6), and under laboratory conditions, on undisturbed soil columns with simulated rainfalls (Table 7). Although most of these works showed greater losses under conservation tillage (Isensee and Sadeghi, 1997; Isensee et al., 1990; Masse et al., 1998; Singh et al., 2002; Weber et al., 2006), results are contrasted, some of them indicating no effect of tillage (Clay et al., 1998; Fomsgaard et al., 2003; Gaynor et al., 2000; Granovsky et al., 1993; Weed et al., 1995), or even greater losses under conventional tillage (Düring and Hummel, 1993; Gish et al., 1995; Levanon et al., 1993).

On one hand, mulch on the soil surface absorbs rainfall energy, thus avoiding the formation of soil crust (Baumhardt and Lascano, 1996; Blevins and Frye, 1993) and pore sealing (Ela et al., 1992). On the other hand, the continued deposition of crop residues on the soil surface appears to contribute to macropore development by stimulating earthworm activity (Bouché, 1972; Edwards et al., 1988; Rovira et al., 1987; Satchell, 1983). Under conservation tillage, and more particularly under no-tillage, the macropore network formed by earthworm burrows, root channels and cracks is not disrupted by tillage and thus may allow downward flows of water and solutes at a higher rate than if movements occurred only through the soil matrix. Because of this bypass of the soil matrix, this type of water and solute movement is called ‘preferential flow through macroporosity’. It occurs mainly during saturated conditions. In the soil matrix, solutes move by convection-dispersion and, due to significant contact between the liquid and solid phases, opportunities for pesticide retention are greater than in macropores (Shipitalo and Edwards, 1996). Ogden et al. (1999) showed that soil tillage destroyed the connectivity of the macropores and thus increased fluxes through the soil matrix.

Proportionally to their quantity, crop residues can limit losses due to leaching. By reducing the infiltration rate at the soil surface, i.e. at the crop residues/soil interface, the mulch promotes pesticide fluxes within the soil matrix, thus avoiding preferential flows through macropores (Sigua et al., 1993). On the other hand, at the residues/soil interface or when residues

Table 6 Field studies of pesticide leaching (% of applied dose) under different tillage practices

Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Depth cm	Water sampling system	Soil properties			Leaching % applied dose	Reference
					Clay	Organic carbon g kg^{-1}	pH		
Alachlor	2.2	NT	0–120	–	–	17.3–21.4	5.7–7.0	0.0002–0.10[2]	(Weed et al., 1995)
		RT		–	–	17.6–20.8	5.9–6.7		
		MT$_{Ch}$		–	–	18.6–21.3	5.7–6.8		
		CT		–	–	19.7–20.4	6.3–6.6		
Atrazine	2.2, 1.5, 1.8, 1.9[3]	NT	0–100	D[4]	–	–	–	0.09, 0.08, 0.15, 0.05[3]	(Masse et al., 1996)
Desethylatrazine		CT			–	–	–	0.04, 0.02, 0.12, 0.02	
		NT			–	–	–	0.10, 0.06, 0.19, 0.09	
		CT			–	–	–	0.07, 0.03, 0.15, 0.04	
Atrazine	2.8	NT	0–120	–	–	17.3–21.4	5.7–7.0	0.02–0.35	(Weed et al., 1995)
		RT		–	–	17.6–20.8	5.9–6.7		
		MT$_{Ch}$		–	–	18.6–21.3	5.7–6.8		
		CT		–	–	19.7–20.4	6.3–6.6		
Atrazine	1.3	MT$_{Ch}$	–	–	120–300	15.4–24.2	5.4–4.9	0.07–0.11, 0.08–0.22	(Fortin et al., 2002)
		CT	–	–	120–300	15.7–23.5	5.3–5.8	0.03–0.07, 0.11–0.12	
Atrazine	1.7	NT	0–120	L[4]	324–421	2.0–12.0	5.5–6.7	0.15–0.86, 0.21–9.60	(Hall et al., 1989)
		CT						<0.01–0.19, 0.75–0.85	
Atrazine, Cyanazine	1.7, 2.2, 1.7	MT mulch	0–120	L	–	–	–	3.0–5.1	(Watts and Hall, 1996)
Simazine		CT			–	–	–	0.69–0.93	
Cyanazine	2.2	NT	0–120	L	324–421	2.0–12.0	5.5–6.7	0.03–0.23, <0.10–4.73	(Hall et al., 1989)
		CT						<0.01–0.15, 0.32–0.56	
Dicamba	0.56	NT	–	–	–	2.0–12.0	5.5–6.7	1.99, 0.39, 5.56, 1.05, 2.45	(Hall and Mumma, 1994)
		CT	–	–	–			<0.01, 0.20, 0.20, 0.0, 0.58	
Fluometuron	1.6	NT	0–90	L	130	1.3–17.7	5.61	29.9–37.4, 37.9–50.8, 0–10.3, 0.8–54.7	(Essington et al., 1995)
		CT				1.1–10.2	5.85	11.9–69.3, 30.0–79.5, 6.1–28.7, 3.4–73.3	
Glyphosate	0.8	NT (20)[5]	0–110	L	134–227	1.1–19.2	5.9–7.6	0.022	(Fomsgaard et al., 2003)
AMPA		NT						0.018	
Glyphosate		CT			83–268	0.5–11.3	6.4–8.7	0.0305	
AMPA		CT						0.0205	

(continued)

Table 6 (continued)

					Soil properties				
Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Depth cm	Water sampling system	Clay	Organic carbon g kg^{-1}	pH	Leaching % applied dose	Reference
Metolachlor	2.6, 2.6, 2.6, 2.4	NT	0–100	D	–	–	–	0.00, 0.02, 0.02, 0.04	(Masse et al., 1996)
		CT			–	–	–	0.00, 0.00, 0.02, 0.01	
Metolachlor	2.2	MT mulch	0–120	L	–	–	–	2.46	(Watts and Hall, 1996)
		CT			–	–	–	0.37	
Metolachlor	2.5	MT$_{Ch}$	–	–	120–300	15.4–24.2	5.4–4.9	0.02–0.04, 0.06–0.12	(Fortin et al., 2002)
		CT	–	–	120–300	15.7–23.5	5.3–5.8	0.01–0.03, 0.07–0.10	
Metolachlor	4.48	NT (10)	0–97	L	60–290	3.0–6.4	4.4–6.1	1.4, 6.7	(Weber et al., 2006)
		CT						0.7, 4.4	
Metolachlor	2.2	NT	0–120	L	324–421	2.0–12.0	5.5–6.7	0.01–0.47, <0.10–4.19	(Hall et al., 1989)
		CT						<0.01–0.10, 0.25–0.61	
Metribuzine	0.45	NT	0–120	–	–	17.3–21.4	5.7–7.0	0.14–0.87	(Weed et al., 1995)
		RT		–	–	17.6–20.8	5.9–6.7		
		MT$_{Ch}$		–	–	18.6–21.3	5.7–6.8		
		CT		–	–	19.7–20.4	6.3–6.6		
Simazine	1.7	NT	0–120	L	324–421	2.0–12.0	5.5–6.7	0.06–1.76, 0.18–8.36	(Hall et al., 1989)
		CT						0.01–0.18, 1.50–1.63	

[1] Codes of tillage practices are reported in Table 1.
[2] Hyphens are used to indicate the range of variation of values.
[3] Commas are used to separate different years of study.
[4] D: water sampling in drains; L: water sampling in lysimeters.
[5] Numbers in parenthesis indicate the age in years of the tillage system.

Table 7 Laboratory studies on pesticide leaching (% of applied dose) under different tillage practices

Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Depth cm	Soil properties			Rainfall intensity mm h^{-1}	Leaching % applied dose	Reference
				Clay	Organic carbon g kg^{-1}	pH			
Alachlor	2.2	NT (16)[2]	0–30	–	–	–	50	1.6	(Weed et al., 1998)
		CT		–	–	–		0.4	
Alachlor	3.3	NT (8)	0–10	–	–	–	50	29.7	(Clay et al., 1991)
		CT		–	–	–		14.4	
Atrazine	2.8	NT	0–18	170	10.2–15.3[3]	6.4–5.9	–	14.7	(Levanon et al., 1993)
		CT			11.8–12.6	6.4–6.7	–	19.3	
		NT			10.2–15.3	6.4–5.9	–	12.9	
		CT			11.8–12.6	6.4–6.7	–	20.4	
Atrazine	1.3	NT	0–10	–	NT: 7.5–24.4	–	9	58.4	(Sigua et al., 1995)
		CT		–	CT: 7.0	–	9	42.7	
		NT		–		–	9	47.2	
		CT		–		–	9	33.2	
		NT		–		–	9	31.6	
		CT		–		–	9	29.4	
		NT		–		–	9	26.7	
		CT		–		–	9	20.1	
Atrazine	1.3	NT	0–10	–	NT: 24.4–7.5	–	9	50.7	(Sigua et al., 1995)
		NT		–	CT: 7.0	–	9	35.7	
		NT		–		–	9	34.9	
		NT		–		–	9	35.9	
		CT		–		–	9	40.3	
		CT		–		–	9	40.1	
		CT		–		–	9	38.2	
		CT		–		–	9	37.0	
Carbofuran	1.9	NT	0–18	170	10.2–15.3	6.4–5.9	–	4.5	(Levanon et al., 1993)
		CT			11.8–12.6	6.4–6.7	–	18.7	
		NT			10.2–15.3	6.4–5.9	–	4.2	
		CT			11.8–12.6	6.4–6.7	–	7.7	
Diazinon	2.5	NT	0–18	170	10.2–15.3	6.4–5.9	–	0.25	(Levanon et al., 1993)
		CT			11.8–12.6	6.4–6.7	–	0.35	
		NT			10.2–15.3	6.4–5.9	–	0.0	
		CT			11.8–12.6	6.4–6.7	–	0.0	

(continued)

Table 7 (continued)

				Soil properties			Rainfall		
Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Depth cm	Clay	Organic carbon g kg^{-1}	pH	intensity mm h^{-1}	Leaching % applied dose	Reference
Metamitron	4	NT (20)	0–30/40	112–210	9.0–18.0		0.16, 0.38, 0.46	0.03, 3.0, 10.1	(Düring and Hummel, 1999)
		CT		151–217	9.0–1.0			0.3, 5.0, 12.2	
Metolachlor	2.25	NT	0–18	170	10.2–15.3	6.4–5.9	–	8.2	(Levanon et al., 1993)
		CT			11.8–12.6	6.4–6.7	–	10.2	
		NT			10.2–15.3	6.4–5.9	–	6.5	
		CT			11.8–12.6	6.4–6.7	–	9.3	
Metolachlor	10	NT (20)	0–30/40	112–210	9.0–18.0	–	0.25, 0.42	1.8, 5.7	(Düring and Hummel, 1999)
		CT		151–217	9.0–1.0	–		3.6, 12.4	
Metolachlor	20	NT	0–15	280	19.0	7.3	saturation	38	(Singh N et al., 2002)
		CT						27	
Terbuthylazine	5	NT (20)	0–30/40	112–210	9.0–18.0	–	0.25, 0.42	3.5, 9.7	(Düring and Hummel, 1999)
		CT		151–217	9.0–1.0	–		5.2, 12.2	
Terbuthylazine	10	NT	0–15	280	19.0	7.3	saturation	11	(Singh N et al., 2002)
		CT						6	

[1] Codes of tillage practices are reported in Table 1.

[2] Numbers in parenthesis indicate the age in years the tillage system.

[3] Hyphens are used to indicate the range of variation of values.

and soil are mixed, the presence of these two compartments that have different water-holding capacities and hydraulic conductivities can also generate preferential flow (Kasteel et al., 2007; Ma and Selim, 2005).

There is no direct relationship between the number of macropores and the intensity of preferential flow, since some macropores do not participate in the conduction of water (Shipitalo et al., 1990). Preferential flow activation seems to depend, in particular, on the initial soil water content, but in contrasted ways according to studies (Granovsky et al., 1993; Shipitalo and Edwards, 1996). Although preferential flows through macropores occur in both conservation and conventional tillage (Andreini and Steenhuis, 1990; Essington et al., 1995; Gish et al., 1991; Granovsky et al., 1993), this dependency on initial water content was found to be greater under conservation tillage (Flury et al., 1995; Sigua et al., 1995). At low initial water content, the hydrophobicity of organic materials at the soil surface under conservation tillage could limit the entry of water into the soil matrix, creating locally and temporarily saturation conditions, thus favouring macropore fluxes (Edwards et al., 1989, 1992a; Phillips et al., 1989; Shipitalo et al., 1990). Despite earthworm burrows being found to have high pesticide sorption capacities due to high amounts of organic compounds in burrow linings (Edwards et al., 1992b; Stehouwer et al., 1993), water and pesticide transfer rates through macropores are generally higher than for the soil matrix (Shipitalo and Edwards, 1996). Other studies showed a higher leaching of atrazine (Kitchen et al., 1998; Seyfried and Rao, 1987; Sigua et al., 1995) and fluometuron (Essington et al., 1995) under wet soil initial conditions. A better understanding of the effect of the initial water content on the dynamics of macropore flow is thus needed to improve the control of pesticide leaching and to complement modelling efforts. An illustration is given by Sigua et al. (1995), who observed under laboratory conditions that a decrease in soil matric potential from −1 kPa (near saturation) to −33 kPa led to a decrease of 15% in atrazine leaching.

In addition, the temporal dynamics of preferential flow through macropores needs to be taken into account. During wetting, the number of macropores hydraulically active tends to increase, thus increasing the possibilities of preferential flow (Jaynes et al., 2001; Kung et al., 2000; Malone et al., 2001). However, under unsaturated conditions, macropores are inactive but still the observed movements of solutes through the soil matrix may be faster than those estimated by the convection-dispersion equation.

To describe solute movements in the soil matrix better, the Mobile–Immobile water Model (MIM) has been developed (Coats and Smith, 1964). This model considers that the water-filled pore space is partitioned into two domains: a mobile domain where water can move and solute transport is due to convection and dispersion, and an immobile domain where water is stagnant and solutes move only by diffusion (Coats and Smith, 1964; van Genuchten and Wierenga, 1976). In this model, it is possible to distinguish different degradation and retention kinetics between the two domains (van Genuchten and Wagenet, 1989), allowing a better description of preferential fluxes under unsaturated conditions (Gaston and Locke, 1996; Gaston and Locke, 2000; Pot et al., 2005). This type of preferential flow has been identified for pesticides under both conventional and conservation tillage (Gaston and Locke, 1996; Gaston and Locke, 2000; Singh N et al., 2002), but laboratory studies on undisturbed soil columns showed that immobile water fractions were higher under conservation tillage than under conventional tillage, with 56 vs. 49% (Singh and Kanwar, 1991) and 56 vs. 35% (Singh N et al., 2002), respectively. As for macropore fluxes, preferential flows within the soil matrix also seem to increase under wet soil conditions (Shipitalo and Edwards, 1996). Despite its strong impact on solute transfers, little data about the impact of tillage on MIM-type preferential flow has been published and thus further experimental studies are needed.

Intensity and timing of rainfall after treatment are major factors affecting pesticide leaching (Granovsky et al., 1993; Heatwole et al., 1997; Isensee and Sadeghi, 1994; Masse et al., 1996). Their effects may be greater than those generated by tillage (Gaynor et al., 1995; Granovsky et al., 1993; Otto et al., 1997). During small, low-intensity rainfalls, pesticides intercepted by the mulch can be washed off and then can penetrate slowly into the soil matrix where sorption processes can reduce their leaching (Shipitalo et al., 1990). On the contrary, during high-intensity rainfalls, wash-off is significant and the hydrophobicity of organic residues can slow down infiltration and activate preferential flow in macropores (Isensee and Sadeghi, 1994). By removing the mulch under conservation tillage, Sadeghi and Isensee (1997) observed that leaching was reduced and finally, lower losses under conservation tillage than under conventional tillage were measured. Furthermore, high-intensity rainfall generally leads to greater water and solute fluxes than low-intensity rainfall (Quisenberry et al., 1994; Trojan and Linden, 1992), with most pesticide leaching occurring during the first 2 or 3 rains (Fortin et al., 2002). For atrazine, Sigua et al. (1993) showed on undisturbed soil columns collected under no-tillage plots that the intensity of rainfall determines the percentage of losses: 33% of the applied dose for an intensity of 3 mm h^{-1} and 52% at 9 mm h^{-1}. In addition, if a small and low-intensity rainfall (1.5 mm for Sadeghi and Isensee, 1997, or 5 mm for Shipitalo et al., 1990) preceded a leaching event, then pesticide transport could be reduced by 50% compared with treatments that did not receive this preliminary rain. Moreover, these studies also showed that allowing time for sorption and diffusion in the soil matrix to occur can reduce pesticide movements in soils.

Some questions remain about how rapidly soil hydraulic properties change and preferential flow paths develop when tillage is modified. Very few data have been published on this aspect. In their study, Isensee and Sadeghi (1996) reversed the tillage of 7-year-old no-tillage and conventional tillage plots on which preferential flows had been previously observed (Isensee and Sadeghi, 1994; Isensee et al., 1990; Sadeghi and Isensee, 1992). They found that, immediately after ploughing, preferential flows under the new conventional tillage had disappeared and that sufficient macropore pathways were developed under the new no-tillage in only one year without tillage, leading to significant leaching differences. However, it seemed that two years were required before preferential transport systems became well established. Moreover, in the new conventional tillage, the macropore network inherited from 7 years of no-tillage and situated below the newly ploughed horizon appeared to be functional and preferential flow could still occur (Isensee and Sadeghi, 1997). Soil structural stability, which depends on soil texture, also greatly influences the dynamics of preferential flow (Singh et al., 2002). For example, in four soils of various textures under no-tillage, clay content enhanced macropore stability, leading to a leaching of atrazine 40% higher than for a sandy soil (Sadeghi et al., 2000).

Most of the studies about the effects of tillage on leaching compare contrasted techniques such as ploughing and no-tillage. Other conservation techniques such as mulch tillage or ridge tillage are still poorly studied in terms of pesticide leaching. In some studies of mulch tillage systems, greater leaching of atrazine, cyanazine, simazine and metolachlor was measured compared with conventional tillage (Watts and Hall, 1996). To the contrary, other studies did not find any differences in atrazine and metolachlor leaching between mulch tillage and conventional tillage, concluding that the differentiation between these systems was too weak (Fortin et al., 2002). Between mulch tillage and ridge tillage systems, differences in the migration of atrazine were also poorly marked (Kitchen et al., 1998). However, localisation of the herbicide in seed rows under ridge tillage allowed a decrease by a factor of 3 in the applied doses and could significantly reduce losses by leaching (Lamb et al., 1998; Lowery et al., 1998). In their study comparing three different tillage systems, Mazzoncini et al. (1998) observed a deeper migration of diclofop-methyl after a heavy rainfall under no-tillage, while the pesticide was found mainly in the seedbed layer (0–5-cm depth) under mulch and conventional tillage.

4.3 Runoff

Agricultural runoff is the primary mechanism contributing to pesticide contamination of surface waters. The main objective of conservation tillage is to reduce runoff and soil erosion (Gebhardt et al., 1985).

Runoff is also affected by pesticide properties. Sorption properties directly act on the dominant mode of transport. For pesticides that have a high sorption capacity on organo-mineral particles, such as glyphosate, trifluralin, paraquat or organochlorine pesticides, surface transport is associated with erosion and soil particle transport (Wauchope, 1978). Potter et al. (2004) indicated that 55% of pendimethalin (K_{OC} = 5000 L kg^{-1}) losses were bound to sediment. However, for most pesticides, transport to surface water is realised in solution in water runoff (Wauchope, 1978). Losses in solution represented from 88 to 97% of total losses of alachlor and cyanazine (Hansen et al., 2001), 99.8% of atrazine losses (Basta et al., 1997) and, according to tillage practices, from 89 to 98% of fluometuron losses (Potter et al., 2004). For herbicides transported only in solution, such as chlorimuron and nicosulfuron, the interest of conservation techniques, especially no-tillage used to control erosion, is very limited (Afyuni et al., 1997). For some authors, however, pesticide transport in solution may be overestimated due to desorption from suspended sediments during runoff or sample storage and thus the importance of the solid phase as a source of pesticides in runoff would be underestimated (Hansen et al., 2001).

Logan et al. (1994) compared the effects of conventional tillage and no-tillage on the transport of atrazine, alachlor, metolachlor and metribuzin by runoff. No difference between tillage systems was found but runoff losses were positively correlated with the half-lives of the molecules. In other studies, water solubility directly determines the loss by runoff and explains differences in behaviour between alachlor and cyanazine (Hansen et al., 2001) or between atrazine and metribuzin (Gaynor et al., 2001). Isoxaflutole has a low solubility in water but its hydrolysis forms a diketonitrile metabolite (active ingredient) which is 50 times more soluble and thus transported by runoff (Rector et al., 2003). However, mainly because of analytical costs, metabolite monitoring in runoff is not systematic even in research studies. For fluometuron, Potter et al. (2004) indicated that 50% of the herbicide losses occurred as desmethylfluometuron metabolite. Results of runoff losses for some molecules, such as atrazine or fluometuron, are often contrasted under conservation tillage despite similar water solubility and retention by organic compounds. Potter et al. (2003) suggested a relationship to predict the mode of transport of pesticides (in solution or adsorbed) based on their K_{OC} and their solubility.

Pesticide solubility may vary according to formulation. For example, micro-encapsulation of alachlor increases its solubility, allowing a faster migration within the soil matrix and a decrease in its runoff compared with the commercial formulation (Isensee and Sadeghi, 1993). Metolachlor solubility is higher than that of atrazine but it may be reduced by its formulation, which may then limit its runoff compared with atrazine (Sadeghi and Isensee, 2001).

Concerning soil properties, increasing soil organic matter content in topsoil improves soil aggregate stability and cohesion, leading to a significant decrease in soil loss (Rhoton et al., 2002). It appears, however, that tillage effects on pesticide transfer by runoff or erosion are, as for leaching, mixed (Table 8). Many works highlighted a decrease in pesticide losses by runoff under conservation tillage due to a decrease in water runoff volume as the degree of tillage is reduced (Seta et al., 1993; Tebrügge and During, 1999; Watts and Hall, 1996; Webster and Shaw, 1996). However, in some studies, pesticide concentrations in runoff from conservation tillage plots, especially no-tillage plots, were higher than in runoff from conventional tillage plots. As a consequence, conservation tillage plots may generate greater pesticide losses despite a lower water runoff volume (Heatwole et al., 1997; Kenimer et al., 1987; Shipitalo and Owens, 2006; Webster and Shaw, 1996). In other studies, water runoff volume was higher under conservation tillage (Gaynor et al., 1995; Myers et al., 1995). A review of tillage effects by Fawcett et al. (1994) concluded that, under natural rainfall, conservation tillage was efficient in controlling runoff, erosion and pesticide losses. Under simulated rainfall, the amount and intensities of water applied shortly after treatment resulted in very mixed results in conservation tillage. For Fawcett et al. (1994), intensive rainfall was more an indicator of the limits of the efficiency of conservation tillage to control runoff rather than an accurate representation of their effects under real field conditions. Modelling was used to identify and classify the most suitable practices to control atrazine runoff in a watershed (Harman et al., 2004). In this study, conservation tillage was found to be marginally effective. The most effective practices were: sediment retention ponds, grass filter strips, atrazine application at planting time in bands and wetlands construction. According to these contradictory results, it seems necessary to clarify the main conditions and mechanisms involved in pesticide transfer by runoff that may lead to an efficient or inefficient control of losses by conservation tillage.

Rainfall is the most important parameter controlling water runoff, soil erosion and pesticide loss. Depending on its arrival time after treatment, its intensity and the interval between two rainfall events, rainfall can lead to very contradictory results for the same study site, the same molecule or even the same practice (Baker and Johnson, 1979). Pesticide transport in soils directly depends on the interval between treatment and the first rainfall (Baker and Johnson, 1979; Potter et al., 2004; Shipitalo and Owens, 2003). Several studies indicated that conservation tillage was inefficient in controlling runoff when heavy rainfall occurs quickly after pesticide application (Rector et al., 2003; Shipitalo and Owens, 2003; Shipitalo and Owens, 2006). Moreover,

Table 8 Field studies of water and pesticide runoff under simulated (S) or natural (N) rainfall under different tillage practices

			Soil properties					Runoff			
Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Clay	Organic carbon (OC) g kg^{-1}	pH	Slope %	Rainfalls mm	Type	water % applied dose	pesticide % applied dose	Reference
Alachlor	2.24	NT	–	5.8–17.4[2]	–	12–18[2]	657, 487, 463, 425[3]	N[4]	13, 4, 12, 8[3]	7.1, 0.45, 1.8, 0.1[3]	(Baker and Johnson, 1979)
		RT	–		–				17, 4, 8, 8	8.0, 0.8, 0.45, <0.1	
		CT	–		–				17, 8, 18, 9	14.3, 0.7, 4.1, 0.2	
Alachlor	2.80	NT	220	17.4	–	6	136	N	34, 41	0.32, 4.8	(Sauer and Daniel, 1987)
		MT$_{Ch}$							28, 54	0.25, 2.1	
		RT							35, 54	0.36, 6.0	
		CT							44, 48	0.29, 3.0	
Alachlor	1.79	NT	–	7.4–23.7	–	3–5	–	–	4–6, 3–4[1]	0.22–0.24, 0.15–0.24	(Isensee and Sadeghi, 1993)
		CT	–	6.6–6.9	–		–	–	5–6, 3–5	0.48–0.57, 0.15–0.28	
Alachlor	3.36	NT C[5]	–	12.8	–	7–11	67	S[4]	19	0.06	(Felsot et al., 1990)
		NT U/D[5]	–		–		66		34	0.08	
		CT C	–		–		67		27	0.1	
		CT U/D	–		–		56		58	0.2	
Alachlor	3.36	NT C[5]	–	12.8	–	8	63	S	6	0.09	(Felsot et al., 1990)
		NT U/D[5]	–		–	8	64		23	2.1	
		ST C	–		–	9	64		4	0.06	
		ST U/D	–		–	9	63		10	0.6	
		RT C	–		–	10	64		24	0.75	
		RT U/D	–		–	10	63		29	1.3	
		MT$_{Ch}$C	–		–	8	64		33	1.7	
		MT$_{Ch}$ U/D	–		–	8	64		32	1.2	
		CT C	–		–	10	63		33	2.3	
		CT U/D	–		–	10	64		43	6.5	
Alachlor	2.24–3.36	NT 1	–	–	–	11	629–1012	N	1–10	Tr-0.23	(Shipitalo et al., 1997)
		NT 2	–	–	–	10	686–1025		2–14	0.01–0.19	
		MT$_{Ch}$ 1	–	–	–	13	609–981		<0.01–1	Tr-<0.01	
		MT$_{Ch}$ 2	–	–	–	7	663–1007		1–15	Tr-0.22	
Alachlor	2.2	MT$_{Ch}$	–	14.0	7.1	8–10	240, 480	N	–	0.4, 0.2	(Hansen et al., 2001)
	2.2	RT	–	17.4					–	0.1, 0.1	
	2.2	CT	–	13.4					–	1.0, 0.6	
	0.7 Loc[6]	MT$_{Ch}$	–	14.0					–	0.1, 0.06	
	0.7 Loc	RT	–	17.4					–	0.1, 0.06	
	0.7 Loc	CT	–	13.4					–	0.2, 0.06	

Alachlor[7]	1.68	NT	–	–	–	10–11	976	N	8	0.28	(Shipitalo and Owens, 2006)
	3.36	MT$_{Ch}$	–	–	–	7–13	953		7	0.11	
		MT$_{Dk}$	–	–	–	6–9	960		11	0.12	
Atrazine	1.7	NT	–	–	–	3–5	–	–	–	0.06, –	(Watts and Hall, 1996)
		MT mulch	–	–	–	–	–	–	–	–, 0.28	
		CT	–	–	–		–	–	–	0.18, 0.77	
Atrazine	1.68	NT	67	9.7	5.64	2	–	–	–	0.7	(Heatwole et al., 1997)
		CT	59	4.4	5.91		–	–	–	1.5	
Atrazine (+ DEA + DIA)	2.24	NT 1	–	–	–	13	–	–	4–7	0.027–0.82	(Shipitalo and Owens, 2003)
	2.24	NT 2	–	–	–	7	–	–	6–15	0.049–4.95	
	2.24	MT$_{Ch}$ 1	–	–	–	11	–	–	0.1–2	Tr-0.011	
	2.24	MT$_{Ch}$ 2	–	–	–	10	–	–	9–18	0.0045–2.62	
	1.12	MT$_{Dk}$ 3	–	–	–	6	–	–	7–17	0.034–0.64	
	1.12	MT$_{Dk}$ 4	–	–	–	7	–	–	4–8	0.0010–0.59	
	1.12	MT$_{Dk}$ 5	–	–	–	9	–	–	10–22	0.0043–6.37	
Atrazine	2.24	NT	–	5.8–17.4	–	12–18	657, 487	N	13, 4	7.1, 0.75	(Baker and Johnson, 1979)
		RT	–		–				17, 4	19.2, 1.8	
		CT	–		–				18, 8	20.1, 0.09	
Atrazine	2.80	NT	220	17.4	–	6	136	–	34, 41	0.32, 7.3	(Sauer and Daniel, 1987)
		MT$_{Ch}$						–	28, 54	0.21, 3.0	
		RT						–	35, 54	0.34, 8.7	
		CT						–	44, 48	0.25, 4.3	
Atrazine	2.24	NT	–	–	–	–	941, 1158, 824, 926, 1029, 860		5, 6, 7, 18, –, 1	2.5, 1.9, 1.0, 5.1, –, 2.0	(Ghidey et al., 2005)
		MT	–	–	–	–			1, 4, 7, 18, 18, 1	1.1, 0.3, 0.6, 1.5, 4.0, 2.1	
Atrazine	1.34	NT	–	7.4–23.7		3–5	–	–	4–6, 3–4	1.39–1.51, 0.56–0.90	(Isensee and Sadeghi, 1993)
		CT	–	6.6–6.9			–	–	5–6, 3–5	0.81–0.97, 0.21–0.47	
Atrazine	1.7	NT	–	12.0	6.7	3–5	–	–	–, 0.1, <0.01, 0.02	–, 0.10, <0.01, <0.01	(Hall et al., 1991)
		CT	–			3–4	–	–	0.3, 0.3, 0.03, 0.04	0.36, 0.33, 0.02, <0.01	

(continued)

Table 8 (continued)

			Soil properties						Runoff		
Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Clay	Organic carbon (OC) g kg^{-1}	pH	Slope %	Rainfalls mm	Type	water % applied dose	pesticide % applied dose	Reference
Atrazine	2.24	NT	–	–	–	9	132	S	6	0.7	(Seta et al., 1993)
		MT	–	–	–				22	1.3	
		CT	–	–	–				34	1.8	
Atrazine	2.24	NT	232	21.5	–	10.3	100	S	0.2	0.28	(Kenimer et al., 1987)
		CT				10.6			3	2.85	
Atrazine	0.56	NT (1)[8]	–	9.5	4.9	4	80	S		7.9	(Basta et al., 1997)
		MT$_{Ch}$								10.5	
		MT$_{Dk}$								7.5	
Atrazine	2.24	NT 1	–	–	–	11	629–1012	N	1–10	<0.01–0.08	(Shipitalo et al., 1997)
		NT 2	–	–	–	10	686–1025		2–14	<0.01–1.10	
		MT$_{Ch}$ 1	–	–	–	13	609–981		<0.01–1	<0.01	
		MT$_{Ch}$ 2	–	–	–	7	663–1007		1–15	<0.01–0.91	
Atrazine	1.6	NT (2) – M.[9]	210	9.1	5.9	2–6	31.7–50.8	S	31–50, 35–38	2.2–9.4, 2.1–4.7	(Myers et al., 1995)
		NT – Sil.[8]							31–56, 35–48	1.7–8.1, 1.9–2.5	
		CT							16–24, 34–47	0.5–1.6, 1.7–3.0	
Atrazine	0.9	NT (4)	–	13.4	6.0	1–2	–	N/N + S	–	4.99/10.51	(Rector et al., 2003)
	0.9 Inc.[10]	CT					–		–	1.26/2.73	
	0.9	CT					–		–	2.48/7.72	
	1.8	NT (4)					–		–	2.64/7.52	
Atrazine	0.55	MT$_{Ch}$ + Cov.C[11]	–	14.5	–	<1	995, 688	N	8, 7	0.1, 1.8	(Gaynor et al., 2001)
		MT$_{Ch}$							14, 9	0.4, 1.5	
		CT + Cov.C							8, 6	0.4, 0.7	
		CT							12, 7	0.6, 1.8	
Atrazine[7]	2.24	NT	–	–	–	10–11	976	N	8	1.29	(Shipitalo and Owens, 2006)
		MT$_{Ch}$	–	–	–	7–13	953		7	0.66	
		MT$_{Dk}$	–	–	–	6–9	960		11	0.92	
Carbofuran	1.12	NT C	–	12.8	–	7-11	67	S	19	0.2	(Felsot et al., 1990)
		NT U/D					66		34	0.6	
		CT C					67		27	0.1	
		CT U/D					56		58	1.21	
Chlorimuron	0.014	NT	80	5.3	5.9	0–1	–	–	–	2.0	(Afyuni et al., 1997)
		CT								1.3	
Chlorpyrifos	1.34	NT	220	17.4	–	6	136	–	34, 41	0.04, 0.10	(Sauer and Daniel, 1987)
		MT$_{Ch}$							28, 54	0.05, 0.29	
		RT							35, 54	0.06, 0.28	
		CT							44, 48	0.08, 0.20	

Cyanazine	2.24	NT	–	5.8–17.4	–	12–18	463, 425	N	12, 8	4.5, 0.2	(Baker and Johnson, 1979)
		RT							8, 8	2.0, 0.1	
		CT							18, 9	8.0, 0.3	
Cyanazine	1.34	NT	–	7.4–23.7	–	3–5	–	–	4–6, 3–4	1.30–1.91, 0.50–0.68	(Isensee and Sadeghi, 1993)
		CT	–	6.6–6.9	–				5–6, 3–5	0.52–0.84, 0.15–0.27	
Cyanazine	2.2	NT	–	12.0	6.7	3–5	–	–	–, 0.1, <0.01, 0.02	–, 0.06, <0.01, <0.01	(Hall et al., 1991)
		CT				3–4			0.3, 0.3, 0.03, 0.04	0.13, 0.30, 0.01, <0.01	
Cyanazine	2.1	MT_{Ch}	–	14.0	7.1	8–10	240, 480	–	–	2.0, 0.9	(Hansen et al., 2001)
	2.1	RT	–	17.4					–	0.38, 0.8	
	2.1	CT	–	13.4					–	4.4, 1.0	
	0.7 Loc[6]	MT_{Ch}	–	14.0					–	0.8, 0.2	
	0.7 Loc	RT	–	17.4					–	0.6, 0.2	
	0.4 Loc	CT	–	13.4					–	0.8, 0.2	
Cyanazine	2.2	NT	–	–	–	3–5	–	–	–	0.02, –	(Watts and Hall, 1996)
		MT mulch							–	–, 0.17	
		CT							–	0.11, 0.39	
Dicamba	0.56	NT	–	12.0	6.7	3–5	–	–	–, 0.1, <0.01, 0.02	–, 0.12, <0.01, <0.01	(Hall and Mumma, 1994)
		CT				3–4			0.3, 0.3, 0.03, 0.04	0.46, 0.81, <0.01, <0.01	
Dimethipin	0.35	ST (1)	32	5.1	6.5	3–4	–	S	23	5.0	(Potter et al., 2003)
		CT							23	1.6	
Fluometuron	1.7	NT	–	18.6	6.4	3	376, 480	N + S	6	8.0, 5.3	(Baughman et al., 2001)
		RT							5	8.2, 3.2	
		CT							6	9.9, 4.4	
Fluometuron	2.0	ST	32	5.1	6.5	3–4	63.8	S-I^{12}_{Var}	20	1.4	(Potter et al., 2006)
		ST					64.3	S-I^{12}_{cons}	23	1.9	
		CT					62.7	S-I_{Var}	45	0.8	
		CT					60.0	S-I_{cons}	50	0.6	
Fonofos	1.12	NT	–	5.8–17.4	–	12–18	487, 463, 425		4, 12, 8	0.18, 1.3, 0.05	(Baker and Johnson, 1979)
		RT							4, 8, 8	0.09, 0.25, nd	
		CT							8, 18, 9	0.36, 1.0, 0.07	

(continued)

Table 8 (continued)

Pesticide	Dose	Tillage[1]	Soil properties			Slope	Rainfalls	Type	Runoff		Reference
	kg a.i. ha^{-1}		Clay	Organic carbon (OC) g kg^{-1}	pH	%	mm		water % applied dose	pesticide % applied dose	
Imazaquin	0.14	SS (3)	660	16.9	6.4	NA	–	–	–	1.22, 3.39	(Seifert et al., 2001b)
		CT							–	1.53, 3.63	
Isoxaflutole/diketonitrile	0.11	NT (4)	–	13.4	6.0	1–2	–	N/N + S	–	2.82/9.24	(Rector et al., 2003)
	0.11 Inc.[10]	CT							–	0.93/3.35	
	0.11	CT							–	1.63/6.75	
	0.05	NT (4)							–	2.35 / 6.84	
Linuron	1.12	NT 1	–	–	–	11	629–1012	N	1–10	<0.01–0.01	(Shipitalo et al., 1997)
		NT 2	–	–	–	10	686–1025		2–14	<0.01–1.04	
		MT_{Ch} 1	–	–	–	13	609–981		<0.01–1	<0.01	
		MT_{Ch} 2	–	–	–	7	663–1007		1–15	<0.01–0.46	
Linuron[6]	1.12	NT	–	–	–	10–11	976	N	8	0.70	(Shipitalo and Owens, 2006)
		MT_{Ch}	–	–	–	7–13	953		7	0.29	
		MT_{Dk}	–	–	–	6–9	960		11	0.21	
Metolachlor	3.4	NT − S/W[13]	–	18.6	6.4	3	213, 191, 188	N+S	8, 7, 9	0.4, 0.2, 0.6	(Webster and Shaw, 1996)
		NT − Soy[13]							–, 14, 18	–, 1.4, 0.3	
		CT − S/W							9, 9, 15	0.8, 1.1, 0.6	
		CT − Soy							9, 5, 10	0.4, 0.5, 0.8	
Metolachlor	1.9	NT (2) – M.[9]	210	9.1	5.9	2–6	31.7–50.8	S	31–50, 35–38	1.2–9.0, 0.9–4.3	(Myers et al., 1995)
		NT – Sil.[9]							31–56, 35–48	1.4–8.0, 0.8–1.8	
		CT							16–24, 34–47	0.4–1.5, 1.1–2.7	
Metolachlor	2.2	NT	–	12.0	6.7	3–5	–	–	–, 0.1, <0.01, 0.02	–, 0.06, <0.01, <0.01	(Hall et al., 1991)
		CT							0.3, 0.3, 0.03, 0.04	0.16, 0.25, 0.01, <0.01	
Metolachlor	2.2	NT	–	–	–	3–5	585, 748, 486, 562	N	–	0.03, –	(Watts and Hall, 1996)
		MT mulch							–	–, 0.22	
		CT							–	0.29, 0.55	
Metolachlor	2.24	NT	67	9.7	5.64	2	–	–	–	0.5	(Heatwole et al., 1997)
		CT	59	4.4	5.91				–	1.2	
Metolachlor	1.12–1.87	NT	–	–	–	–	941, 1158, 824, 926, 1029, 860	N	5, 6, 7, 18, –, 1	5.5, 1.0, 0.9, 2.1, –, 0.3	(Ghidey et al., 2005)
		MT	–	–	–	–			1, 4, 7, 18, 18, 1	2.0, 0.4, 1.2, 1.6, 5.4, 0.4	

Metolachlor	0.84	MT$_{Ch}$ + Cov.C[11]	–	14.5	–	<1	995, 688	N	8, 7	0.06, 0.8	(Gaynor et al., 2001)
		MT$_{Ch}$							14, 9	0.2, 1.0	
		CT + Cov.C							8, 6	0.3, 0.5	
		CT							12, 7	0.4, 1.4	
Metolachlor	0.7	NT (4)	–	13.4	6.0	1–2	–	N/N + S	–	4.22/8.28	(Rector et al., 2003)
	0.7 Inc.[10]	CT							–	1.01/1.97	
	0.7	CT							–	2.22/5.98	
	1.4	NT (4)							–	2.44/6.01	
Metribuzin	0.38	NT 1	–	–	–	11	629–1012	N	1–10	<0.01–0.79	(Shipitalo et al., 1997)
		NT 2	–	–	–	10	686–1025		2–14	0.08–0.22	
		MT$_{Ch}$ 1	–	–	–	13	609–981		<0.01–1	<0.01–0.02	
		MT$_{Ch}$ 2	–	–	–	7	663–1007		1–15	<0.01–0.01	
Metribuzin[7]	0.38	NT	–	–	–	10–11	976 953 960	N	8	0.83	(Shipitalo and Owens, 2006)
		MT$_{Ch}$	–	–	–	7–13			7	0.04	
		MT$_{Dk}$	–	–	–	6–9			11	0.55	
Metribuzin	0.25	MT$_{Ch}$ + Cov.C[11]	–	14.5	–	<1	995, 688	N	8, 7	0.04, 1.1	(Gaynor et al., 2001)
		MT$_{Ch}$							14, 9	0.3, 1.4	
		CT + Cov.C							8, 6	0.3, 0.6	
		CT							12, 7	0.3, 1.7	
Metribuzin	0.4	NT − S/W[13]	–	18.6	6.4	3	213, 191, 188	N + S	8, 7, 9	0.7, 0.5, 0.7	(Webster and Shaw, 1996)
		NT − Soy[13]							–, 14, 18	–, 1.9, 0.6	
		CT − S/W							9, 9, 15	1.7, 1.6, 0.9	
		CT − Soy							9, 5, 10	0.9, 0.8, 1.4	
Metribuzin	0.816	NT	120	13.0	–	10	294	N	0.2	0.01	(Malone et al., 1996)
		CT							15	0.64	
Nicosulfuron	0.014	NT	80	5.3	5.9	0–1	–	–	–		(Afyuni et al., 1997)
										2.3	
		CT							–		
										1.1	
Norflurazon	1.7	NT	–	18.6	6.4	3	376, 480	N+S		4.2, 4.2	(Baughman et al., 2001)
									6		
		RT								4.2, 2.5	
									5		
		CT								4.8, 3.6	
									6		
Pendimethalin	1.0	ST	32	5.1	6.5	3–4	63.8	S-I$_{Var}$[12]	20	0.4	(Potter et al., 2006)
		ST					64.3	S-I$_{cons}$[12]	23	0.4	
		CT					62.7	S-I$_{Var}$	45	5.0	
		CT					60.0	S-I$_{cons}$	50	4.1	

(continued)

Table 8 (continued)

Pesticide	Dose kg a.i. ha^{-1}	Tillage[1]	Soil properties Clay	Organic carbon (OC) g kg^{-1}	pH	Slope %	Rainfalls mm	Type	Runoff water % applied dose	Runoff pesticide % applied dose	Reference
Simazine	1.7	NT	–	12.0	6.7	3–5	–	–	–, 0.1, <0.01, 0.02	–, 0.18, <0.01, <0.01	(Hall et al., 1991)
		CT				3–4			0.3, 0.3, 0.03, 0.04	0.62, 0.51, 0.03, <0.01	
Simazine	1.7	NT	–	–	–	3–5	–	–	–	0.02, –	(Watts and Hall, 1996)
		MT mulch							–	–, 0.13	
		CT							–	0.11, 0.35	
Terbufos + Terbufos metabolites	1.12	NT C	–	12.8	–	8	63	S	6	0.03	(Felsot et al., 1990)
		NT U/D				8	64		23	0.3	
		ST C				9	64		4	0.03	
		ST U/D				9	63		10	0.2	
		RT C				10	64		24	0.4	
		RT U/D				10	63		29	2.3	
		MT$_{Ch}$C				8	64		33	1.4	
		MT$_{Ch}$ U/D				8	64		32	1.3	
		CT C				10	63		33	1.6	
		CT U/D				10	64		43	7.5	
Thidiazuron	0.05	ST (1)[8]	32	5.1	6.5	3–4	–	S	23	13.7	(Potter et al., 2003)
		CT							23	12.2	
Tribufos	0.30	ST (1)	32	5.1	6.5	3–4	–	S	23	12.8	(Potter et al., 2003)
		CT							23	14.5	
2,4-D	0.56	NT	232	21.5		10	100	S	0.2	0.02	(Kenimer et al., 1987)
		CT				11			3	0.27	

[1] Codes of tillage practices are reported in Table 1.
[2] Hyphens are used to indicate the range of variation of values.
[3] Commas are used to separate different years of study.
[4] N: natural rainfall; S: simulated rainfall.
[5] C: contoured slope; U/D: up-and-down slope.
[6] Loc: seed row localisation of the pesticide treatment.
[7] Average results for 9 years of study.
[8] Numbers in parenthesis indicate the age in years of the soil tillage.
[9] M.: maize = residues restituted; Sil.: silage = no crop residues.
[10] Inc.: preplant soil incorporation of herbicide.
[11] Cov.C: cover crop.
[12] I_{Var}: variable rainfall intensity; I_{cons}: constant rainfall intensity.
[13] Soy: continuous soybean; S/W: soybean/wheat rotation.

most pesticide loss by runoff occurred during the first few runoff events after application (Seifert et al., 2001a; Shipitalo and Owens, 2006). In a 9-year-study comparing three types of conservation tillage (NT, MT_{Ch} and MT_{Dk}), Shipitalo and Owens (2006) indicated that 60 to 99% of herbicide (alachlor, atrazine, linuron, and metribuzin) losses were due to the five largest transport events. After a rainfall representing 3% of annual rainfall and occurring 2 days after treatment, atrazine losses reached 4.7% of the applied dose (Shipitalo and Owens, 2003). Triplett et al. (1978) proposed a linear relationship to predict atrazine concentrations in runoff ($[\text{atrazine}]_{\text{runoff}}$) based on the number of days after treatment ($\ln([\text{atrazine}]_{\text{runoff}} = a + b.\ \ln$ (number of days after treatment)). This relationship has been tested and used for several pesticides such as alachlor (Shipitalo et al., 1997), atrazine and its metabolites (deethylatrazine and deisopropylatrazine) (Gaynor et al., 1995; Shipitalo and Owens, 2003; Shipitalo et al., 1997), cyanazine (Franti et al., 1998), linuron (Shipitalo et al., 1997), dimethipin (Potter et al., 2003), metolachlor (Gaynor et al., 1995; Ghidey et al., 2005; Webster and Shaw, 1996), metribuzin (Shipitalo et al., 1997), thidiazuron and tribufos (Potter et al., 2003). However, Hansen et al. (2001) indicated that the relationship between ln $[\text{pesticide}]_{\text{runoff}}$ and ln (number of days after treatment) was sensitive to annual variability in weather and may not be a good variable for simple estimates of herbicide concentration in runoff. They recommended the use of cumulative rainfall as a variable for estimating concentration in runoff: $\ln[\text{pesticide}]_{runoff} = \text{a} + \text{b.ln}$ (cumulative rainfall). The intensity of the first rainfall also influences the fate of the applied molecules. A small, low-intensity rainfall occurring a few days after treatment allows the incorporation of pesticide within the topsoil horizon, thus reducing losses during subsequent runoff events (Afyuni et al., 1997; Gaynor et al., 1995; Olson et al., 1998).

As for leaching, the initial soil water content is also a key factor controlling runoff. Runoff due to exceedance of infiltration capacity was found to occur earlier under no-tillage than under conventional tillage (Isensee and Sadeghi, 1993; Rector et al., 2003; Sadeghi and Isensee, 2001). In hydromorphic soils, saturation occurred more rapidly under no-tillage and runoff was more significant than under conventional tillage (Ghidey et al., 2005). Soil tillage, even superficial, increases soil infiltration capacity and drainage, which can limit runoff (Olson et al., 1998). The time interval between two rainfalls may significantly affect runoff volumes under no-tillage. Isensee and Sadeghi (1993) indicated that, for a time interval lower than 7 days between two rainfalls, the high soil water content remaining under no-tillage, favoured by the mulch, led to a significantly higher runoff than under conventional tillage.

The mulch increases soil surface roughness, thus reducing runoff (Isensee and Sadeghi, 1993; Selim et al., 2003). However, the mulch effectiveness in controlling runoff and erosion depends on the nature and quantity of plant residues. For example, Olson et al. (1998) observed that after a soybean crop, the abundance of residues under no-tillage was not sufficient to limit (water and atrazine) runoff and higher runoff volumes than under mulch tillage were measured. For quantities of residues increasing from 0 to 1.5 t ha^{-1} either remaining on the soil surface in no-tillage or mixed in the ploughed horizon in conventional tillage, runoff was reduced by 96 and 40%, respectively (Kenimer et al., 1987).

Strip tillage is a conservation tillage well-developed in the US which is starting to be used for spring productions by farmers in Europe. On one hand, strip tillage was found to reduce runoff compared with conventional tillage but, on the other hand, to increase lateral subsurface flow (Bosch et al., 2005). Potter et al. (2003) indicated that, after only one year of strip tillage, no effect on runoff volumes was measured compared with conventional tillage but erosion rates were lower. On the same study site, a few years later, runoff volumes were decreased by a factor of 4 compared with conventional tillage (Potter et al., 2004). Concerning pesticide losses, fluometuron losses were 2 to 3 times higher and pendimethalin losses were 12 times lower under strip tillage than under conventional tillage (Potter et al., 2006). Under strip tillage, herbicide treatments are applied only on the seed row representing only 1/3 of the field surface. In inter-row positions, the soil surface is most often covered by a cover crop, which reduces the risk of runoff (Hansen et al., 2001). Ridge tillage can also be used to reduce runoff but, in some cases, pesticides could migrate from the ridge tops to the furrows and then be transferred by runoff (Gaynor et al., 1987; Olson et al., 1998). Very few studies reported results on the effects of sub-soiling on runoff. Seifert et al. (2001a) did not observe any effect of this tillage practice on runoff and pesticide loss compared with conventional tillage. Pesticide incorporation into soil through superficial tillage was found to significantly reduce losses by runoff (Franti et al., 1998; Olson et al., 1998; Rector et al., 2003) but this technique is not suitable for no-tillage systems and may lead to an increase in losses by leaching. Finally, whatever the tillage system, soil tillage along isotopographic lines rather than up-and-down slope was efficient in reducing transfers by runoff (Felsot et al., 1990).

4.4 Summary and Recommendations

Concerning pesticide transfer, it clearly appears that initial soil conditions (water content and temperature) and climatic conditions (rainfall intensity, interval between treatment and the first rainfall) play a large role in the dynamics of water and solutes and may explain the contrasted effects of tillage on pesticide transfers. Improving the understanding of pesticide transport related to tillage operations implies clarifying the temporal and spatial dynamics of solute flows, especially preferential flows, which are in this case particularly relevant. Most of the studies mentioned in this review highlighted the transient and local nature of transport mechanisms, making them difficult to characterise. In the same order of ideas, the lack of efficient and robust ways to quantify soil structure and its dynamics is an impediment to the prediction of pesticide fate and transport, especially in topsoil layers.

The significant effect of rainfall characteristics on pesticide transfers suggests that tillage effects are highly dependent on the type of climate, and its eventual modifications to come. Regional studies should be of great help in developing generic models for more robust predictions. Tillage is certainly a relevant means of controlling agricultural pesticide impacts on the environment. Pesticide fate in soil implies numerous processes in complex interactions. If one is to improve tillage systems to mitigate these impacts, one needs first to understand how the various tillage practices modify the functional characteristics of soil (water retention, hydraulic conductivity, solute transport, etc.) through detailed, comprehensive studies. Such studies should consider the diversity of tillage systems, which are currently insufficiently explored and documented. Such research efforts would imply long-term studies (>10 years) to allow significant and well-established differentiation between the cropping systems being compared and would thus involve stability in funding research programmes.

5 Conclusion

This review article outlines the four main following points.

1. Pesticide interception is enhanced under conservation tillage practices. The intensity of this process depends on (a) the amount and nature of crop residues which have sorption capacities 10 to 60 times higher than soil, and (b) the climatic conditions, such as the timing between pesticide treatment and the first rainfall and its intensity.
2. Pesticide retention, which is generally positively correlated with organic carbon content, is increased in the topsoil layer under conservation tillage. Desorption of pesticides is also affected by tillage and greater proportions of pesticides remained sorbed on soil particles and on mulch under conservation tillage.
3. As a consequence of points 1 and 2, a lower fraction of pesticide remains available for biological degradation under conservation tillage and, in several cases, pesticide persistence in soils increases. In addition to this lower bio-availability due to retention processes, the presence of crop residues, a lower temperature and a higher acidity under conservation tillage can disrupt or slow down microorganisms' activity. Moreover, a greater availability of carbon under conservation tillage can defer the use of pesticides as a source of carbon.
4. Transport of pesticides is affected by tillage management and by its interactions with climatic conditions – more particularly by the intensity of rainfall, its arrival time after treatment, the interval between two rainfall events – and pesticide properties – e.g. water solubility, retention, half-life and formulation. In a general way, conservation tillage is more efficient in reducing runoff than leaching. Indeed, a higher soil surface roughness due to the presence of crop residues and a greater aggregate stability lead to a significant decrease in pesticide

loss in water runoff or bound to eroded sediment, while reducing tillage intensity maintains a well-connected macropore network through which leaching of pesticides is enhanced.

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Sustainable Cow-Calf Operations and Water Quality

Gilbert C. Sigua

Contents

Abstract As animal agriculture has evolved to larger production operations in subtropical regions of United States, the problems associated with manure handling, storage and disposal have grown significantly. Understanding the interaction effects of sustainable cow farming with water-table management, nutrient dynamics and water quality in pastures could be the key to reducing nutrients in runoff. Soils do not contribute equally to nutrient export from watersheds or have the same potential to transport nutrient to runoff nor would soil test levels accurately predict total dissolved nutrients. Better understanding of soil nutrient dynamics and crop nutrient changes resulting from different management systems should allow us to predict potential impact on adjacent surface waters. In many states, these issues are critical and of increasing importance among environmentalists, ranchers, and public officials particularly in the case of N and P. One of the first steps in assessing N or P level on any farm is to consider total N or total P inputs and outputs. In Florida, reduction of P transport to receiving water bodies is the primary focus of several studies because P has been found to be the limiting nutrient for eutrophication in many aquatic systems. Long-term monitoring of the changes in soil nutrients, especially soil P would enable us to predict soil chemical or physical deterioration under continuous forage-livestock cultivation and to adopt measures to correct them before they actually happen. Despite substantial measurements using both laboratory and field techniques, little is known about the spatial and temporal variability of nutrient dynamics across the entire landscape, especially in agricultural landscapes with cow-calf operations.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_36,

from Sigua, Agron. Sustain. Dev. 30 (2010) 631–648. DOI: 10.1051/agro:2010001

Keywords Bahiagrass • Cow-calf • Groundwater • Surface water • Groundwater • Sustainability • Nutrient cycling • Plant uptake • Water quality • Subtropics • BMPs • Eutrophication • Trophic state index (TSI)

1 Introduction

Beef cattle (*Bos taurus*) pastures in subtropical regions of United States and other parts of the world are typically dominated by subtropical and tropical grasses such as bahiagrass (*Paspalum notatum,* Flügge) or bermudagrass (*Cynodon dactylon,* L). The establishment and maintenance of persistent grass-legume pastures is a key option to increase productivity and profitability of beef cattle production systems. The development of effective grass-legume pastures for beef cattle production is a good option to improve nutritional value of the pastures, minimize N or P fertilizers input, and better manage nutrient cycling to enhance water quality. The greatest environmental concern with many grazing areas in Florida is level of soil P due to P accumulation in soil, and the subsequent loss of sediment-bound and soluble P in runoff. Our ability to estimate the levels and changes of soil P and other crop nutrients in subtropical beef cattle pastures has the potential to improve our understanding of P dynamics and nutrient cycling at the landscape level.

Throughout the southeastern United States, grazinglands have considerable variability in soils, climate, and growing season, which not only affect the types of forage that can be grown, but also the overall environmental and biodiversity management. Grazing animals affect the movement and utilization of nutrients through the soil and plant system, and thus the fertility of pasture soils (Haynes and Williams, 1993). Different pasture species may affect nutrient use and turnover due to seasonal timing of growth, root type, and forage types (Haynes, 1981; Wedin and Tilman, 1990; Stout et al., 1997). Nutrient availability at watershed scale may control pasture growth and thus the number of domestic animals that can be supported. Increased nutrient availability, through fertilizer applications, and the subsequent increases in pasture production offer the potential for increased animal production (Haynes and Williams, 1993). Society relies on adequate freshwater resources to support populations of people, agriculture, industry, wildlife habitat, aquatic ecosystems, and a healthy environment. Consequently, the interaction of pasture management and hydrology is important issue to environmentalists, ranchers, and public officials because it may affect nutrient dynamics and water quality.

Forage-beef cattle research programs must adopt an integrated approach that will lead to the development of appropriate sustainable pasture technologies that optimize beef cattle ranching profitability. Thus, both actual and perceived environmental problems associated with beef cattle production systems need to be addressed when new management systems are being developed. A key issue to be evaluated is how different livestock management practices impact the environment, including water quality, flora and fauna bio-diversity, and soil and landscape integrity. Another equally important issue concerns the balance of fertility management for forage-livestock agro-ecosystem that may result in increased nutrient use efficiency and, therefore, less likelihood of nutrient loss to the environment due to leaching and/or runoff. Additionally, there is a heightened likelihood of P losses from over fertilized pastures through surface water runoff or percolation past the root zone (Gburek and Sharpley, 1998; Stout et al., 2000).

Reduction of P transport to receiving water bodies has been the primary focus of several studies because P has been found to be the limiting nutrient for eutrophication in many aquatic systems (Botcher et al., 1999; Sigua et al., 2000; Sigua and Tweedale, 2003). Elsewhere, studies of both large (Asmussen et al., 1975) and small watersheds (Romkens et al., 1973; Hubbard and Sheridan, 1983) have been performed to answer questions regarding the net effect of agricultural practices on water quality with time or relative to weather, fertility, or cropping practices.

Recent assessments of water quality status have identified eutrophication as one of the major causes of water quality "impairment" not only in the United States, but also around the world. In most cases, eutrophication has accelerated by increased inputs of P and/or N due to intensification of crop and animal production systems since the early 1990's. The current high demand for quality protein and fiber production because of increasing world population has resulted in an intensification of agricultural production systems. As animal-based agriculture has evolved

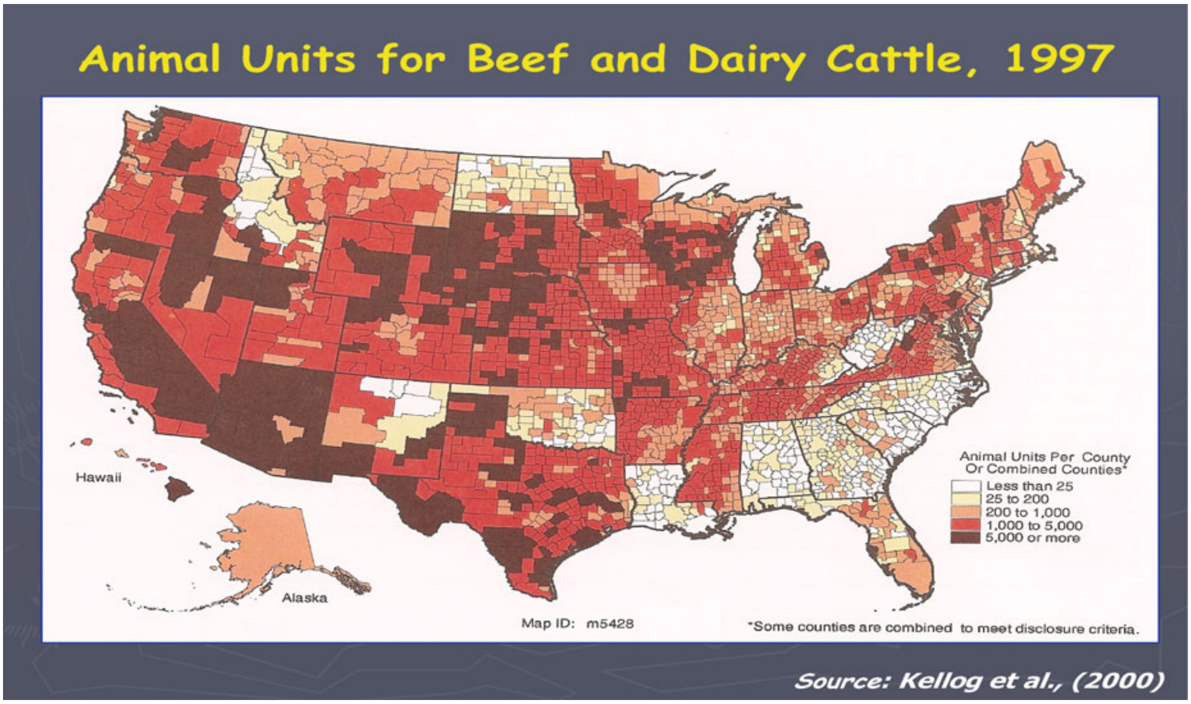

Fig. 1 Spatial distribution of animal units for beef and dairy cattle in the United States (*Source: Kellog et al., 2000*)

to larger production operations in subtropical region of United States, the problems associated with manure handling, storage and disposal have grown significantly. This review article examines the following two key questions: (1) are forage-based animal production systems as suggested by regulators the major sources of non-point source nutrients pollution that are contributing to the degradation of water quality in lakes, reservoirs, rivers, and ground water aquifers? and (2) is properly managed cow-calf operations in subtropical agro-ecosystem would not likely be the major contributors to excess loads of N or P in surface water and/or shallow groundwater?

1.1 Animal Numbers, Livestock Operations and Animal Manure Production in the United States

Data from the census of agriculture were used by Kellog et al. (2000) to make estimates of livestock populations in the United States. A census of agricultural producers is being conducted every five years by the United States Department of Agriculture National Agricultural Statistics Service. The basic building block of the estimation process is an animal unit. For the purposes of this paper, an animal unit represents 1000 pounds or 450 kilograms of live animal weight. An example of animal units for beef and dairy cattle spatial distribution in the United States is shown in Figure 1. For more detailed information related to spatial and temporal trends of animal units in the United States, the published report by Kellog et al. (2000) is highly suggested. They also reported the estimated amount nutrients (i.e., N and P excreted) based on manure production associated for livestock animals units.

The amount of manure N and P excreted in the United States are shown in Figures 2 and 3. The annual amount of manure N and P production from beef cattle operations remained constant from 1982 to 1997 while manure N and manure P production for dairy cattle had shown slightly decreasing trend from 1982 to 1997. On the contrary, the manure N and manure P production from poultry operations showed a remarkable increasing trend from 1982 to 1997 (Figs. 2 and 3).

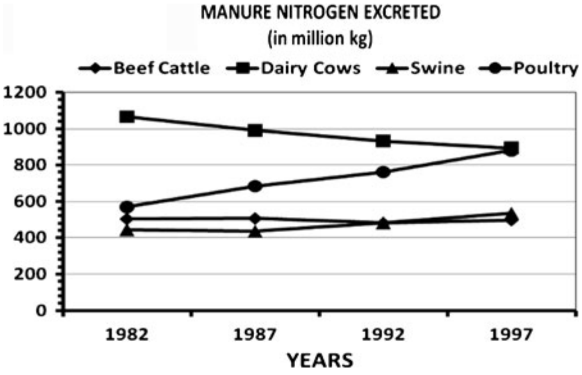

Fig. 2 Temporal distribution of manure nitrogen excreted in the United States (*Source: Kellog et al., 2000*)

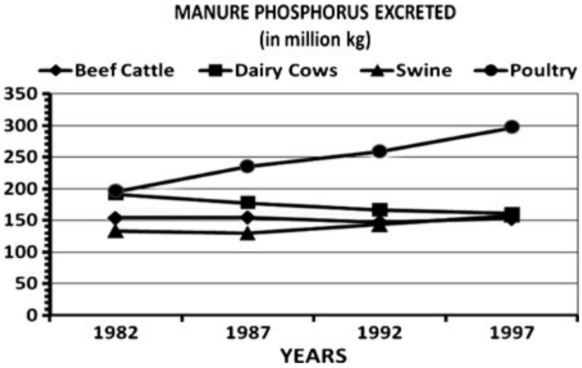

Fig. 3 Temporal distribution of manure phosphorus excreted in the United States (*Source: Kellog et al., 2000*)

1.2 Overview: Cow-Calf Operation and Management in Florida

Eleven million ha of grazingland in the subtropical (23.5–30° N Lat) United States supports about 30% of the U.S. beef cow herd. Florida's beef production ranks 10th among beef producing states in the United States and 4th nationally among states in number of herds with more than 500 brood cows. Florida's beef cattle had sales of more than $443 million in 2004. The majority of Florida's cow herd is located in the Kissimmee/Lake Okeechobee watershed, a place where the public is becoming more concerned about high levels of P entering the lake and subsequently flowing out into waterways and the Everglades.

Florida is a large state with a considerable variability in soils and climate. In north Florida, there are some clay-loam soils with good moisture-holding capacity that are quite productive (Chambliss et al., 2001). Coming down the peninsula, soils are dominated by sandy ridges and flatwoods. In general, the flatwood soils with their higher moisture-holding capacity are more productive than the upland deep, droughty sands. The warm growing season is longer in south Florida than in north Florida while winter temperatures are usually lower in north Florida than in south Florida. These differences in climate, soils and length of growing season affect the types of forage that can be grown. Nevertheless, Florida's relatively mild climate, together with more than 127 cm of annual rainfall, affords a better opportunity for nearly 12 months of grazing than in any other state except Hawaii (Chambliss et al., 2001).

1.2.1 Pasture Management: Grazing and Fertilization (Typical in Florida Ranches)

Bahiagrass is a common pasture used for beef cattle across Florida. Fertility and management practices have been based on University of Florida's

Table 1 Monthly grazing activity and estimates of nitrogen contributions from cattle feces and urine (*Source: Sigua et al., 2009*)

	Average days grazed per pasture	Animal unit per month[1]	Total feces excreted[2]	Total feces nitrogen excreted[3]	Total urine nitrogen excreted[4]	Total nitrogen after losses[5]
Months		kg ha^{-1} month^{-1}				
January	13.8	1.0	913	5.0	2.8	2.3
February	9.4	0.8	669	3.7	2.0	1.7
March	13.5	0.9	753	4.1	2.5	1.9
April	12.0	0.7	619	3.4	1.9	1.6
May	12.7	0.7	654	3.6	1.9	1.7
June	12.0	0.9	748	4.1	2.5	1.9
July	6.9	0.7	628	3.4	1.9	1.6
August	9.1	0.8	734	4.0	2.5	1.9
September	8.2	1.1	947	5.2	3.0	2.5
October	6.7	1.1	976	5.3	3.1	2.5
November	6.6	0.8	705	3.9	2.2	1.8
December	9.4	1.2	1037	5.7	3.4	2.7
Totals	–	**10.8**	**9347**	**51.2**	**27.8**	**24.1**

[1] Animal units per month (450 kg cow/calf unit).

[2] Total feces excreted (kg as excreted) = [(number of AUM × total annual animal feces excretion/12) × total feces excretion (as excreted) per animal per year] = 10.4 metric tons (Kellog et al., 2000).

[3] Total nitrogen excreted = total feces excreted × percent total nitrogen in feces (0.55%; Kellog et al., 2000). Total urine nitrogen excreted = based on urine N (g day per animal with live weight of 381 kg). Values on this table were adjusted for 450 kg cow-calf unit (Yan et al., 2007).

[4] Total nitrogen after losses = based on volatilization correction of 70% (during and after animal excretion).

recommendations as described by Chambliss (1999). Pastures are being grazed during spring of the year. After the start of summer rainy season, pastures that are to be hayed are being dropped out of the grazing cycle (usually starting in July). Pasture fields with bahiagrass are normally fertilized in the spring with 90 kg N ha^{-1} and 45 kg K$_2$O ha^{-1}. Grazing cattle at the United States Department of Agriculture Subtropical Agricultural Research Station in Brookville, FL and other Florida ranches are being rotated among pastures on a 3-day grazing interval with 24 days of rest between pastures. The average number of grazing cattle was about 2.9 animal units per hectare and grazing days of about 5.5 on monthly basis as shown in Table 1. In addition, the number of days grazed each month, average number of animals per hectare and estimated total feces excreted along with the estimated total N in feces and from urine are also shown in Table 1.

1.3 Eutrophication Associated with Animal-Based Agriculture: Overview

Eutrophication is frequently a result of nutrient pollution, such as the release of sewage effluent, urban stormwater run-off, and run-off carrying excess fertilizers into natural waters. Nutrients (e.g., N or P) and other pollutants may enter from a number of sources (Fig. 4). However, it may also occur naturally in situations where nutrients accumulate (e.g. depositional environments) or where they flow into systems on an ephemeral basis. Eutrophication generally promotes excessive plant algal growth and decay, favors certain weedy species over others, and is likely to cause severe reductions in water quality (Schindler, 1974).

In aquatic environments, enhanced growth of choking aquatic vegetation or phytoplankton (that is, an algal bloom) disrupts normal functioning of the ecosystem, causing a variety of problems such as a lack of oxygen in the water, needed for fish and shellfish to survive (Fig. 5). The water then becomes cloudy, colored a shade of green, yellow, brown, or red. Human society is impacted as well; eutrophication decreases the resource value of rivers, lakes, and estuaries such that recreation, fishing, hunting, and aesthetic enjoyment are hindered. Health-related problems can occur where eutrophic conditions interfere with drinking water treatment. Many drinking water supplies throughout the world may experience periodic massive surface blooms of *cyanobacteria* (Kotak et al., 1993). These blooms contribute to a wide range of water-related

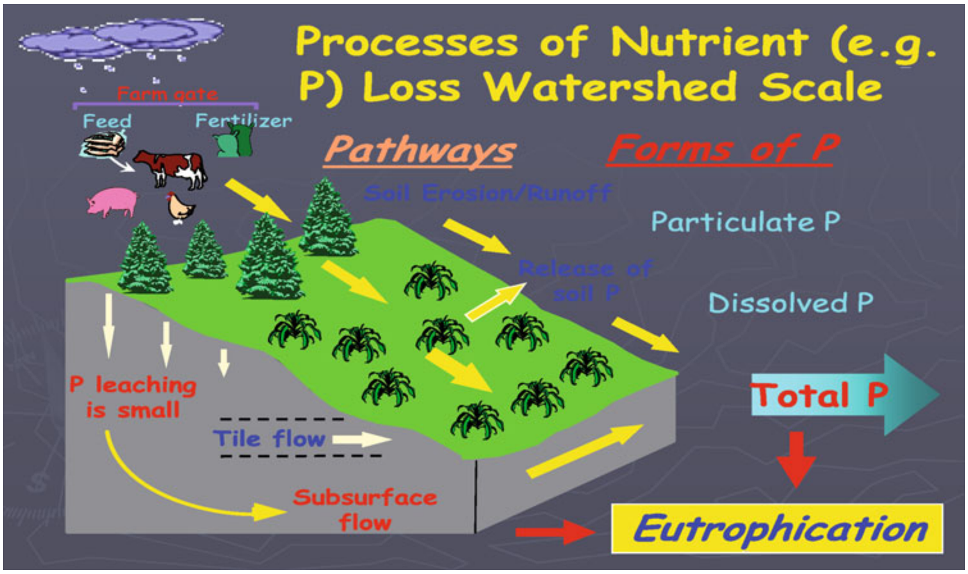

Fig. 4 Nutrient losses from farm gate on watershed scale causing eutrophication

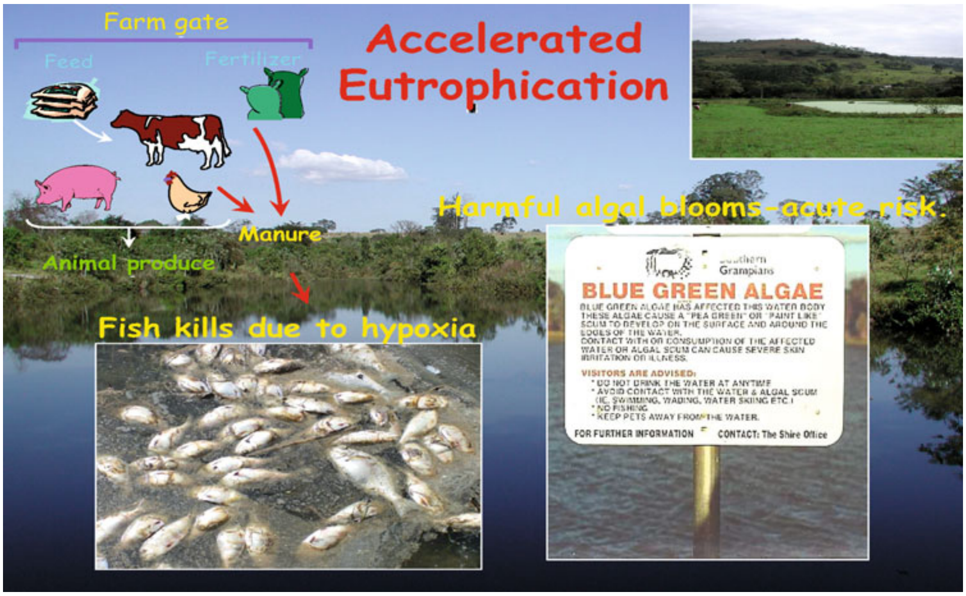

Fig. 5 Effect of accelerated eutrophication from farm gate to harmful algal blooms and fish kills due to hypoxia

problems including summer fish kills (Fig. 5), and unpalatability of drinking water (Palmstrom et al., 1988).

Increased loss of nutrients in agricultural runoff has potentially serious ecological and public health implications (Hooda et al., 2000). Nitrogen and P are particularly important as both are implicated in aquatic eutrophication (Levine and Schindler, 1989). Eutrophication and the associated ecological effects result in a general decline in overall water quality, restricting its use for general and drinking purposes (USEPA, 1988; Sharpley and Withers, 1994).

2 Forage-Based Cow-Calf Operation: Effect on the Environment and Water Quality

Beef cattle operations have been suggested as one of the major sources of non-point source P and N pollution that are contributing to the degradation of water quality in lakes, reservoirs, rivers, and ground water aquifers in Florida (Allen et al., 1976, 1982; Bogges et al., 1995; Edwards et al., 2000). Cattle manure contains appreciable amounts of N and P (0.6% and 0.2%, respectively), and portions of these components can be transported into receiving waters during severe rainstorms (Khaleel et al., 1980). Work in other regions of the country has shown that when grazing animals become concentrated near water bodies, or when they have unrestricted long-term access to streams for watering, sediment and nutrient loading can be high (Thurow, 1991; Brooks et al., 1997). Additionally, there is a heightened likelihood of N and P losses from overfertilized pastures through surface water runoff or percolation past the root zone (Schmidt and Sturgul, 1989; Gburek and Sharpley, 1998; Stout et al., 1998, 2000).

Reduction of P transport to receiving water bodies has been the primary focus of several studies because P has been found to be the limiting nutrient for eutrophication in many Florida aquatic systems (Botcher et al., 1999; Sigua et al., 2000; Sigua and Steward, 2000; Sigua and Tweedale, 2003). Recently, Sigua et al. (2004, 2006) found that the levels of soil P varied widely with different pasture management. Water quality in lakes associated with cattle production was "good", equivalent to 30–46 trophic state index (TSI) based upon the Florida Water Quality Standard. These findings indicate that properly managed livestock operations may not be major contributors to excess loads of nutrients (especially P) in surface water (Sigua et al., 2006). In another study in south Florida, Arthington et al. (2003) reported that the presence of beef cattle at three stocking rates (1.5, 2.6, and 3.5 ha per cow) had no impact on nutrient loads (P and N) in surface runoff water compared with pastures containing no cattle. However, these studies should not be considered as definitive for the region because of the wide range in management options (fertilization, stocking rate, grazing system, forage type, etc.) that are used in beef cattle production systems. Whether or not nutrient losses from grazed pastures are significantly greater than background losses and how these losses are affected by soil, forage management, or stocking density are not well defined (Gary et al., 1983; Edwards et al., 2000; Sigua et al., 2004). Concern for losses of soil P by overland flow were noted when soil P exceeded 150 mg kg^{-1} in the upper 20-cm of soil (Johnson and Eckert, 1995; Sharpley et al., 1996). Sharpley (1997) noted that all soils do not contribute equally to P export from watersheds or have the same potential to transport P to runoff. In their studies, Coale and Olear (1996) observed that soil test P levels did not accurately predict total dissolved P. Better understanding of soil P dynamics and other crop nutrient changes resulting from different management systems should allow us to better predict potential impact on adjacent surface waters. These issues are critical and of increasing importance among environmentalists, ranchers, and public officials in the state (Sigua et al., 2006).

2.1 Impact of Grazing Cattle, Cattle Movement, and Grazing Behavior on Water Quality and the Soil Nutrient Dynamics Around and Beneath Cattle Congregation Sites

Understanding cattle movement in pasture situations is critical to assess their impact on agro-ecosystems. Movement of free-ranging cattle varies due to spatial arrangement of forage resources within pastures (Senft et al., 1985) and the proximity of water (Holechek, 1988; Ganskopp, 2001), minerals (Martin and Ward, 1973), and shade to grazing sites. The breed of the animal also affects livestock distribution pattern (Herbel et al., 1967). Hammond and Olson (1994) and Bowers et al. (1995) reported that temperate British breeds (Angus and Hereford) of *Bos taurus* cows grazed less during the day than tropically adapted Senepol cows, but compensated for reduced grazing activity during the hotter parts of the day by increasing time spent grazing at night. Grazing animals congregate close to the shade and watering areas during the warmer periods of the day (Mathews et al., 1994, 1999). White et al. (2001) claimed that there was a correlation between time spent in a particular area and the number of excretions and this behavior could lead to an increase in the concentration of soil nutrients close to shade and water. Sigua (2004) demonstrated that concentrations of total inorganic N, total P and the degree of soil compaction varied significantly among different animal congregation sites. The highest concentrations of total inorganic N and total P were found at the shade and mineral feeder sites, respectively. The most compacted soil was at the mineral feeders' site. Although the levels of total inorganic N and total P were high near the center of the congregation sites, their levels did not increase with soil depth and their concentrations decreased almost linearly away from the center of the congregation sites. Soil compaction tended to decrease away from the center of the mineral feeder sites, but not at the water trough or shade sites. This study suggest that congregation sites in beef cattle operations in Florida are not as nutrient rich as suspected, and may not contribute more nutrients to surface and groundwater supply under Florida conditions because P levels at the center of sites were below 150 mg kg^{-1} (Sigua, 2004). This concentration of soil P should not be considered an absolute maximum number for soil P to become harmful to water quality and the environment, but rather a good indicator of P accumulation in the soil. Furthermore, since there was no evidence of a vertical build up or horizontal movement of inorganic N or total P in the landscape, Sigua et al. (2005) surmised that cattle congregation sites may not be considered a substantial source of nutrients at the watershed level.

Grazing animals impact the movement and cycling of nutrients through the soil and plant system, and thus on the fertility of pasture soils (Haynes, 1981; Haynes and Williams, 1993). Grazing can accelerate and alter the timing of nutrient transfers, and increase amounts of nutrients cycled from plant to soil (Klemmendson and Tiedemann, 1995). Long periods of time, position of shade and water resources for grazing cattle can influence the spatial distribution of soil biochemical properties including soil organic C and N, particulate organic C and N, microbial biomass, and net N mineralization (Ruess and McNaughton, 1987; Kieft, 1994; Kieft et al., 1998; Franzluebbers, 2000). Long-term intensive grazing may decrease the input of organic matter into soils in the immediate vicinity of individual plants and eventually reduce nutrient concentrations beneath plants by limiting availability of photosynthesis and/or meristematic tissues necessary for growth (Milchunas and Lauenroth, 1993; Briske and Richards, 1995). Thrash (1997) reported that concentration of large herbivores around the troughs negatively impacted the infiltration rate of the soils, with implications for the rate of soil loss and the soil moisture regime. Elsewhere, grazing, trampling, and dung deposition by large herbivores often result in a zone of decreasing impact on many vegetation and soil parameters including herbaceous vegetation basal cover, soil bulk density, and penetrability away from water points (Andrew and Lange, 1986; Thrash et al., 1991). The effect of trampling appears to be less severe on vegetated grasslands than on poor or bare soil (Warren et al., 1986). Studies on grazing and soil compaction generally find that exposure to livestock grazing compacts soil and that soil compaction increases with grazing intensity. This pattern is reflected in reviews of the scientific literature on the subject (Fleischner, 1994; Lauenroth et al., 1994; Kauffman and Krueger, 1984).

2.2 Effects of Forage Type and Harvest Method on Above Ground Net Production, N and P Uptake, and Soil C, P, and N Dynamics in Subtropical Pastures

The productivity of any ecosystem depends on the amounts of nutrients stored in various compartments, such as vegetation, litter, soil, and animal biomass, and on the rates of nutrient cycling and transfer among those compartments. The cycling of nutrients in a given ecosystem is affected by a combination of biological and physical processes (Holt and Coventry, 1990). The relative importance of these processes varies considerably between ecosystems as a result of differences between climate, soils, vegetation, and management practices. Nutrient dynamics in various agro-ecosystems are continually evolving in response to changing management practices. Soil dynamics may continue to change in response to external abiotic perturbations such as global changes in temperature, precipitation, and CO_2 concentration (Janzen et al., 1997, 1998).

Different pasture species affect nutrient use and turnover due to seasonal timing of growth, root type, and forage types (Wedin and Tilman, 1990; Stout et al., 1997). Other researchers (Anderson and Coleman, 1985; Dormaar, 1992) also have attributed the amount of grassland soil organic matter to the amount of root biomass. Vegetation grazed by livestock is rapidly

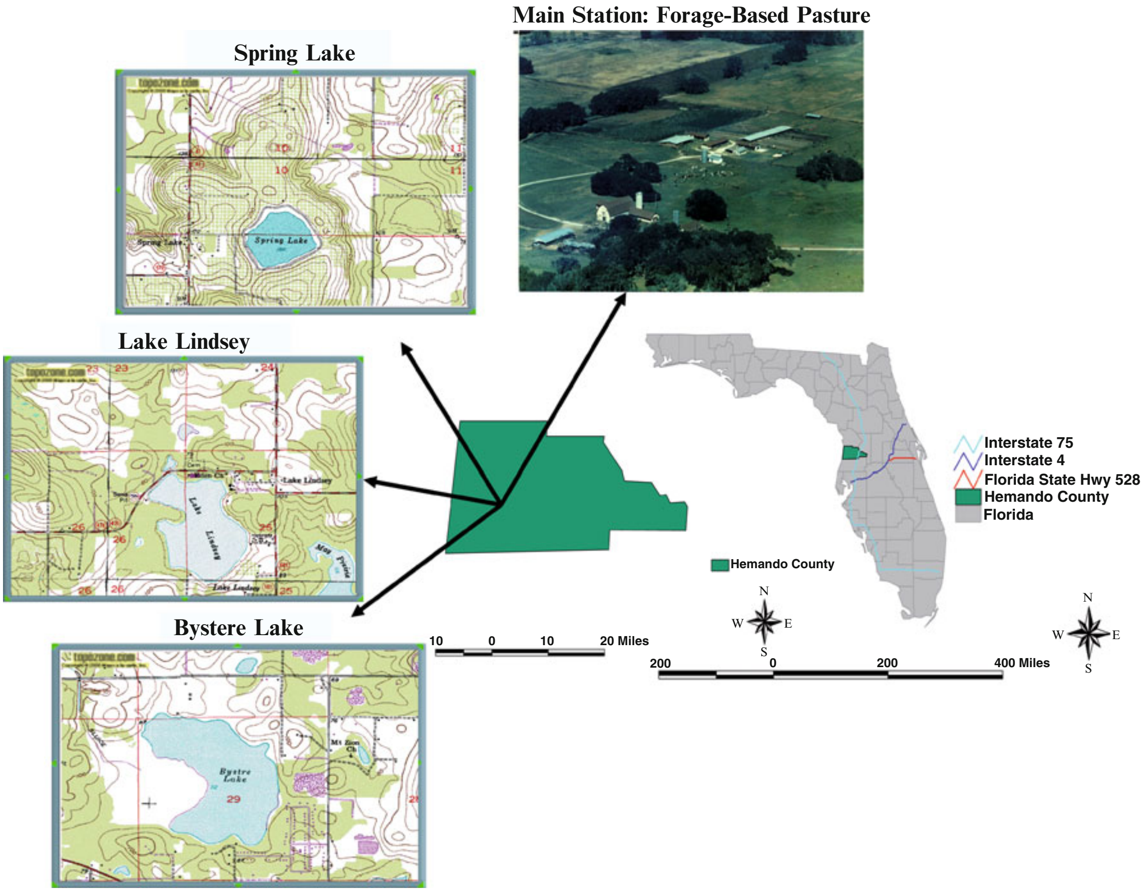

Fig. 6 Location of the study sites and aerial view of the Subtropical Agricultural Research Station, Brooksville, Hernando County, Florida, USA

decomposed during digestion and many nutrients are returned to the soil in readily available forms in feces and urine (Lauenroth et al., 1994).

3 Case Studies: Effects of Grazing on Surface Water/Shallow Groundwater Quality and Soil Quality (Florida Experiences)

3.1 Case Study #1: Surface Water Quality

3.1.1 Experimental Methods

The lakes that we studied were adjacent to or within about 14-km away from the USDA-ARS, Subtropical Agricultural Research Station (STARS), Brooksville, FL (Fig. 6). These lakes are associated with forage-based beef cattle operations. The lakes were (1) Lake Lindsey (28°37.76'N, 82°21.98'W), adjacent to STARS; (2) Spring Lake (28°29.58'N; 82°17.67'W), about 10 km away from STARS; and (3) Bystre Lake (28°32.62'N; 82°19.57'W), about 14 km away from STARS.

Monthly water quality monitoring of lakes associated with beef cattle pastures was begun in 1993 and continued until 2002 by the field staff of the Southwest Florida Water Management District (SWFWMD). Monthly water samples were taken directly from the lakes using a water (Van Dorn) grab sampler. Water quality parameters monitored were Ca, Cl, $NO_2 + NO_3$-N, NH_4-N, total N, total P, K Mg, Na, Fe, and pH. All sampling, sample preservation and transport, and chain of custody procedures were performed in accordance with an EPA-approved quality assurance plan with existing quality assurance requirements (USEPA, 1979; APHA, 1992). The SWFWMD Analytical Laboratory, using EPA-approved analytical methods, performed the chemical analyses of water samples from the lakes (USEPA, 1979).

Trophic state index development and calculation. Lake trophic state index (TSI) is understood to be the biological response of a lake to forcing factors such as nutrient additions. Nutrients promote growth of microscopic plant cells (phytoplankton) that are fed upon by microscopic animals (zooplankton). The TSI of (Carlson, 1983) uses algal biomass as the basis for trophic state classification (e.g. oligotrophic, mesotrophic, eutrophic, and hypereutrophic).

The Florida trophic state index of (Brezonik, 1984), which was derived using data from 313 Florida Lakes, was modified in the study. The first step involved in assigning a TSI value was to assess the current nutrient status based on TN/TP ratio of the lake. The TN to TP ratios were classified into three categories namely: N limited (TN/TP $< 10{:}1$); P limited (TN/TP $> 30{:}1$); and balanced ($10{:}1 \leqslant$ TN/TP $\leqslant 30{:}1$). The TSI of each lake that we studied was calculated by entering key water quality parameters: total P (TP, ug L^{-1}); total N (TN, mg L^{-1}); chlorophyll a (CHL, mg m^{-3}) for the measurements of planktonic algae density, and Secchi depth (SD, m) for measuring water transparency into an empirical formula (Eqs. (1) to (3)). Equation (1) (P-limited) was used to calculate the TSI values for Lake Lindsey and Spring Lake while equation (2) (N-limited) was used to calculate the TSI value for Bystere Lake. A novel paper on trophic state index for lakes written by (Carlson, 1977) was an excellent reference to explain the mathematical derivations of the different equations listed below.

I. **PHOSPHORUS - LIMITED LAKES**
(TN/TP > 30:1) **(1)**
TSI (AVG) = 1/3 [TSI (CHL) + TSI (SD) + TSI (TP)]
where: TSI (CHL) = 16.8 + 14.4 * ln (CHL), (mg m^{-3})
TSI (SD) = 60.0 – 30.0 * ln (SD), (m)
TSI (TP) = – 23.8 + 23.6 * ln (TP), (ug L^{-1})

II. **NITROGEN - LIMITED LAKES**
(TN/TP < 10:1) **(2)**
TSI (AVG) = 1/3 [TSI (CHL) + TSI (SD) + TSI (TN)]
where: TSI (CHL) = 16.8 + 14.4 * ln (CHL), (mg m^{-3})
TSI (SD) = 60.0 – 30.0 * ln (SD), (m)
TSI (TN) = 59.6 + 21.5 * ln (TN), (ug L^{-1})

III. **NUTRIENT - BALANCED LAKES**
(10:1 ⩽ TN/TP ⩽ 30:1) **(3)**
TSI (AVG) = 1/3 [TSI (CHL) + TSI (SD) + (0.5 TSI (TP) + TSI (TN))]
where: TSI (CHL) = 16.8 + 14.4 * ln (CHL), (mg m^{-3})
TSI (TN) = 56 + 19.8 * ln (TN), (ug L^{-1})
TSI (TP) = – 18.4 + 18.6 * ln (TP), (ug L^{-1})
TSI (SD) = 60.0 – 30.0 * ln (SD), (m)

3.1.2 Highlights of Research Results

Status of water quality in lakes. Assessment of water quality data from 1993 to 2002 confirms that water–quality variations (temporal and spatial) existed in lakes associated with beef cattle pasture systems in Central Florida. The lakes were found to differ from each other in Ca, NO_2+NO_3-N, TN, TP, K, Mg, Na, and Fe. Significant temporal variations were observed for NH_4-N, TP, and Fe while significant interaction effects (lakes × year) were only noted for NH_4-N, TP, Mg, Na, and Fe.

The levels of NO_2+NO_3-N and NH_4-N in lakes did not show any significant differences from 1993 to 2002 while TN of Bystere Lake declined from 1.12 to 0.76 mg L^{-1} between 1993 and 2002. Lake Lindsey's TN in 1993 was about 0.82 mg L^{-1} and 0.80 mg L^{-1} in 2002 while Spring Lake's TN in 1993 and 2002 were 0.65 and 0.78 mg L^{-1}, respectively. The levels of TP in Bystere Lake between 1993 and 2002 increased from 0.08 to 0.34 mg L^{-1} while levels of TP in Lake Lindsey did not change from 1993 to 2002. A decline of TP was noted in Spring Lake from 1993 (0.19 mg L^{-1}) to 2002 (0.01 mg L^{-1}). With the continuous conversion of cropland and pastureland to residential use (although at slow pace), contribution of nutrients from anthropogenic sources is becoming a big concern environmentally over time for lakes associated with forage-based pasture systems.

Total nitrogen (TN)/total phosphorus (TP) ratio. Nitrogen and P are the primary crop nutrients that can impact the environment. When applied in excess of crop needs, nutrients can run off into surface waters resulting in excessive aquatic plant growth and toxicity

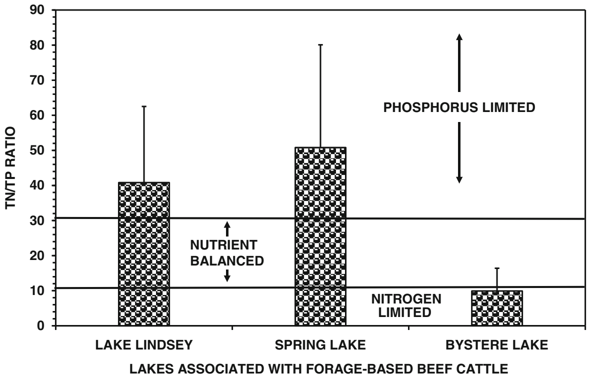

Fig. 7 Total nitrogen (TN) to total phosphorus (TP) ratio of lakes associated with forage-based beef cattle pasture system (*Source:* *Sigua et al., 2006c*)

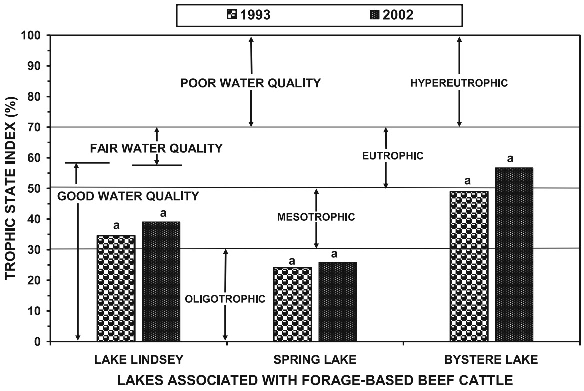

Fig. 8 Trophic state index for lakes with forage-based beef cattle pasture system. Trophic state index is sginficantly different ($P \leqslant 0.05$) when superscripts located at top bars are different (*Source:* *Sigua et al., 2006c*)

to certain fish species. The TN/TP ratio may be a useful method to establish the N and P reduction targets in the environment (Sigua et al., 2000). The ratio of TN to TP is one of the important components in calculating the TSI of lakes.

Several studies have shown that a TN/TP ratio $\leqslant$10:1 appears to favor algal blooms, especially blue-green algae, which are capable of fixing atmospheric N (Schindler, 1974; Chiandini and Vighi, 1974; Sakamoto, 1966; Sigua et al., 2000). Figure 7 shows the TN/TP ratio of the lakes that were associated with beef cattle operations. Lake Lindsey and Spring Lake can be classified as P-limited lakes with TN/TP ratios of 41:1 and 51:1, respectively. Bystere Lake with a TN/TP ratio of 9:1 was classified as an N-limited lake and may have higher probability for algal bloom compared with Lake Lindsey and Spring Lake because of its higher P levels. From 1993 to 2002, the TP in Bystere Lake increased from 0.08 mg L^{-1} to 0.34 mg L^{-1}.

Trophic state index of lakes (TSI). The Florida TSI was devised to integrate different but related measures of lake productivity or potential productivity, into a single number that ranges from 0 to 100. The measures included in the calculation of TSI are water transparency (Secchi depth), chlorophyll *a* (measurement of algae content), TN, and TP. The Florida TSI for Lake Lindsey, Spring Lake, and Bystre Lake were 35, 30, and 46, respectively (Fig. 8). Based on this, the TSI of these lakes can be classified as "good" according to Florida water quality standard (TSI of 0–59 = "good"; TSI of 60 to 69 = "fair"; and TSI of 70 to 100 = "poor"). Although the TSI levels of the three lakes did not show any significant change from 1993 to 2002, TSI levels increased numerically for all lakes (Fig. 8). This is reflected in a change in the trophic status of Bystere Lake. Lake Lindsey with TSI of 31 and 38 in 1993 and 2002, respectively, remained within the mesotrophic classification, while Spring Lake with TSI of 25 and 26 in 1993 and 2002, respectively, remained in the oligotrophic category. Lake Lindsey (mesotrophic lake) would normally have moderate nutrient concentrations with moderate growth of algae and/or aquatic macrophytes and with clear water (visible depth of 2.4 to 3.9 m).

Oligotrophic lake such as Spring Lake would normally have less abundance of aquatic macrophytes and algae, or both because nutrients are typically in short supply. Oligotrophic lakes tend to have water clarity greater than 3.9 m due to low amounts of free-floating algae in the water column.

Bystere Lake, which was at the upper end of the mesotrophic range in 1993 (TSI of 49), shifted into the slightly eutrophic state in 2002 with a TSI value slightly above 50. Eutrophic lakes normally have green, cloudy water, indicating lots of algal growth in the water. Water clarity of most eutrophic lakes generally ranges from 0.9 to 2.4 m. Generally, water quality in Lake Lindsey and Spring Lake was consistently good (1993–2002) while water quality of Bystere Lake ranged from good in 1993 to fair in 2002 (Fig. 8).

3.2 *Case Study #2: Shallow Groundwater Quality*

3.2.1 Experimental Methods

Instrumentation and water sample collection. Two adjacent 8-ha pasture fields were instrumented with a pair of shallow wells placed at different landscape positions. The different landscape positions are top slope (TS; 10–20% slope, 2 ha; middle slope (MS; 5–10% slope, 2 ha and bottom slope (BS; 0–5% slope, 2 ha). The wells were constructed of 5 cm schedule 40 PVC pipe and had 15 cm of slotted well screening at the bottom. During installation of wells, sand was placed around the slotted screen, and bentonite clay was used to backfill to the soil surface to prevent surface water or runoff from moving down the outside of the PVC pipe and contaminating groundwater samples. A centralized battery-operated peristaltic pump was used to collect water samples. Wells were completely evacuated during the sampling process to ensure that water for the next sampling would be fresh groundwater. Water samples were collected from the groundwater wells every two weeks. However, there were periods when ground water levels were below the bottom level of the wells and samples could not be obtained. In addition to ground water samples, surface water samples were collected in the pasture bottoms or the seep area when present, by taking composite grab samples on the same schedule. The seep area, which is located at the lower end of BS is a remnant of a sinkhole formation and became a small scale lake with varying levels of surface water. The seep area of about 2 ha in size is where runoff and seepage from higher parts of pasture converge.

Water sample handling and analyses. Water samples were transported to the laboratory following collection and refrigerated at 4°C. Water samples were analyzed for NO_3-N and NH_4-N using a Flow Injector Analyzer according to standard methods (APHA, 1989).

3.2.2 Highlights of Research Results

Concentration of NH_4-N, NO_3-N and TIN in Surface and Ground Water

Concentrations of NH_4-N, NO_3-N, and TIN in shallow groundwater did not vary with landscape positions (Fig. 9). However, concentrations NH_4-N, NO_3-N, and TIN in the water samples collected from the seep area were significantly ($P \leqslant 0.05$) higher when compared to their average concentrations in water

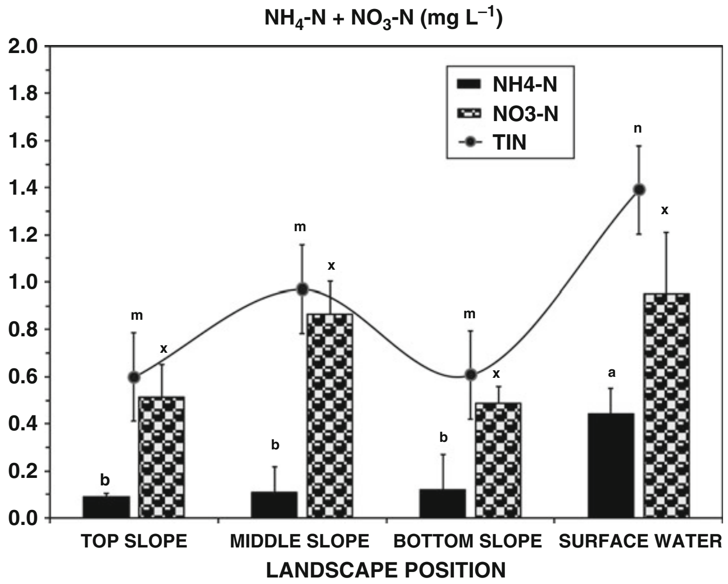

Fig. 9 Average concentrations of NH_4-N, NO_3-N and TIN in shallow groundwater at different landscape positions. Line above the bars and across the line represents standard error of the mean. Means of NH_4-N (a & b), NO_3-N(x & y) and TIN (m & n) in shallow groundwater are significantly different ($P \leqslant 0.05$) when superscript located at top bar and line is different (*Source:* *Sigua et al., 2009a*)

Table 2 Summary statistics for the concentration of inorganic nitrogen in shallow groundwater under bahiagrass pastures associated with cow-calf operations

Statistical parameters	NO_3-N	NH_4-N	Total inorganic N
	2004		
Number of samples (n) (N)	40	40	40
Mean (mg L^{-1})	0.7	0.1	0.8
Median (mg L^{-1})	0.4	0.04	0.6
Mode (mg L^{-1})	0.2	0.04	0.2
Maximum (mg L^{-1})	4.7	1.3	5.9
Minimum (mg L^{-1})	0.2	0.03	0.2
Std. error mean	0.2	0.03	0.2
Variance	0.9	0.04	0.9
Skewness	3.2	4.5	3.2
	2005		
Number of samples (n)	45	45	45
Mean (mg L^{-1})	0.8	0.3	1.03
Median (mg L^{-1})	0.3	0.1	0.7
Mode (mg L^{-1})	0.1	0.1	0.3
Maximum (mg L^{-1})	4.1	2.8	6.9
Minimum (mg L^{-1})	0.1	0.1	0.2
Std. error mean	0.1	0.1	0.1
Variance	0.8	0.2	1.0
Skewness	2.1	4.6	2.1
	2006		
Number of samples (n)	10	10	10
Mean (mg L^{-1})	0.7	0.2	0.8
Median (mg L^{-1})	0.4	0.1	0.6
Mode (mg L^{-1})	–	0.1	–
Maximum (mg L^{-1})	2.7	0.4	3.1
Minimum (mg L^{-1})	0.1	0.1	0.2
Std. error mean	0.2	0.03	0.2
Variance	0.6	0.01	0.6
Skewness	0.8	1.9	1.8

Source: Sigua et al., 2009a.

samples collected from the different landscape positions (Fig. 9). Averaged across year, concentration of TIN ranged from 0.5 to 1.5 mg L^{-1}. The highest TIN concentration occurred ($P \leqslant 0.05$) in the surface water while the concentrations from the shallow groundwater wells (BS-0.6 mg L^{-1}, MS-0.9 mg L^{-1}, and TS-0.6 mg L^{-1}) were similar to each other and lower than the seepage area (Fig. 9).

Average concentrations of NO_3-N (0.4 to 0.9 mg L^{-1}) among the different sites were well below the maximum, of 10 mg L^{-1}, set for drinking water (Fig. 9). On the average, the concentrations of NO_3-N did not vary significantly with landscape positions, and as with TIN, the levels were significantly lower than surface water from seepage area (Fig. 9). The maximum NO_3-N concentrations (averaged across landscape position) in shallow groundwater for 2004, 2005 and 2006 were also below the drinking water standards for NO_3-N. Other summary statistics for the levels of NO_3-N, NH_4-N and TIN in shallow groundwater are shown in Table 2.

Similar trends in landscape positions were found for average concentrations of NH_4-N (Fig. 9). Again, the concentrations of NH_4-N in shallow groundwater did not vary significantly among top slope, middle slope, and bottom slope wells. These levels of NH_4-N were lower than that of the surface water (0.5 mg L^{-1}). Annual average concentrations of NO_3-N, NH_4-N and TIN in shallow groundwater of pastures associated with beef cattle operations did not vary significantly with the different year of sampling. The highest concentration of TIN was in 2005 followed by 2006 and 2004 (Table 2).

Average concentrations of NO_3-N in surface water and shallow groundwater (0.4 to 0.9 mg L^{-1}) among the different sites did not exceed the drinking water standard for NO_3-N (10 mg L^{-1}). Nitrate levels in excess of 10 mg L^{-1} in drinking water can cause health problems for human infants, infant chickens and pigs, and both infant and adult sheep, cattle and horses. The relatively constant concentration of NO_3-N in the shallow groundwater over the three years (2004–2006) could be due to the combined effects of precipitation, N fertilization, and leaching from plant decomposition and animal feces and urine. Uptake of N by the actively growing bahiagrass reduced the amount of mineral N remaining vulnerable to leaching during the growing season (Decau et al., 2003).

Concentration of Total Phosphorus and Degree of Phosphorus Saturation in Soils

Concentrations of total P in soils varied significantly ($P \leqslant 0.001$) with landscape position and sampling depth, but there was no interaction effect of landscape position and sampling depth (Table 3). Soil samples from the seep area had the lowest concentration of total P when compared with other landscape positions. Soils from the middle slope (9.2 ± 1.8 mg kg^{-1}) had the greatest concentration of total P followed by top slope (5.9 ± 1.8 mg kg^{-1}) and bottom slope (5.7 ± 1.5 mg kg^{-1}). Averaged across years, total P in the soil was about 9.1 mg kg^{-1}.

Degree of P saturation in the soils varied significantly ($P \leqslant 0.001$) with landscape position and sampling depth, but was not affected significantly by the interaction of landscape position and sampling depth (Table 3). The middle slope position (19.9 ± 4.9 %) had the highest degree of P saturation followed by top slope, bottom slope and seep area. Soils collected at sampling depth of 0–20 cm (20.9 ± 6.1 %) had significantly higher degree of P saturation than soils collected between 20 and 100 cm.

There was a significant ($P \leqslant 0.05$) decrease in the average concentrations of total P with increasing sampling depth (Table 3). The upper two depths (0–20 cm and 20–40 cm) had the highest concentrations while the lowest amount of total P was found in the lowest sampling depth of 60–100 cm (Table 3). These results suggest that there had been little movement of total P into the soil pedon since average degree of P saturation in the upper 20 cm was 21% while degree of P saturation at lower soil depth (60–100 cm) was about 3%.

Results indicate that current pasture management including cattle rotation in terms of grazing days and current fertilizer (inorganic + manures + urine)

Table 3 Average concentration (± std. error of mean) and F-values of total phosphorus and degree of phosphorus saturation in soils at various landscape positions and soil depths

Soil parameters	Total phosphorus	Degree of phosphorus saturation
	— mg kg^{-1}—	—— % ——
A. Landscape position		
1. Top slope	5.91 ± 1.77b*	14.79 ± 4.57a
2. Middle slope	9.19 ± 1.77a	19.92 ± 4.97a
3. Bottom slope	5.67 ± 1.53b	7.86 ± 2.14b
4. Seep area	0.38 ± 0.13c	1.25 ± 0.73c
$LSD_{(0.05)}$	2.62	6.35
B. Soil depth (cm)		
1. 0–20	7.05 ± 1.86a	20.93 ± 6.11a
2. 20–40	9.05 ± 2.18a	13.77 ± 3.22b
3. 40–60	3.24 ± 0.94b	5.64 ± 1.67c
4. 60–100	1.81 ± 0.62b	3.47 ± 1.06c
$LSD_{(0.05)}$	2.62	6.35
	F-values	**F-values**
Landscape position (LP)	17.37***	14.77**
Soil depth (SD)	14.66**	14.24**
LP × SD	2.13^{ns}	2.28^{ns}

*Means in columns within each subheading followed by common letter(s) are not significantly different from each other at $P \leqslant 0.05$.
**Significant at $P \leqslant 0.001$; ns – not significant.

application rates for bahiagrass pastures offer little potential for negatively impacting the environment. Properly managed livestock operations contribute negligible loads of total P to shallow groundwater and surface water. Overall, there was no buildup of soil total P in bahiagrass-based pasture. Therefore, results of this study may help to renew the focus on improving fertilizer efficiency in subtropical beef cattle systems, and maintaining a balance of P removed to P added to ensure healthy forage growth and minimize P runoff.

3.3 Changes in Soil P, K, Ca, Mg and pH Associated with Cow-Calf Operation

During the last 15 years (1988–2002), concentrations for P, K, Ca, Mg, and soil pH have declined by about 7%, 38%, 46%, 61%, and 23% in pastures with bahiagrass and 27%, 55%, 76%, 56%, and 22% for pasture fields with bahiagrass + Rhizoma peanuts that were grazed in spring and hayed in early fall, respectively (Table 4). However, the levels of Ca:Mg ratio in fields with grazed and hayed bahiagrass and grazed and hayed Rhizoma peanuts had increased by about 42% and 29%, respectively. It is worth mentioning that depletion rates of P, K, Ca, and Mg were greater in pasture fields with bahiagrass + Rhizoma peanuts than in pastures with bahiagrass suggesting that the former has greater nutritional demands.

Long-term monitoring on the changes in soil P and other crop nutrients in subtropical foraged-based beef cattle pastures enabled to predict soil chemical deterioration and/or soil accumulation of nutrients that could occur under continuous forage-livestock cultivation and to adopt measures to correct them before they actually happen. In addition, long-term monitoring also provided answers about the nutrient dynamics/cycling and turnover in legume-grass mixtures and the efficiency of fertilizer use. The knowledge that was gained on the relationship of temporal and spatial changes in soil nutrient levels in forage-based beef cattle pasture should indeed provide insights for improved grazing management, which could be both economically and environmentally safe.

Table 4 Changes (%) in Mehlich-1 extractable soil P and other nutrients under different pasture management in STARS, Brooksville, FL in 2002 relative to the 1988 levels of Mehlich-1 extractable soil P and other nutrient (*Source: Sigua et al., 2006b*)

	Pasture management	
Soil nutrients	BG+GZHY (%)	RP-G+GZHY (%)
P	−6.6	−26.9
K	−38.2	−55.1
Ca	−45.8	−75.6
Mg	−61.2	−56.0
Ca/Mg ratio	+42.1	+29.0
pH	−22.1	−21.9

BG – bahiagrass; RP-G – rhizoma peanuts-mixed grass; GZHY – grazing + haying.

4 Management Options: Managing Properly Cow-Calf Operations to Improve Water Quality and Pasture Sustainability

4.1 Improving Pasture Sustainability

The cow-calf (*Bos taurus*) industry in subtropical United States and other parts of the world depends almost totally on grazed pasture areas. Thus, the establishment of complete, uniform stand of bahiagrass in a short time period is economically vital. Failure to obtain a high-quality bahiagrass stand early means the loss of not only the initial investment costs, but also both production and its cash value. Forage production often requires significant inputs of lime, N fertilizer and less frequently of P and K fertilizers. Domestic wastewater sludge or sewage sludge, composted urban plant debris, waste lime, phosphogypsum and dredged materials are examples of materials that can be used for fertilizing and liming pastures. Beef cattle producers throughout the United States need better forage management systems to reduce input costs and protect environmental quality.

The ability to reuse lake-dredged and domestic sewage sludge materials for agricultural purposes is important because it reduces the need for offshore disposal and provides an alternative to disposal of these materials in landfills that are already overtaxed. Often these materials can be obtained at little or no cost to the farmers or landowners. Thus, forage production offers an alternative to waste management since nutrients in the lake-dredged materials and biosolids are recycled into crops that are not directly consumed by humans. Results have shown the favorable influence that biosolids and lake-dredged materials had on bahiagrass during its early establishment in sandy subtropical beef cattle pasture areas in south central Florida. Some of the promising effects of added biosolids and lake-dredged materials on soil quality and on early establishment of bahiagrass are summarized below.

4.1.1 Biosolids as Nutrient Source

Biosolids usually are applied at agronomic rates designed to supply crops with adequate N nutrition. Biosolids contain a substantial amount of N (typically 3 to 6 % by weight). The N is not immediately available to crops, but is released slowly by biological activity. Since biosolids are produce and handled by different processes at different treatment plants, it is important to know if those treatment processes affect how much N becomes available to plants. Nutrients in municipal residuals produced annually in the United States account for about 2.5% of the total N, 6% of the P, and 0.5% of the K applied on farms each year (Muse et al., 1991).

The field experiment was conducted at the University of Florida Agricultural Research and Education Center, Ona, FL (27°26'N, 82°55'W) on a Pomona fine sandy soil. With the exception of the control, bahiagrass plots received annual biosolids and chemical fertilizers applications to supply 90 or 180 kg total N ha^{-1} yr^{-1} from 1997 to 2000. Land application of biosolids and fertilizer ceased in 2001 season. In early April 1998, 1999, and 2000, plots were mowed to 5-cm stubble and treated with the respective N source amendments. The experimental design was three randomized complete blocks with nine N-source treatments: ammonium nitrate (AMN), slurry biosolids of pH 7 (SBS7), slurry biosolids of pH 11 (SBS11), lime-stabilized cake biosolids (CBS), each applied to supply 90 or 180 kg N ha^{-1}, and a nonfertilized control (Control). Application rates of biosolids were calculated based on the concentration of total solids in materials as determined by the American Public Health Association SM 2540G methods (APHA, 1989) and N in solids. The actual amount of biosolids applications was based on the amount required to supply 90 and 180 kg N ha^{-1}. Sewage sludge materials were weighed in buckets and uniformly applied to respective bahiagrass plots.

Forage yield of bahiagrass was significantly ($P \leqslant 0.001$) affected by the different biosolids in all years (1998 to 2002), but not by the interaction effects of year × treatments. Although yield trend was declining from 1988 to 2002, forage yield of bahiagrass that received biosolids were consistently and significantly ($P \leqslant 0.05$) greater than the forage yield of the unfertilized bahiagrass (Table 5). The bahiagrass fertilized with SBS11-180 had the greatest forage yield in 1998 (5.1 ± 0.4 Mg ha^{-1}), 1999 (4.6 ± 0.2 Mg ha^{-1}), 2000 (4.5 ± 0.2 Mg ha^{-1}), and in 2002 (3.3 ± 0.6 Mg ha^{-1}). Forage yield of bahiagrass fertilized with AMN-90 and AMN-180 was significantly greater than

Table 5 Comparison on forage yield (Mg ha^{-1}; mean ± S.D.) of bahiagrass among years with repeated application of biosolids (1998, 1999, and 2000) and with no biosolids application (2002) (*Source: Sigua, 2008*)

	With sewage sludge			Without sewage sludge
Nitrogen sources*	1998	1999	2000	2002
Control	2.4 ± 0.5d**	1.8 ± 0.2c	1.4 ± 0.3d	1.2 ± 0.2c
AMN-90	4.3 ± 0.2ab	3.7 ± 0.1b	2.1 ± 0.1cd	2.1 ± 0.3bc
AMN-180	4.7 ± 0.4a	4.7 ± 0.02a	3.2 ± 0.3b	2.2 ± 0.4b
SBS7-90	4.4 ± 0.4ab	3.1 ± 0.3b	2.2 ± 0.4bcd	2.5 ± 0.5ab
SBS7-180	5.0 ± 0.5a	5.1 ± 0.2a	2.6 ± 0.2bc	2.3 ± 0.5b
SBS11-90	4.1 ± 0.5abc	3.3 ± 0.3b	1.9 ± 0.3cd	1.9 ± 0.2bc
SBS11-180	5.1 ± 0.4a	4.6 ± 0.2a	4.5 ± 0.2a	3.3 ± 0.6a
CBS-90	2.9 ± 0.4cd	2.2 ± 0.2c	1.8 ± 0.6cd	2.5 ± 0.5ab
CBS-180	3.3 ± 0.3bcd	3.3 ± 0.1b	2.7 ± 0.2bc	2.5 ± 0.5ab

*AMN – ammonium nitrate; SBS7 – slurry biosolids of pH 7; SBS11 – slurry biosolids of pH 11; CBS7 – limed-stabilized cake biosolids; 90–90 kg N ha^{-1}; 180–180 kg N ha^{-1}.

**Mean values in each column followed by the same letter(s) are not different ($P > 0.05$) according to the Duncan's multiple range test.

those of the unfertilized bahiagrass in 1998 and 1999, but not in 2000 and in 2002. Although SBS11-180 had the greatest residual effect (170%) in 2002, CBS-90 and CBS-180 had more pronounced effects when compared with the other sewage sludge sources because their relative impact on forage yield compared with the control between years with (1997–2000) and without (2002) sewage sludge applications increased from 30% to 110% and 70% to 110%, or net increases of 267% and 57% in forage yield change, respectively.

The residual effects of applied sewage sludge on bahiagrass yield expressed as percent forage yield change over the unfertilized bahiagrass are shown in Figure 8. Residual effects of AMN-90 (–6%), AMN-180 (–31%), SBS7-80 (–21%), and SBS7-180 (–17%) declined (negative) with time, but the residual effects of applied SBS11-180 (+13%), CBS-90 (+267%), and CBS-180 (+57%) were positive over time although sewage sludge application ceased after harvest in 2000. The percent forage yield change of bahiagrass fertilized with SBS11-180, CBS-90, and CBS-180 during years when sewage sludges were applied (1998–2000) were 150%, 30%, and 70% compared with percent forage yield change of 170%, 110%, and 110% in 2002 (when sewage sludge applications ceased), respectively.

The residual effects on forage yield of applied CBS-90 (+267%) and CBS-180 (+57%) relative to the control increased with time although biosolids applications ceased after the 2000 harvest season. This was probably due to the higher concentration of organic N in addition to the liming property of CBS. Liming the field could have some direct and indirect effects on forage productivity and on the nutrient status of the soils. Perhaps the single direct benefit of liming is the reduction in acidity and solubility of aluminum and manganese (Peevy et al., 1972). Some of the indirect benefits of liming pasture fields among others would include: enhancing P and microelement availability, nitrification, N fixation, and improving soil physical conditions (Nelson, 1980; Tisdale and Nelson, 1975; Russell, 1973). Dried and composted biosolids have slower rates of N release and in case of CBS with much higher solids concentration (500 000 mg L^{-1}); more N will be released in the second, third, or even the fifth year after the initial application due to higher amount of organic N than ammonium N. The proportions of ammonium and organic N in biosolids vary with the stabilization process.

4.1.2 Lake-Dredged Materials as Nutrient Source

The field study was adjacent to the Coleman Landing spoil disposal site in Sumter County, FL. Each plot (961 m^2) was excavated to a depth of about 28 cm, and existing natural soil (NS) and organic materials were completely removed. Excavated NS materials were placed at the south end of the test plots. Existing vegetation from each plot was totally removed prior to backfilling each plot with different ratios of NS and lake-dredge materials (LDM): (100% NS + 0% LDM); (75% NS + 25% LDM); (50% NS + 50% LDM); (25% NS + 75% LDM); and (0% NS + 100% LDM). These ratios of NS to LDM represent the treatment combinations of LDM0; LDM25; LDM50; LDM75; and LDM100, respectively. Natural soils that were excavated were backfilled to each plot along with lake-dredged materials that were hauled from the adjacent settling pond. The total amount of lake-dredged materials and natural soils that was placed back on each test plot was in accordance with the different ratios of lake-dredged materials and natural soils that were described above. After mixing the natural soils and lake-dredged materials, each of the test plots was disked to a uniform depth of 28 cm. Plots were disked in an alternate direction until lake-dredged materials and natural soils were uniformly mixed. Each plot was seeded with bahiagrass at a rate of 6 kg $plot^{-1}$, followed by dragging a section of chain link fence across each test plot to ensure that bahiagrass seeds were in good contact with the natural soils and lake-dredged materials. Field layout was based on the principle of a completely randomized block design with four replications.

The forage yield of bahiagrass at 112, 238, and 546 Julian days after seeding are shown in Fig. 10. Forage yield of bahiagrass varied significantly ($P \leqslant 0.001$) among plots with lake-dredged materials additions. The greatest forage yield of 673 ± 233 kg ha^{-1} at Julian day 112 was from plots amended with 50% lake-dredged materials while bahiagrass in plots amended with 100% lake-dredged materials and 75% lake-dredged materials had the highest forage yield at Julian days 238 and 546 with average forage yield of 3349 ± 174 and 4109 ± 220 kg ha^{-1}, respectively (Fig. 10). The lowest forage yield of 89 ± 63, 1513 ± 166, and 1263 ± 116 kg ha^{-1} were from the control plots for Julian days 112, 238, and 546, respectively (Fig. 10). The average forage yield increase of

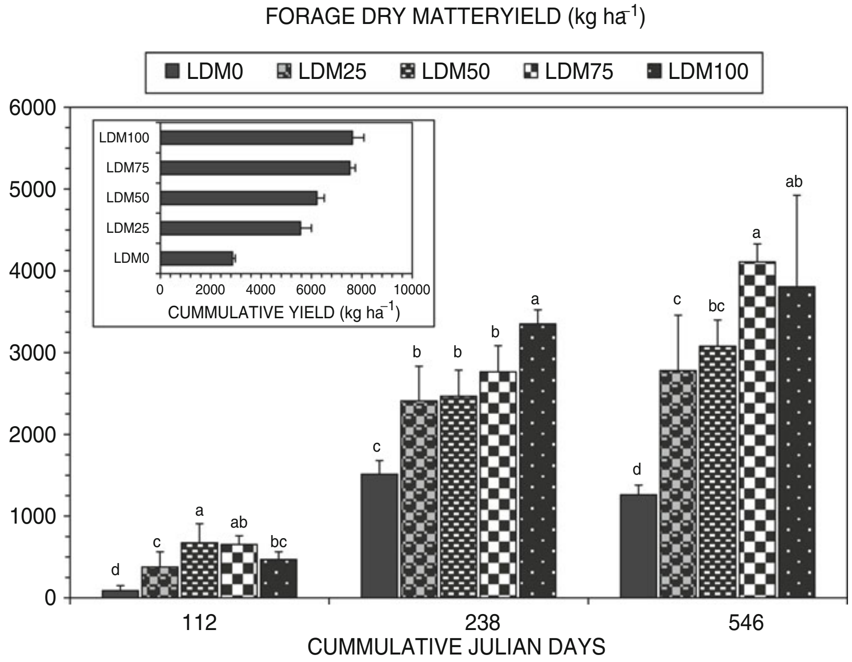

Fig. 10 Forage yield of bahiagrass (Julian days 112 to 546) as affected by varying levels of dredged materials application. Forage yield from plots with or without lake-dredged materials are significantly different ($P \leqslant 0.05$) at Julian days 112, 238, and 546 when superscripts located at top of bars are different (*Source: Sigua, 2008*)

bahiagrass in plots amended with lake-dredged materials (averaged across treatments) was 512%, 82%, and 173% when compared with bahiagrass in control plots with 0% lake-dredged materials for Julian days 112, 238, and 546, respectively (Fig. 10). These data show the favorable influence that lake-dredged materials had on forage yield of bahiagrass during its early establishment in subtropical beef cattle pastures.

Mean forage yield of bahiagrass during Julian day 112 in plots with 50% lake-dredged materials of 673 ± 233 kg ha^{-1} was not significantly different from that in plots with 75% lake-dredged materials (654 ± 106 kg ha^{-1}), but was greater than that in plots with 25% lake-dredged materials (378 ± 185 kg ha^{-1}) and 0% lake-dredged materials (Fig. 10). For Julian day 238, the greatest forage yield among plots amended with lake-dredged materials was from plots with 100% lake-dredged materials (3349 ± 174 kg ha^{-1}). The lowest forage yield of 1513 ± 166 kg ha^{-1} was from plots with 0% lake-dredged materials. Mean forage yield of bahiagrass in plots with 50% lake-dredged materials of 2467 ± 320 kg ha^{-1} was not significantly different from that in plots with 75% lake-dredged materials (2467 ± 320 kg ha^{-1}) and 25% lake-dredged materials (2409 ± 423 kg ha^{-1}), but was greater than that in plots with 0% lake-dredged materials (Fig. 10).

4.1.3 Using Legumes to Enhance Nitrogen Fertility in Bahiagrass Pastures

Beef cattle pastures in the United States and other parts of the world are typically dominated by tropical grasses such as bahiagrass or bermudagrass; drawbacks to these grasses include limited growth during the winter, relatively low nutritional value, and high N fertilizer costs. The development of effective grass-legume pastures for beef cattle production would improve nutritional value of the pastures, minimize N fertilizer input, and better manage nutrient cycling to enhance water quality. Improving the nutritional value of these grass pastures by the addition of legume will improve beef cattle gains in this region and impact both quantity and cost of the red meat supply in the United States and other parts of the world.

The establishment and maintenance of persistent grass-legume pastures is a key option to increase productivity and profitability of beef cattle production systems. Despite the great potential to improve sustainability of animal production, the adoption of technologies has been limited and slow. The integration of legumes (*Arachis glabrata*, Benth) and non-legumes (bahiagrass, *Paspalum notatum*, Flugge) can produce synergistic effects that can minimize external inputs, particularly N fertilizers in agricultural ecosystems. There is a need to carry out research that will help to understand the different factors affecting the fixation and release of biologically fixed N to non-legume crops. The integration of legumes and non-legumes (grass) can produce synergistic effects that can minimize external inputs, particularly N fertilizers in agricultural ecosystems (Saikia and Jain, 2007; Rochester et al., 2001; Omay et al., 1998; Keisling et al., 1994; Oyer and Touchton, 1990).

Symbiotic N_2 fixation may provide N for the growth of legumes and eventually to intercropped plants, such as grasses through the transfer of N from the legumes. An increased understanding of the mechanisms of N transfer in grass-legume mixtures will help increase forage productivity. Nitrogen transfer if properly documented and quantified can lead to improved management systems and increased productivity of grass-legume mixtures (e.g., bahiagrass-peanut mixtures), while minimizing the use of inorganic N fertilizers. This will increase profits and decrease the impact of agriculture on the environment.

4.1.4 Managing Nutrients Across Paddocks to Improve Water Quality

Nutrient Balance

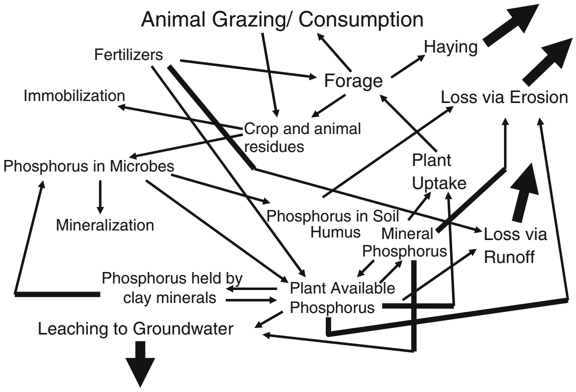

Fig. 11 Generalized schematic showing different phosphorus compartments in forage-based pasture system (*modified from Sigua et al., 2006c*) (➨ Loss or export from the system)

Nutrient balance in the ecosystem involves profitability of the agricultural enterprise and commitments to resource management to maintain quality of air, water and land resources. The role of nutrient management in livestock systems takes on new meaning as producers and the public together consider economic and noneconomic issues (Nelson, 1999). The intensification of livestock production with its associated increased demand for fodder has encouraged farmers to rely more heavily on chemical fertilizers and imported feeds, and very often the waste is considered as a disposal problem rather than useful source of plant nutrients (Hooda et al., 2000). It should be noted that for a farm to be sustainable, its P or N budget should balance, at least after soil reserves are brought up to desired levels for sustainable production. If there is a net loss of P or N, the farm's soils will eventually become depleted and if there is an excess, the likelihood of pollution is greater (Van Horn et al., 1996). Effective use and cycling of P or N is critical for pasture productivity and environmental stability. Phosphorus or N (not shown) cycling in pastures is complex and interrelated and pasture management practices influence the interactions and transformations occurring within the P cycle (Fig. 11).

Increased loss of nutrients in agricultural runoff has potentially serious ecological and public health implications (Hooda et al., 2000). Nitrogen and P are particularly important as both are implicated in aquatic eutrophication (Levine and Schindler, 1989). Any approach that controls N and/or P losses from agriculture to water must begin with the long-term objective of increasing N and/or P efficiency by attempting to balance N or P inputs with N or P outputs within a watershed. Reducing N or P loss in agricultural runoff may be brought about by Best Management Practices (BMPs) that control the source and transport of N and/or P (Table 6).

A review paper published by Shigaki et al. (2006) on animal-based agriculture options for the future contained excellent discussion on the source and transport management of nutrients in the watershed. Source management attempts to minimize the buildup in the soil above levels sufficient for optimum crop growth while transport management refers to efforts to control the movement of nutrients from soils to sensitive locations such as bodies of fresh water. As shown in Table 6, there are several measures available to minimize the potential for nutrient losses in agricultural runoff, which address sources and transport of P and/or N. Important measures to be considered are those that attempt to decrease the surplus of nutrients (P or N)

Table 6 Modified best management practices for the control of diffuse sources of agricultural nutrients (*Source: Shigaki et al., 2006*)

I. Source BMPs – Practices that minimize nutrients loss at the origin

1. Attempts to match animal requirements for N or P with feeds P or N
2. Enzyme added to feeds to increase nutrient utilization by animals
3. Test soils and manures to optimize N and P management
4. Chemically treat manure to reduce P solubility (e.g., alum, fly ash, etc.)
5. Biologically treat manure (e.g., microbial enhancement)
6. Calibrate fertilizer and manure application equipment
7. Apply proper amount of fertilizer
8. Careful timing of fertilizer application to avoid imminent heavy rainfalls

II. Transport BMPs – Practices that minimize transport of nutrients

1. Minimize erosion, runoff and leaching of nutrients
2. Use cover crops to protect soil surface from erosion
3. Install filter strips and other conservation buffers to trap eroded P
4. Manage riparian zones, grassed waterways and wetlands to trap eroded nutrients
5. Stream bank fencing to exclude animal from water course

III. Source and transport BMPs – Systems approach to minimize nutrient losses

1. Retain crop residues and reduce tillage to minimize erosion and runoff
2. Proper grazing management to minimize erosion and runoff
3. Install and maintain manure handling systems
4. Implement nutrient management plan for the farms

in localized areas. Shigaki et al. (2006) suggested the following measures: (1) dietary P reduction; (2) feed additives that enhance P utilization by animals; (3) alternative uses for manure other than land application; and (4) transporting manure to P- or N-deficient areas.

Water Quality Best Management Practices (BMP) for Florida Cow-Calf Operations

Although cow-calf operations in Florida are generally low-intensity agriculture with relatively low levels of pollutant discharged off-site, certain sites and management practices may contribute to violations of State water quality standards. Under these situations, cattle ranches may contribute elevated levels of P, N, sediments, bacteria and oxygen-demanding organic materials. The potential for discharges from cow-calf operations to cause water quality violations varies greatly, depending on soil type, slope, drainage features, stocking rate, nutrient management and other factors. In general, areas where cattle tend to congregate or have access to water bodies may have the greatest potential to contribute to water pollution (Sigua and Coleman, 2007). Spearheaded by the Florida Cattlemen's Association (FCA) and drawn up in a unique partnership between producers and regulators, the BMP manual serves as both roadmap and vehicle to enhance and protect water quality in Florida.

In 1997, the Florida Cattlemen Association began drafting a common sense, economically-viable guidelines for production practices designed to protect water bodies and maintain compliance with the State of Florida water quality standards. The manual describes the water quality best management practices (BMPs) for beef cow-calf operations in Florida. The different practices are specifically targeted for beef cow-calf operations in Florida and the activities that normally occur in conjunction with beef cattle production. These are not rules or regulations, but voluntary best management practices for Florida ranches that consider good water quality conditions.

The manual is heralded as a unique consensus document that outlines common sense, economically and technically feasible production and management practices that enhance and protect Florida's water resources. It is designed specifically for Florida's cow-calf operations; it does not apply to concentrated animal feeding operations (CAFOs), which generally require a permit (see the 107-page BMP manual at http://jefferson.ifas.ufl.edu/old/agpages/cow_calf_bmp/BMPManual.pdf). Although the manual was designed for statewide use in Florida, the general premise and principles can be adopted elsewhere.

5 Summary and Conclusions

Current pasture management including cattle rotation in terms of grazing days and current fertilizer (inorganic + manures + urine) application rates for bahiagrass pastures in subtropical regions of USA offer little potential for negatively impacting the environment. Properly managed livestock operations contribute negligible loads of total P and N to shallow groundwater and surface water. Overall, there was no buildup of soil total P and N in bahiagrass-based pasture. These observations may help to renew the focus on improving fertilizer efficiency in subtropical beef cattle systems, and maintaining a balance of P and/or N removed to P and/or N added to ensure healthy forage growth and minimize P or N runoff.

Contrary to early perception, forage-based animal production systems with grazing are not likely one of the major sources of non-point source P pollution that are contributing to the degradation of water quality in lakes, reservoirs, rivers, and ground water aquifers, but perennially grass-covered pastures are associated with a number of environmental benefits. Continuous grass cover leads to the accumulation of soil organic matter, sequestering carbon in the soil and thereby reducing the potential CO_2 accumulation in the atmosphere. The increase in soil organic matter is also related to soil quality, with improvements in soil structure, aeration and microbial activity. Effective use and cycling of N or P is critical for pasture productivity and environmental stability. In addition to speeding up N or P recycling from the grass, grazing animals can also increase N or P losses in the system by increasing leaching potential due to concentrating N into small volumes of soil under dung and urine patches, redistributing N or P around the landscape, and removal of N or P in the form of animal products.

The overall goal efforts to reduce N or P losses from animal-based agriculture should be to balance off-farm P inputs in feed and fertilizer with outputs to the environment. Source and transport control strategies can provide the basis to increase N and P efficiency in agricultural systems.

Overall,

- Forage-based animal production systems as suggested by regulators are not the major sources of non-point source nutrients pollution that are contributing to the degradation of water quality in lakes, reservoirs, rivers, and ground water aquifers; and
- Properly managed cow-calf operations in subtropical agro-ecosystem would not likely be the major contributors to excess loads of N or P in surface water and/or shallow groundwater.

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Biogeography of Soil Microbial Communities: A Review and a Description of the Ongoing French National Initiative

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Contents

Abstract Microbial biogeography is the study of the distribution of microbial diversity on large scales of space and time. This science aims at understanding biodiversity regulation and its link with ecosystem biological functioning, goods and services such as maintenance of productivity, of soil and atmospheric quality, and of soil health. Although the initial concept dates from the early 20th century (Beijerinck (1913) De infusies en de ontdekking der backterien, in: Jaarboek van de Knoniklijke Akademie van Wetenschappen, Muller, Amsterdam), only recently an increasing number of studies have investigated the biogeographical patterns of soil microbial diversity. A such delay is due to the constraints of the microbial models, the need to develop relevant molecular and bioinformatic tools to assess microbial diversity, and the non-availability of an adequate sampling strategy. Consequently, the conclusions from microbial ecology studies have rarely been generally applicable and even the fundamental power-laws differ because the taxa-area relationship and the influence of global and distal parameters on the spatial distribution of microbial communities have not been examined. In this article we define and discuss the scientific, technical and operational limits and outcomes resulting from soil microbial biogeography together with the technical and logistical feasibility. The main results are that microbial communities are not stochastically distributed on a wide scale and that biogeographical patterns are more influenced by local parameters such as soil type and land use than by distal ones, e.g. climate and geomorphology, contrary to plants and animals. We then present the European soil biological survey network, focusing on the French national initiative and the "ECOMIC-RMQS" project. The objective

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_37,

from Ranjard et al., Agron. Sustain. Dev. 30 (2010) 359–365. DOI: 10.1051/agro:2009033

of the ECOMIC-RMQS project is to characterise the density and diversity of bacterial communities in all soils in the RMQS library in order to assess, for the first time, not only microbial biogeography across the whole of France but also the impact of land use on soil biodiversity (Réseau de Mesures de la Qualité des Sols = French Soil Quality Monitoring Network, 2200 soils covering all the French territory with a systematic grid of sampling). The scientific, technical and logistical outputs are examined with a view to the future prospects needed to develop this scientific domain and its applications in sustainable land use.

Keywords Soil biogeography • Microbial communities • Soil survey • Microbial ecology • Diversity

1 Introduction

Although microorganisms are the most diverse and abundant type of organism on earth (Gans et al., 2005; Curtis and Sloan, 2005), the determinism of microbial diversification and the distribution of microbial diversity from small to large scales has been poorly documented and is little understood. Most studies of prokaryote diversification have focused on variations due to mutations and/or lateral gene transfers and subsequent selection resulting from environmental stresses and competition for resources. Few have considered more neutral mechanisms, such as genetic drift due to physical isolation, thus revealing the crucial lack of integration of the spatial scale into microbial community assembly (Ranjard and Richaume, 2001; Papke and Ward, 2004).

Ecologists have long recognised that beta-diversity (how community composition changes across a landscape) is pivotal to understanding how environmental factors affect the magnitude and variability of biodiversity. This conceptual vision is also relevant to microorganisms since beta-diversity patterns offer valuable insights into the relative influence of dispersal limitations, environmental heterogeneity, and environmental and evolutionary changes in shaping the structure of ecological communities (Green et al., 2004). Although the spatial patterning of microbial diversity is known to have important consequences on plant community structure and ecosystem functioning, microbial beta-diversity patterns have been poorly investigated and remain largely unknown. The empirical relationship between the number of species and the area sampled (taxa-area relationship) has not been empirically examined in microorganisms, as it has in plants and animals (Horner Devine et al., 2004; Green and Bohannan 2006). This may partly be explained by the characteristics of microorganisms, namely (i) their small size, which makes access within environmental matrices difficult, (ii) their high density (e.g. more than one billion per gram of soil) and (iii) their huge diversity (from 1000 to 1 000 000 species per gram of soil, Torsvik and Øvreås, 2002), not to mention the complexity of precisely defining their species. Progress is also hampered by difficulties in designing an adequate sampling strategy. Such a strategy needs to integrate a large scale of sampling (region, territory, etc.), with precise squaring that is representative of any landscape modifications, which therefore implies a very large number (several thousands) of samples.

Our aim in this paper is to present the results of the first analytical studies of the biogeography of soil microbial communities, the concept applied and the technical feasibility of this novel scientific domain in environmental microbiology. We shall then describe the European strategy and the French national soil survey in which different research teams with scientific expertise in soil science, statistics, microbial ecology and geochemistry are, for the first time, working to assess the inventory and mapping of soil microbial diversity on a national territorial scale.

2 Scientific Outcomes of Microbial Biogeography

Biogeography is the study of the distribution of biodiversity over space and time (Martiny et al., 2006). In other words, the aim in biogeography is to reveal where organisms live and their abundance, and to determine those environmental factors that select or maintain the presence of these organisms. This scientific approach was first applied during the eighteenth century in studies of the geographic distribution of plant and animal diversity. These investigations provided insights into the mechanisms that generate and maintain diversity in macroorganisms e.g., speciation, extinction, dispersal and species interactions (Brown and Lomolino, 1998).

Despite the key role of microorganisms in a wide range of biogeochemical cycles, few studies (in comparison with macroorganisms) have examined the distribution of microbial diversity on a broader scale than field plots. The first study describing and investigating microbial biogeography was conducted by Beijerinck (1913), who stated that "everything is everywhere, but, the environment selects" (see Sect. 3). Since then, few authors have examined the full extent of microbial diversity or described the biogeographical patterns in an attempt to assess this statement and specify which environmental factors exert the strongest influence on indigenous microbial communities. Even though recent advances in molecular biology have led to the development of tools to assess bacterial diversity in environmental samples without culturing, most studies have focused on cataloguing the bacterial diversity in particular sites or describing how bacterial communities have been affected by environmental perturbations (for review see Ranjard et al., 2000). Thus, the conclusions of such microbial ecology investigations cannot be generally applied as the data from different studies are difficult to compare and the trends deduced are often inconsistent.

2.1 Phylogeography of Particular Populations

To date, most studies dealing with microbial biogeography have been limited to the phylo-geography of particular populations, particularly of pathogenic or symbiotic organisms. One of the main results is that many host-associated microorganisms exhibit genetic and functional patterns that are related to the distribution of their hosts (for review see Martiny et al., 2006). As regards the free-living microorganisms, most recent investigations have been focused on individual soil bacterial strains (Cho and Tiedje, 2000). These studies have tended to demonstrate that the genetic distance between microorganisms is related to geographic distance, and have highlighted correlations between assembly composition and environmental or geographic characteristics (for review see Martiny et al., 2006). Few publications have considered the soil microbial community as a whole and how it is structured on a large spatial scale.

2.2 Biogeography of Microbial Communities

In one of the rare studies available, Green et al. (2004) genotyped fungal community structure in numerous Australian soils (about 1500) and were able to demonstrate that despite the high local diversity of microorganisms, the regional diversity was only moderate. Fierer and Jackson (2006) produced a continent-scale description of soil bacterial diversity by considering about 100 different soils sampled from the north to south of America. By applying a DNA fingerprinting method, they demonstrated that bacterial diversity was unrelated to site temperature, latitude and other variables that, in contrast, strongly influence plant and animal diversity, and that community composition was largely independent of geographic distance. The environmental factor most influencing bacterial diversity was soil pH, with the highest diversity occurring in neutral soils and the lowest in acidic soils. These studies also demonstrated that taxa-area relationships in soil microorganisms were weak, thus indicating that microbial biogeography differs fundamentally from that of "macroorganisms". By applying a pyrosequencing technique to ribosomal sequences in the same set of soil samples, Jones et al. (2009) defined the ecological attributes of particular populations such as *Acidobacteria* and confirmed the importance of soil pH in their dissemination.

In contrast, Johnson et al. (2003) demonstrated that variations in the genetic structure of bacterial communities from numerous agricultural soils were not correlated with pH but with soil texture and electrical conductivity. The overall inconsistency of these reports may possibly be ascribed to the inadequate sampling strategy in terms of number and representativeness of the soils sampled. However, it underlines the fact that the number of studies dealing with microbial-biogeography needs to be increased to understand the determinism of microbial diversity better, especially as this latter directly affects a wide range of ecosystem processes and therefore the quality of our environment.

2.3 The First Concept of Microbial Biogeography: "Beijerinck, 1913"

Microbial ecologists describing biodiversity on a large spatial scale, i.e. microbial biogeography, generally invoke one of the oldest fundamental paradigms in microbial ecology, "everything is everywhere, but, the environment selects", proposed by Beijerinck (1913). This tenet was used as the starting point for studies of prokaryotic biodiversity and their biogeographical patterns but was frequently misinterpreted. Baas Becking (1934), and more recently de Wit and Bouvier (2006), rehabilitated the original meaning of this statement, which reflects an apparent contradiction between empirical observations that specific microorganisms are observed in their characteristic environments and the idea that all microorganisms are cosmopolitan. Despite the technical difficulty of verifying the first statement, due to the detection limits of the approaches used to characterise microbial diversity, this premise implies that the genetic cohesiveness of prokaryotic populations can never be broken by physical isolation but solely by adaptation. These basic concepts were only implicit in the publication of Beijerinck (1913) but were tacitly accepted (Baas Becking, 1934).

The concept "everything is everywhere" is supported by several particularities of the microbial model: microorganisms (i) are small and easily transported, (ii) are able to form a resistant physiological stage that allows them to survive in hostile environments, and (iii) have extremely large population sizes with a high probability of dispersal and a low probability of local extinction (Fenchel, 2003). The fact that more than 10^{18}–10^{20} microorganisms are estimated to be transported annually through the atmosphere between continents supports the hypothesis of a wide dispersion of microbes (Gans et al., 2005). Further evidence is that bacteria can be isolated from places where "they should not be", e.g. thermophilic bacteria from cold sea water (Isaksen et al., 1994).

In contrast, "the environment selects" might seem to challenge the concept that "everything is everywhere", and suggests that geographic isolation of populations coupled with limited dispersal leads to allopatric speciation. An increasing number of studies have demonstrated that the physical isolation of free-living microorganisms may be more widespread than previously thought (for review see Papke and Ward, 2004). However, most recent studies have been limited to characterisation of the culturable populations which are known to represent only a very small fraction of the whole community (Amann et al., 1995). To date, few studies have focused on microbial communities, possibly because of the technical limitations associated with identifying the huge microbial diversity in natural ecosystems and the difficulties in detecting minor populations. These technological limitations have now been partly resolved thanks to the recent development of molecular tools that circumvent the isolation and culture of organisms, and allow microbial community structure and diversity to be characterised without a priori knowledge (for review see Ranjard et al., 2000; Christen, 2008). Furthermore, these tools are now generally automated and allow the moderate throughput essential to studies involving the characterisation of numerous environmental samples.

3 Soil Biodiversity Monitoring in Europe

3.1 International Context

Although soil inventory programmes exist in all European countries, there are not many fully operational soil monitoring systems in Europe (Morvan 2008). Few include more than one sampling point in time so that most are mere inventories. The only EU-wide soil monitoring network is the ICP forest level 1 grid, which was partly re-sampled in 2006–2007 within the Forest Focus BioSoil project (Lacarce et al., 2009). The data requirements for this re-sampling did not include microbial diversity. Progress in recent years has been hampered by a lack of perception of the importance of soil, data ownership issues and data incompatibility resulting from the multiplicity of different sampling and analytical procedures. The ENVASSO project addressed the need to characterise soils by setting up a series of interlinked objectives to harmonise the soil data sets that currently exist in EU Member States (Kibblewhite et al., 2008). Eight threats to soil (erosion, declining organic matter, contamination, compaction, salinisation, loss of biodiversity, soil sealing, landslides and flooding) have been identified in the European Commission's official Soil Communication part of the Thematic Strategy for Soil Protection in Europe. The aim of the ENVASSO project was to develop a system to harmonise existing, mostly national, data sets and provide a central reference point to assess current soil status and ensure sustainable management in the future. This project identified the physical and chemical parameters to monitor, most of which were already being monitored by the national soil surveys conducted in different European countries.

3.2 The French Soil Survey: RMQS

It was apparent from the initial data collected during the ENVASSO project that biodiversity was not fully included in any national soil monitoring network except in the Netherlands. In 2001, a new structure was created in France to reorganise soil mapping and soil monitoring programmes and to provide relevant insights into the spatial distribution of soils and the evolution of their properties. This structure, called the Scientific Group on Soils (GIS Sol) includes the Ministries of Agriculture and Environment, the Environment and Energy Management Agency (ADEME), the Research Institute for Development (IRD), the National Forest Inventory (IFN) and the National Institute of Agronomic Research (INRA). The main soil monitoring programme is the French Soil Quality Network ("Réseau de Mesures de la Qualité des Sols", RMQS), that is based on a 16 km by 16 km grid, with 2200 sites representative of the main soil systems and land uses in France. This configuration has the advantage of being fully compatible with the sites of the ICP forest level 1 network (now called BioSoil) which monitors forested soils across Europe. The RMQS is a complete and balanced network of 2200 sites which will be sampled every 10 years to monitor soil quality. The primary objectives of the network are to characterise and quantify diffuse contamination in trace elements (Saby et al., 2006) and to evaluate and monitor organic carbon stocks. For this purpose, a set of analyses (particle-size distribution, bulk density, C, N, pH, trace elements, etc.) and a complete description of the soil profile at each site are obtained together with information about past activities, the environment, etc. Apart from these objectives, the network is responsible for many other soil quality evaluations and monitoring, e.g. persistent organic pollutants (pesticides, dioxins, organochlorides, PAH, etc.).

3.3 An Ongoing Project: The ECOMIC-RMQS Project

Based on this soil survey, the scientific project "ECOMIC-RMQS" was set up in 2006, offering the first opportunity to implement biological diversity in a soil monitoring network. This project is coordinated by the Microbiology of the Soil and Environment Centre ("Centre de Microbiologie du Sol et de l'Environnement" i.e. CMSE, INRA Dijon, Burgundy, France) and is one of the first steps to demonstrate the technical feasibility and scientific relevance of federating European initiatives to monitor soil biodiversity monitoring.

The objective of the ECOMIC-RMQS project is to characterise the density, genetic structure and diversity of bacterial communities in all soils in the RMQS library (2200 soils sampled up to 2009) in order to assess, for the first time, not only microbial biogeography across the whole of France but also the impact of land use on soil biodiversity. The strategy for characterising the density and diversity of the bacterial communities relies on molecular tools such as quantitative PCR, DNA micro-array and DNA fingerprinting on soil DNA extracts (Fig. 1).

This integrated project will provide cognitive insights into the ecological theory on the community assembly by:

- elucidating the relative contribution of geographic isolation versus wide dispersal in limiting bacterial diversification,
- allowing better examination of the taxa-area relationship for bacteria,
- deciphering the hierarchy of the environmental parameters (plant cover, physico-chemical characteristics, climate factors, etc.) that most contribute to bacterial community diversity and composition.

This project should also have more applied outcomes as a result of:

- determining the state of bacterial diversity in French soils,
- better estimation of the impact of land use and human activities on microbial diversity and distribution,
- identifying bacterial bio-indicators specific to land management and human activities.

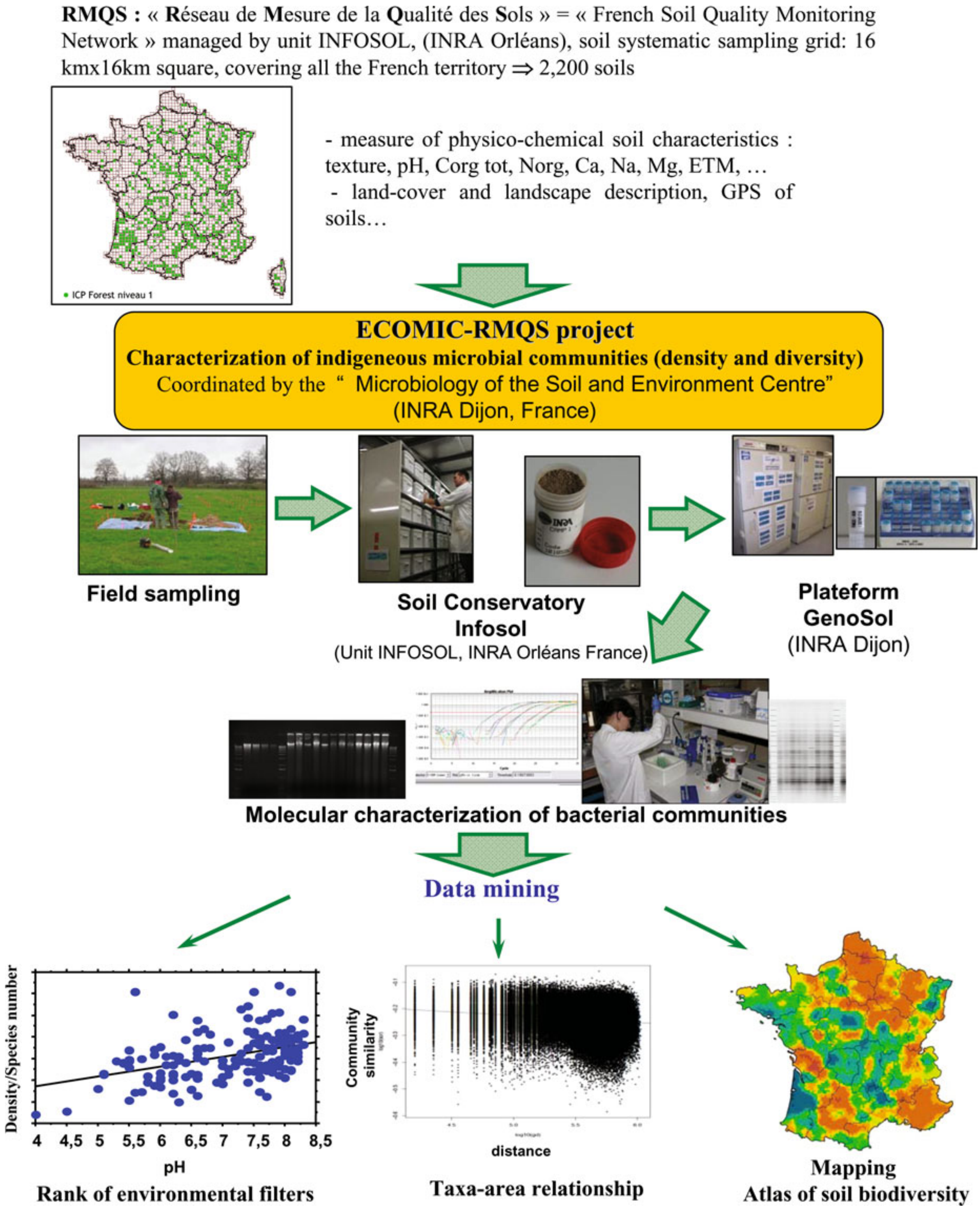

Fig. 1 Schematic description of strategy and outputs of the ECOMIC-RMQS project

The ECOMIC-RMQS project provides an appropriate means of (i) assessing the microbial-biogeography by use of the RMQS and (ii) elucidating the determinisms of bacterial community diversification in soils better. This project should provide answers to some of the questions originating from mechanistic hypotheses:

- Are microbial communities a "black box" with no spatial structure or, like macroorganisms, do they exhibit a particular distribution with predictable, aggregated patterns from local to regional scales? In other words, does a taxa-area relationship exist in microbial-biogeography? (Green et al., 2004; Horner Devine et al., 2004).
- Are spatial variations due to contemporary environmental factors or to historical land use and contingencies?
- Which environmental factors (edaphic, climatic, land use, anthropogenic) most contribute to the structure and diversity of bacterial communities in soil on very broad geographic scales?

The first results obtained in this programme have demonstrated:

- a non-stochastic distribution of bacterial diversity which is spatially structured in biogeographical patterns on a regional scale (Dequiedt et al., 2009),
- a taxa-area relationship for soil bacterial communities,
- a positive correlation between bacterial diversification and landscape diversity and fragmentation,
- a greater influence of local environmental local parameters (pedoclimatic and land use) as opposed to global parameters (climate and geomorphology) on bacterial community density and diversity,
- the strongly deleterious effects that specific land use (especially agriculture) can have on indigenous bacterial communities.

3.4 Need for Technical and Logistic Supports

One of the main prerequisites of a project such as ECOMIC-RMQS, which is aimed to assess microbial diversity on a wide scale, is the development of suitable logistics for storing and managing very large numbers of biological samples and associated data. In addition, the soil samples need to be characterised under medium-throughput conditions with high levels of reproducibility and quality of the applied procedures. The GenoSol Platform, which was set up to store and manage soil samples as well as to ensure molecular characterisation of the microbial communities, meets these requirements (Ranjard et al., 2009). Thus, two complementary structures described as Soil Biological Resource Centres have been developed in France: the INFOSOL Conservatory and GenoSol Platform.

The INFOSOL unit in Orléans (France) houses the soil conservatory. This soil library has been designed for the management, storage, preparation and dissemination of soil samples from the RMQS programme in the long term. Thousands of large samples (5 to 10 kg each) are stored air-dried, under controlled conditions of temperature and hygrometry. The aim is to provide (i) a memory of the state of French soil, (ii) reference samples for re-analysis after other campaigns, and (iii) a soil bank for research development.

The GenoSol Platform (http://www.dijon.inra.fr/plateforme_genosol) was created in 2008 by the "Centre de Microbiologie du Sol et de l'Environnement" (CMSE, INRA Dijon, Burgundy, France). The aim is to provide an appropriate logistic structure for the acquisition, storage and characterisation of soil genetic resources obtained by extensive sampling (several hundred to several thousand soils), on very large space and/or time scales (national soil survey, long-term experimental sites), and to make these resources readily available to the scientific community and to policy-makers. The ultimate goal is to produce a reliable reference system based on molecular characterisation (taxonomic and functional features) of soil microbial communities, that facilitates the scientific interpretation of sample analyses on large scales of time and space sampling. Another aim of the platform is the long-term storage of a library of soil genetic resources (soil DNA) to be made available to national and international scientific communities (Ranjard et al., 2009). In summary, the GenoSol Platform can be considered as a logistic and technical tool and therefore as a strategic partner for research units who wish to benefit from large-scale soil sampling without needing to develop cumbersome or circumstantial methodologies or organisations.

In the context of the ECOMIC-RMQS project, the GenoSol Platform has:

- built up and maintains a national soil DNA library which is to be made available to the scientific community as a whole in order to assess microbial diversity in the future with more powerful tools and/or other molecular analysis,
- developed in collaboration with the INFOSOL conservatory a database of the genetic structure and taxonomic diversity of microbial communities in French soils, so that an Atlas of Soil Biodiversity can be compiled on a national scale,
- established a reference frame for interpreting these analyses by (i) treating the determinants of variability of the microbial communities in soils on a hierarchical basis, and (ii) quantifying the impact of human activities on these communities.

4 Conclusion

Various lines in the studies of microbial biogeography currently available could be further developed:

- the scale of investigation could be increased by considering a whole territory or continent to permit better comparison of the different pedoclimatic regions,
- widescale soil sampling could be conducted in the long term to evaluate the influence of land use management and global changes on the evolution of soil biodiversity,
- the characterisation of soil microbial diversity could be improved by applying recent and innovative techniques such as pyrosequencing of ribosomal genes to permit exhaustive evaluation of the taxonomic diversity of indigenous communities (Christen, 2008; Roesch et al., 2007),
- soil biodiversity could be linked with soil functioning to improve management and protection of the various resources and services of soil,
- the range of variations in diversity could be described for a given pedoclimatic zone and within this zone, for a given land use. Such information could be used to interpret soil quality analyses for soil users (farmer, industrial or urban), biodiversity erosion, and to improve land use management with a view to sustainable development.

Microbial biogeography is therefore relevant at a landscape level to understanding soil biodiversity erosion brought about by natural and/or anthropogenic activities better. Even if the relationship between soil biodiversity and soil or ecosystem functions remains incomplete, such a research strategy should improve soil management in the current context of increasing ecosystem goods and services. Consequently, at the European level, a better coordination of the different national soil surveys and of the strategy employed to characterise biodiversity constitutes a challenge for the future soil protection policy in terms of sustainable development.

Acknowledgements The ECOMIC-RMQS programme was supported by the French National Research Agency (ANR) and ADEME. Sampling was supported by a French Scientific Group of Interest on soils: the "GIS Sol", involving the French Ministry for Ecology and Sustainable Development (MEDD), the French Ministry of Agriculture (MAP), the French Institute for the Environment (IFEN), the French Environment and Energy Management Agency (ADEME), and the National Institute for Agronomic Research (INRA). We thank all the soil surveyors and technical assistants involved in sampling the sites.

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Part VII

Alternative Fertilisation

Nitrogen Rhizodeposition of Legumes

Joëlle Fustec, Fabien Lesuffleur, Stéphanie Mahieu, and Jean-Bernard Cliquet

Contents

Abstract Because nitrogen is one of the major elements limiting growth of plants in agrosystems, large amounts of N fertilisers have been used in the second half of the twentieth century. Chemical fertilisers have contributed to increasing crop yields and food supply, but they have induced environmental damage such as nitrate pollution and wasting fossil fuel. The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input agrosystems. Here we review agricultural practices, measurement methods and biological pathways involved in N cycling. We show that plant roots interact intimately with soil microflora to convert the most abundant but relatively inert form of N, atmospheric N_2, into biological substrates available for growth of other plants, through two consecutive processes; namely, N_2 fixation and N rhizodeposition. In intercropping, companion plants benefit from biological fixation by legumes and subsequent transfer of N from legumes to non-legumes. This transfer from legumes to the release of N compounds by legume roots, a process named rhizodeposition, then the uptake by the companion crop. The two main rhizodeposition pathways are (i) decomposition and decay of nodules and root cells, and (ii) exudation of soluble N compounds by plant roots. The contribution of root N and rhizodeposited N to the soil-N pool is difficult to measure, particularly in the field. Firstly, root N is often underestimated because root recovery is problematic. Second, assessment of N rhizodeposition is challenging. Several ^{15}N labelling methods have been performed for different legume species. Rhizodeposition of N, as a percentage of total plant N, varied from 4 to 71%. The high variability of the results illustrates the need for more studies of

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_38,

from Fustec et al., Agron. Sustain. Dev. 30 (2010) 57–66. DOI: 10.1051/agro:2009003

the environmental and genetic factors influencing the amount of N rhizodeposits released by legumes under field conditions.

Keywords N rhizodeposition • Legumes • N_2fixation • ^{15}N • Isotopic methods • Root exudates • Ecological fertilisation

1 Introduction

Even though N is among the most abundant elements on earth, it is also the major element limiting growth of plants in many agricultural systems because of its unavailability for plants (Hartwig, 1998; Vance, 2001). N fertilisers have been considered for many years as a reasonable insurance against yield loss and have been used extensively (Vance, 2001) but contribute substantially to environmental pollution (Deutsch et al., 2006; Umar and Iqbal, 2007). It is now established that excessive use of these fertilisers affects the balance of the nitrogen cycle in soils, causes eutrophication because of nitrate leaching and has contributed to global warming because of gaseous loss as N_2O. The non-stop use of N fertilisers would also accelerate the depletion of stocks of non-renewable energy resources required for fertiliser production. Furthermore, there are vast areas in the developing world where N fertilisers are neither available nor affordable due to weak infrastructure, poor transportation and high cost.

These problems explain why biological alternatives using diazotrophic prokariots have become of increasing interest in agricultural practices in the last few years, particularly for low-input systems. Biological N fixation can act as a sustainable source of N and can complement or replace fertiliser inputs (Garg and Geetanjali, 2007). The two main agricultural practices to benefit from biological N fixation, crop rotation and intercropping legumes (Fabaceae), and non-fixing plants, were practised in ancient times, even if the basis for the benefit derived was not understood (Burris, 1974). Most of the fixed N in legumes is harvested and fed to animals, but evidence from a number of experiments using different methodologies indicates that legumes can deposit significant amounts of N in the soil during growth (Jensen, 1996a, b; McNeill et al., 1998; Khan et al., 2002a; Mahieu et al., 2007; Wichern et al., 2007a, b). Fixed N can also be transferred to intercropped non-legumes in the case of mixed cropping systems, or to following crops in the case of crop rotation.

In addition to the use of legumes in agriculture, other technologies to take advantage of N_2-fixing micro-organisms include the utilisation of the symbiosis between the fern *Azolla azollae* and the N_2-fixing cyanobacterium *Anabaena azollae* as a green manure in rice wetlands, and the use of free-living N_2-fixing bacteria such as *Azopirillum* inoculated into the rhizosphere of grasses.

2 Biological N_2 Fixation of Legumes

2.1 *Processes*

Nitrogen fixation is carried out by a small number of diazotrophic prokaryotic microorganisms, belonging to a wide range of eubacteria and archaebacteria. Diazotrophs are usually divided into free-living and symbiotic forms, though some cyanobacteria are able to fix N either independently or in symbiotic association. Symbiotic diazotrophs include a number of genera of the Rhizobiaceae, which form the well-documented symbiosis with legumes (Gordon et al., 2001; Garg and Geetanjali, 2007), where nitrogen fixation takes place in specialised organs, the nodules. Most of these nodules are formed on legume roots but some rhizobia such as *Azorhizobium caulinodans* are able to form stem nodules. Symbiotic N_2 fixation in legumes is the result of a structurally and physiologically highly organised, host-specific mutualistic interaction between rhizobia and legumes. Biological N fixation is catalysed by an anaerobic enzyme, nitrogenase, which carries a complex metallocluster on its active site. The most abundant nitrogenase contains iron and molybdenum at this site but others contain iron and vanadium, or iron only when molybdenum is not available (Curatti et al., 2006). Because of the economic and ecological benefits of N_2 fixation, the genes associated with this process, designated nif, have been extensively studied and are now well characterised (Gordon et al., 2001). Because nitrogenase is inhibited upon exposure to oxygen, nitrogen-fixing organisms have certain adaptations. In the case of the legume-*Rhizobium* symbiosis, the two main adaptations are the formation of the oxygen diffusion barrier into the nodule and the synthesis of the oxygen carrier protein in the symbiosome, leghaemoglobin (Gordon et al., 2001). A number of other non-legume plants, mainly woody species, also produce N_2-fixing nodules, in symbiosis with the actinomycete, *Frankia* (Uselman et al., 1999).

2.2 Benefits and Use of Legumes in Agrosystems

Because of their ability to fix N_2, legumes are considered to be involved in ecological facilitation processes in all ecosystems (Loreau and Hector, 2001; Rochon et al., 2004; Padilla and Pugnaire, 2006). A wide range of legumes are grown around the world, for production of protein-rich seeds or for harvest of the whole shoot. In agrosystems, legumes contribute nitrogen benefits in two main ways:

(i) Legumes are N-rich plants which can be used in crop rotations to increase the soil-N pool (Chalk, 1998). For this purpose, several legume species such as clovers (*Trifolium* sp.), alfafa and vetches (*Vicia sativa* L. and other *Vicia* genera), fenugreek (*Trigonella foenum-graecum* L.), lupin (*Lupinus angustifolius* L.), velvet bean (*Mucunia pruriens* Bak.), *Crotalaria spectabilis* Roth., or *Sesbania rostrata* Brem. are included in rotations and used as green manure. They contribute to nutrient cycling, soil organic matter conservation, and to the nutrient supply for succeeding crops. However, numerous legumes included in rotations are grain legumes. They are grown worldwide and Crépon (2006) reported production of 241×10^6 t of dry matter in the 2003/2004 season. Soybean (*Glycine max* L.) is mainly produced in North and South America and in Asia. Pea (*Pisum sativum* L.), fababean (*Vicia faba* ssp *minor* L.) and dry bean (*Phaseolus vulgaris* L.) are mainly produced for feed in the northern hemisphere, since in the southern hemisphere, the most common grain legumes are mainly grown for food and are dry bean, chickpea (*Cicer arietinum* L.) and cowpea (*Vigna unguiculata* L.). Lentil (*Lens esculenta* L.), pigeon pea (*Cajanus cajan* L.) and peanut (*Arachis hypogea* L.) are also commonly used for human food. Nitrogen harvest indices of grain legumes such as soybean, pea, fababean or lupin are often high; for instance, N accumulated in the seeds may represent more than 85% of plant N for soybean (Toomsan et al., 1995), and more than 75% for pea plants (Mahieu et al., 2007).

Since roots and rhizodeposits are so rich in N, including a grain legume in rotations may lead to a positive N-preceding effect on the following crop, despite N losses due to harvest. Hence, compared with a cereal grown in the same conditions, greater levels of inorganic N are recorded after harvesting grain legumes, especially in deeper soil layers (Crozat and Fustec, 2006). However, soil inorganic N measurements do not take into account changes in the organic N pool.

(ii) Legumes grown simultaneously and in the same field as non-fixing species (intercropping) lead to a more efficient use of soil resources in time and space (Loreau and Hector, 2001; Hauggaard-Nielsen and Jensen, 2005; Corre-Hellou et al., 2006). The niche separation effect often results in a higher yield in an intercrop than in a sole crop for the non-fixing species. In mixtures with non-fixing plants, N_2 fixation by legumes is higher than in a monoculture regardless of management or location (Carlsson and Huss-Danell, 2003; Corre-Hellou et al., 2006). Experiments undertaken using mixtures of annual crops (for instance, pea-barley intercropping) have shown that this effect is higher in low-input systems than in others, and leads to more stable yields in problematic environments (Jensen, 2006; Corre-Hellou et al., 2007).

Both the niche complementarity effect (Loreau and Hector, 2001) and soil N-pool increase can benefit perennial cover such as legume-based grasslands (Soussana and Machado, 2000; Høgh-Jensen, 2006; Rasmussen et al., 2007). Forage legumes are widespread and have the potential to give high yields over a range of climatic conditions; the four major forage legumes alfafa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), subterraneum clover (*T. subterraneum* L.) and white clover (*T. repens* L.) together cover grassland of hot and dry regions of the earth (Frame et al., 1998). While white clover is the most widespread clover used in agriculture, birdsfoot trefoil (*Lotus corniculatus* L.) is also abundantly sown in temperate and northern areas, as is, to a lesser extent, alsike clover (*T. hybridum* L.).

3 Quantification of N Rhizodeposition

3.1 Estimation of Below-Ground N

When legumes are used as green manure, biological fixation of N can be transferred to the soil through decomposition of above- and below-ground legume residues after harvest (Fujita et al., 1992). This is the reason why legumes are used in organic agriculture and are undersown with cereals for subsequent incorporation into the soil as green manure. Additionally, in intercropping systems, legume roots also release a significant proportion of N into the rhizosphere (rhizodeposited N). However, studies dealing with N balance in rotational farming systems including legume crops have long omitted to consider the below-ground contribution of legumes to the soil-N pool (Unkovich and Pate, 2000). The below-ground N pool can be defined as the sum of visible fibrous macro-root N and the part of soil N derived from rhizodeposition (Høgh-Jensen and Schjoerring, 2001). Estimation of soil N derived from rhizodeposition is greatly influenced by the method of measurement. Sampling of the roots and soil has major consequences on the results. Two kinds of methods are available for measuring below-ground N, with or without the use of a ^{15}N isotope:

(i) The most simple and commonly used approach for assessing below-ground N involves *physical removal of the roots from the soil*. Using this method, values of below-ground N as a percentage of total plant N are often very low compared with those obtained in the greenhouse. This is probably because sampling the entire root biomass is challenging, as many roots are fine and fragile and difficult to recover by wet sieving (Bergersen et al., 1989; Chapman and Myers, 1987; Toomsan et al., 1995; Russell and Fillery, 1996b; Dalal et al., 1997; Rochester et al., 1998; Unkovich and Pate, 2000). Greenhouse experiments undertaken in pots with limited volume allow a higher recovery of the root compartment (Mahieu et al., 2007). In addition, physical recovery of roots does not take rhizodeposited N into account, though this is also a necessary value for assessing below-ground N (Khan et al., 2002a, b).

Crawford et al. (1997) used a sequential coring and summation technique proposed by Hansson and Steen (1984) designed to assess total root production from repeated and simultaneous measurements of living roots, dead roots and old organic material. This method seems more accurate than assessments based solely on physical recovery of intact roots, but total root biomass remains underestimated.

(ii) *Direct labelling of legumes with a tracer such as ^{15}N provides a means to assess the two components of below-ground N* and particularly rhizodeposited N in the soil. ^{15}N is applied to a part of the plant and transferred to all organs by the sap stream, so rhizodeposits are ^{15}N-enriched (Figs. 1 and 2). The percentage of NdfR (N derived from rhizodeposition) is usually calculated using equation (1), proposed by Janzen and Bruinsma (1989):

$$\%\mathrm{NdfR} = [(\mathrm{atom\%\ ^{15}Nexcess\ soil})/(\mathrm{atom\%\ ^{15}Nexcess\ root})] \times 100 \quad (1)$$

The atom% ^{15}N excess values were obtained by correcting the ^{15}N enrichments with background values.

$$\%\mathrm{NdfR} = [(\mathrm{atom\%^{15}Nsoil\ enriched - soil\ background})/(\mathrm{atom\%\ ^{15}Nroot\ enriched - root\ background})] \times 100 \quad (2)$$

The ^{15}N abundance of plants grown in unlabelled soil, or of unlabelled legume or non-legume control plants, has often been used as background (Jensen, 1996a; Russell and Fillery, 1996a, b; Khan et al., 2002a, b; Mayer et al., 2003; Mahieu et al., 2007; Gylfadóttir, 2007). Schmidtke (2005a) has demonstrated that the lower the ^{15}N abundance of the roots, the more important the choice of adequate soil and root background values (Eq. (2)). The best estimation of N derived from rhizodeposition is obtained when the ^{15}N abundance of soil unlabelled N is used for soil background and the ^{15}N abundance of unlabelled roots for root background. Non-fixing plants can also be used for soil and root background values. As N re-absorptions are not taken into account, equations (1, 2) correspond to the assessment of net N rhizodeposition. The amount of total N (mg) derived from rhizodeposition is calculated by multiplying the N amount in this pool with the % NdfR value.

Root/soil sampling methods may also influence the results, since they have direct consequences on ^{15}N enrichment values of roots and soil. In some studies, roots are separated from the soil by dry gentle sieving (2 mm) before being carefully brushed to give a clean root fraction (McNeill et al., 1997, 1998; McNeill and Fillery, 2008). After root/soil sieving, Yasmin et al.

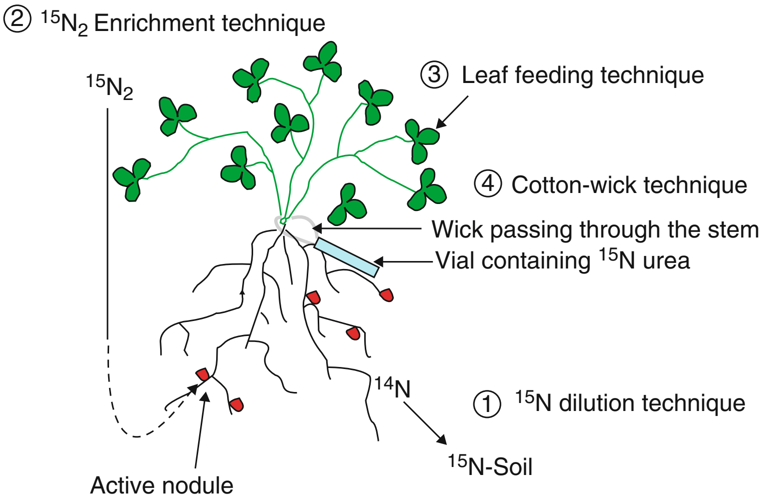

Fig. 1 Some techniques used to assess below-ground contribution of legumes to the soil-N pool in the field. (1) – In the ^{15}N dilution technique, ^{15}N is provided directly to the soil, but the differences in ^{15}N natural abundance between air and soil can also be used. (2) – $^{15}N_2$ enrichment technique: nodulated roots are exposed to $^{15}N_2$. (3) and (4) – In the leaf-feeding techniques and in the cotton-wick technique, ^{15}N is provided as urea, nitrate or ammonium contained in a vial, to the above-ground parts. With the cotton-wick technique, ^{15}N-labelling solution is provided to the plant by means of a cotton-wick passing through a hole in the plant stem. (See Fig. 2 for split-root technique)

(2006) separated the roots from the remaining adhering soil (called 'rhizosphere soil') by –40°C freeze-drying for 2 d. In other studies (Sawatsky and Soper, 1991; Mayer et al., 2003; Schmidtke, 2005a, b; Mahieu et al., 2007; Wichern et al., 2008), after root collection by gentle dry sieving, visible micro-roots were hand-collected with tweezers. Then all roots were shaken in a closed dish with deionised water, and the rinse solution was pooled with the soil sample. Most experiments are undertaken in a sandy substrate to make soil/root sorting easier. In the field or under rain shelters, plants are often planted in columns pushed down into the soil (or mesotrons; Russell and Fillery, 1996b; McNeill et al., 1997; Gylfadóttir, 2007; Table 1), or in microplots delimited with plastic or iron sheets (Rochester et al., 1998; Mahieu et al., 2007).

The use of equation (1) assumes a uniform distribution of ^{15}N label throughout the root system and similar enrichments of both N deposited and of roots, but differences in ^{15}N enrichment between fine roots, coarse roots and nodules are often observed (Khan et al., 2002a, b; Russell and Fillery, 1996a; McNeill and Fillery, 2008).

3.2 ^{15}N Labelling Methods

Isotopic methods should ideally allow a uniform labelling of the whole plant. The ^{15}N label used to assess below-ground N can be provided to the legume in different ways.

(i) In *the ^{15}N dilution technique*, the label is provided directly to the soil and N fixation is estimated by the input of ^{14}N from the atmosphere (Fig. 1). This method is reliable for measurement of N_2 fixation by legumes and transfer to companion plants (Giller et al., 1991; Moyer-Henry et al., 2006; Paynel et al., 2008) but is strongly influenced by small differences in the spatial and temporal distribution of soil ^{15}N when used for measurement of N rhizodeposition (Hétier et al., 1986; Khan et al., 2007). Poth et al. (1986) used a soil with very low nitrogen content and labelled this soil with $^{15}NH_4$ for six years to increase the accuracy of the measurement of rhizodeposition by pigeonpea plants in a greenhouse study.

(ii) *The $^{15}N_2$ enrichment technique*, by which nodulated roots are exposed to $^{15}N_2$, is the more direct way to measure the input of fixed N_2 into the rhizosphere

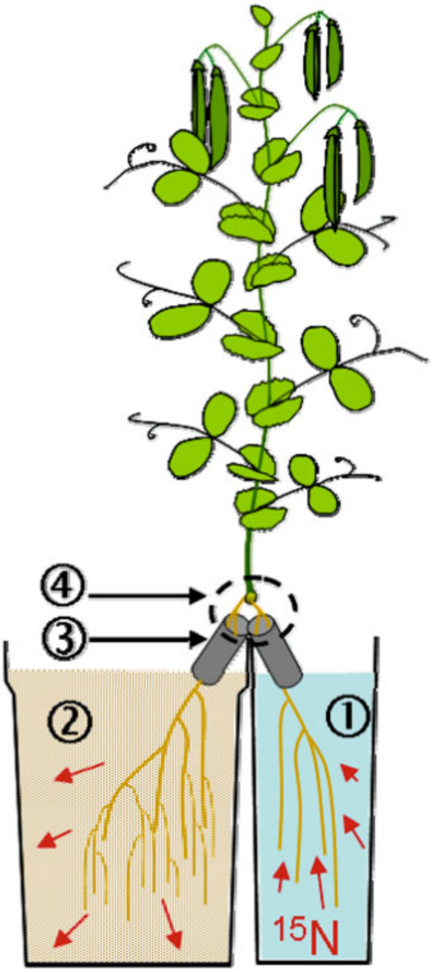

Fig. 2 Split-root method applied to a pea plant. Experimental design used by Mahieu et al. (2007). (1) Hydroponic labelling compartment filled with clay marbles and nutrient solution containing $^{15}NO_3$—$^{15}NH_4$. (2) Compartment filled with soil in which net N rhizodeposition was measured. (3) Rubber tubes protecting the upper part of the roots from desiccation. (4) Putty

(Fig. 1; Warembourg et al., 1982; McNeill et al., 1994; Russelle et al., 1994). However, this technique requires specific equipment and cannot be applied easily in the field. Furthermore, free-living N_2-fixing bacteria can use ^{15}N and complicate interpretation of results.

(iii) *The leaf-feeding technique* involves feeding ^{15}N as a gas (NH_3), or as a solution (urea, ammonium or nitrate; Fig. 1). Janzen and Bruinsma (1989) exposed shoots of wheat plants (*Triticum aestivum* L.) to $^{15}NH_3$ for a relatively short duration (6 h) periodically during the growing season. For this purpose, plants were temporarily placed in a sealed plexiglass enclosure, and the medium was sealed from the atmosphere. This method resulted in a uniform labelling of the above- and below-ground parts (though ^{15}N enrichment in the roots was lower than in the shoots), but has not been used with legumes. Only limited quantities of ^{15}N can be absorbed by the plant material because of short exposure time. Longer periods of exposure would require sophisticated and expensive equipment unsuitable for field measurements (Bazot et al., 2008).

Urea is a good ^{15}N carrier because it is non-polar, highly mobile and has a higher N content than nitrate and ammonium. Leaf-feeding (or leaf-flap) methods involve immersing a part of the foliage in a ^{15}N-labelled solution contained in a sealed vial for several hours. These have been found to be more accurate than spray applications of ^{15}N-labelled urea, because of the loss of $^{15}NH_3$ occurring after ^{15}N-urea hydrolysis and runoff from the labelled leaves to the soil in the case of spray applications (Russell and Fillery, 1996a; Hertenberger and Wanek, 2004). After a spray application of ^{15}N-urea, Zebarth et al. (1991) recovered less than 70% of the ^{15}N applied in the case of alfalfa and 30% in the case of red clover. Using the leaf-feeding technique, Ledgard et al. (1985) labelled pasture legumes by immersing a trifoliolate leaf into a glass vial sealed in a plastic bag and filled with 15 mL of a 10% ^{15}N KNO_3 solution (300 mM) for 72 h and measuring N transfers between neighbouring pasture plants. McNeill et al. (1997, 1998) adapted the leaf-feeding technique (Oghoghorie and Pate, 1972; Pate, 1973), to assess below-ground N of subterraneum clover and serradella (*Ornithopus compressus* L.). They conducted similar experiments in the field and in the greenhouse (McNeill et al., 1997, 1998). After cutting (1997) or not (1998) the 1–2 mm tip, a young expanded leaf was inserted into a 2-mL non-porous vial filled with 1 mL of a 0.25–0.4% (w/v) solution of ^{15}N-labelled urea (99.6 atom% ^{15}N). The system was sealed with inert plastic putty to avoid ^{15}N loss. To avoid leaf damage, the concentration of the urea solution must not be too high. In the field, mean total recovery of the fed ^{15}N in the entire plant-soil system at the late vegetative stage was 85% for subterranean clover and 76% for serradella, but was more than 92% in both species after feeding at maturity (Table 1). In the greenhouse, mean recovery of the fed ^{15}N was 42% in subterranean clover and 64% for serradella. In leaf-feeding methods, ^{15}N

Table 1 N rhizodeposited by various legume species as a percentage of the plant N. Values obtained using different labelling methods. (* injected into labelling compartment soil at the beginning of the experiment, ** continuous labelling in hydroponic compartment, *** injected every two days in vermiculite of labelling compartment, (fr) including fine roots)

Reference	Species	Culture conditions	Method	^{15}N recovery	Rhizodeposited N / plant-N
Zebarth et al. (1991)	*Trifolium pratensis* *Medicago sativa*	Field	Leaf spray	–	–
Sawatsky and Soper (1991)	*Pisum sativum*	Growth chamber	Split-root (^{15}NH$_4$)$_2$S0$_4$ *	–	8–12%
Jensen (1996a, b)	*Pisum sativum*	Growth chamber	Split-root KN0$_3$—^{15}N **	–	7%
Russell and Fillery (1996b)	*Lupinus angustifolius*	Field (mesotrons) under rain shelter	Cotton-wick (^{15}N-urea)	81–102%	18.5%
McNeill et al. (1997)	*Trifolium subterraneum*	Field (mesotrons)	Leaf feeding (^{15}N-urea)	85%	10% (fr)
	Ornithopus compressus			76%	20% (fr)
McNeill et al. (1998)	*Trifolium subterraneum*	Greenhouse (pots)	Leaf feeding (^{15}N-urea)	42%	40% (fr)
	Ornithopus compressus			64%	57% (fr)
Rochester et al. (1998)	*Vicia faba* ssp *minor*, *Glycine max*, *Lens culinaris*, *Lupinus angustifolius*, *Vigna radiata*, *V. angularis*, *V. unguiculata*, *Cajanus cajan*, *Arachis hypogaea*, *Lablab purpureus*, *Pisum sativum*	Field	Petiole feeding (^{15}N-urea)	–	–
Khan et al. (2002a, b)	*Vicia faba*	Greenhouse (pots)	Shoot feeding (^{15}N-urea)	90%	23.5%
	Cicer arietinum,			76%	43.9%
	Vigna radiata,			100%	16.5%
	Cajanus cajan			102%	35.5%
Chalk et al. (2002)	*Sesbania rostrata*	Greenhouse (pots)	Leaf feeding (^{15}N-urea)	35%	–
			Stem injection (^{15}N-urea)	45%	–
			Adventitious root feeding (^{15}N-urea)	101%	–
Mayer et al. (2003)	*Vicia faba*	Cover hall (pots)	Cotton-wick (^{15}N-urea)	84.8%	13%
	Pisum sativum			83.2%	12%
	Lupinus albus			84.5%	16%
Schmidtke (2005a, b)	*Pisum sativum*	Greenhouse	Split-root KN0$_3$—^{15}N ***	–	10.5%
	Lathyrus sativus				9.2%
Yasmin et al. (2006)	*Cicer arietinum*	Greenhouse (pots)	Leaf feeding		–
			Petiole feeding		–
			Cotton-wick		–
Mahieu et al. (2007)	*Pisum sativum*	Greenhouse (pots)	Cotton-wick (^{15}N-urea)	65–85%	9.7–11.7%
		Field		70%	34.2%
	Pisum sativum	Greenhouse (pots)	Split-root ^{15}NO$_3$—^{15}NH$_4$**	–	14.3–17.3%
		Field		–	27.5%
Gylfadóttir et al. (2007)	Mixture	Field (mesotrons)	Leaf feeding (^{15}N-urea)	–	
	Trifolium repens				47%
	Poa pratensis				10% (of total N for both species)
Wichern et al. (2007a)	*Pisum sativum*	Field (mesotrons)	Cotton-wick (^{15}N-urea)	59–77%	32–36%
McNeill and Fillery (2008)	*Lupinus angustifolius*	Field (mesotrons)	Cotton-wick (^{15}N-urea)	69–76%	35–65% (fr)

enrichment of above-ground parts is often higher than that of below-ground parts (McNeill et al., 1997, 1998; Yasmin et al., 2006). ^{15}N leaf-feeding techniques used both by Ledgard et al. (1985) and by McNeill et al. (1998) were also used to measure N compounds deposited in the soil by mixtures of common grassland species in the field and N transfer from legumes to the neighbouring non-fixing plant (Bardgett et al., 1999; Høgh-Jensen and Schjoerring, 2001; Ayres et al., 2007; Rasmussen et al., 2007).

The ^{15}N solution can be fed directly to a *leaf petiole*. Rochester et al. (1998) attached vials containing ^{15}N-urea to petioles of eleven different species of grain legume. Khan et al. (2002b) compared the use of leaf-feeding and petiole-feeding methods in the field with four different species. They concluded that ^{15}N-leaf-flap feeding was best for fababean, mungbean and pigeonpea, but petiole feeding was best for chickpea. The best compromise to enable comparison of results between species was to apply short pulses of labelled urea to the lower third or fourth stem-node using 0.2 mL of 0.5% urea (98 atom% ^{15}N) at each pulse. Leaf and petiole feeding led to higher ^{15}N enrichment of above- than below-ground parts in all tested species except in piegeonpea, where shoot enrichment was about 30% lower than root enrichment (Ledgard et al., 1985; Russell and Fillery, 1996a, b; McNeill et al., 1997, 1998; Khan et al., 2002a; Chalk et al., 2002). In leaf and petiole feeding, although the urea was highly enriched in ^{15}N, the ^{15}N enrichment of the roots was only between 0.11 and 0.90 atom% ^{15}N excess (McNeill et al., 1997; Høgh-Jensen and Schjoerring, 2001; Khan et al., 2002a, b).

(iv) The *cotton-wick technique* was proposed by Russell and Fillery (1996a). ^{15}N-labelling solution is provided to the plant by means of a cotton-wick passing through a hole in the plant stem (Fig. 1). These authors have shown that the transfer of solutions into young lupin plants is more effective using the cotton-wick method than the leaf-feeding method. N uptake by the cotton-wick technique is mainly driven by the transpiration stream, avoiding active mechanisms occurring with root or leaf immersion. Results reported by Russell and Fillery (1996b) and McNeill and Fillery (2008) confirm that this method seems accurate for assessing below-ground N of field-grown lupin and provides a more homogeneous ^{15}N distribution in the plants compared with leaf-feeding techniques (Mayer et al., 2003). It has also been confirmed for fababean, chickpea, mungpea (*Vigna radiata* (L.) R. Wilcz), pigeonpea, pea and white lupin (Russell and Fillery, 1996b; Mayer et al., 2003; Mahieu et al., 2007). Fortnightly pulses of high ^{15}N-urea (99 atom% ^{15}N), were found to be more efficient than a weekly application (Russell and Fillery, 1996a) and provide similar results to pulses applied at given growing stages (6-leaf stage, flowering and pod-filling; Mahieu et al., 2007). In Mayer et al. (2003) the amount of urea applied to pea plants at each pulse was calculated from dilution curves, to keep an average ^{15}N content of 2.5 atom% ^{15}N excess of the plant N during the growing demand. All experiments undertaken on pea showed that ^{15}N recovery was around 90% (84–94%) in the greenhouse and 50–76% in the field (Table 1; Mayer et al., 2003; Mahieu et al., 2007; Wichern et al., 2007a). Furthermore, the longer the experiment, the lower ^{15}N recovery in the plant-soil system (Russell and Fillery, 1996a; Mayer et al., 2003; Mahieu et al., 2007). In cotton-wick, as in leaf-flap and petiole feeding, above-ground parts are markedly more ^{15}N-enriched than roots. Root enrichment ranged between 1.1 and 1.4 atom% ^{15}N excess in Russell and Fillery (1996a), Mayer et al. (2003) and Wichern et al. (2007a) but reached up to 3.6 atom% ^{15}N excess in Mahieu et al. (2007). However, cotton-wick cannot be used with thin-stemmed species such as chickpea (Yasmin et al., 2006). Few attempts have been made to inject ^{15}N-urea directly into the stem with a syringe. Chalk et al. (2002) did not obtain reliable results with *S. rostrata*, probably because of its hollow stem.

(vi) *The split-root technique* was proposed by Sawatsky and Soper (1991) to quantify the amount of N lost from the root system of pea plants. Before the beginning of the experiments, seedlings of pea were raised in moist sand or vermiculite, and the radicle was cut 1 cm below the seed after seedling emergence to enhance the development of adventitious roots. Then the root system was split between two different soil compartments. One of them, filled with soil (Sawatsky and Soper, 1991), with clay marbles (Jensen, 1996a, b; Mahieu et al., 2007) or vermiculite (Schmidtke, 2005a, b) was labelled with a mineral ^{15}N-enriched source, and the other part of the root system growing in the unlabelled soil compartment could be monitored (Fig. 2). Sawatsky and Soper (1991) used a solution of $(^{15}NH_4)_2SO_4$ containing 66.7% ^{15}N: root ^{15}N enrichment was 9.92 atom% ^{15}N excess. Jensen (1996a), Schmidtke (2005a) and Mahieu et al. (2007)

used a 5% or 10% ^{15}N-enriched KNO_3-N, and 10% ^{15}N-enriched NO_3-NH_4, respectively; root enrichments comprised between 0.2 and 3.5% atom% ^{15}N excess.

This technique can also be used to assess N transfer between a legume and a non-fixing species (Jensen, 1996b). It allows continuous labelling during plant growth and N uptake follows a natural pathway. A disadvantage of the split-root method is that it substantially disturbs the root system and plant development, particularly for species with a taproot (McNeill et al., 1997; Khan et al., 2002a). In addition, roots of the labelling compartment tend to keep more than 50% of the applied ^{15}N (Schmidtke, 2005b; Mahieu et al., 2007), leading to lower enrichment in N of the other plant parts. Furthermore, estimation of N derived from rhizodeposition accounts for only a part of the root system. This technique is difficult to adapt to field conditions (Mahieu et al., 2007).

3.3 Amounts of N Rhizodeposited by Legumes

Among all reviewed studies, N derived from rhizodeposition as a percentage of the mature plant N varied from 7% to 57% (Table 1). Using leaf feeding with ^{15}N-urea, the ratio of rhizodeposited N: plant N differed markedly among species (from 10% in subterraneum clover to 57% in serradella); values obtained in subterraneum clover and serradella were markedly higher in the field than in the greenhouse (McNeill et al., 1997, 1998).

Several studies have investigated N rhizodeposition of mature pea crops using split-root or cotton-wick methods (Sawatsky and Soper, 1991; Jensen 1996a, b; Mayer et al., 2003; Schmidtke 2005a, b; Mahieu et al., 2007; Wichern et al., 2007a). Harvesting at different stages indicates that N rhizodeposition increases as plants mature, probably because of the increase in senescing roots and nodules (see Wichern et al., 2008). However, Wichern et al. (2007b) measured high levels of rhizodeposition at early vegetative stages of growth (71% of the plant N at the 3–6 leaf stage). For a pea plant, the ratio of rhizodeposited N: plant N was 4 to 71% and the ratio of the below-ground N: plant N varied from 14 to 74%. At maturity, in greenhouse conditions, rhizodeposited N and below-ground N often represented around 15% and 25% of plant N, respectively (Mahieu et al., 2007; see Wichern et al., 2008). In the field, below-ground N represented around 30% of plant N and rhizodeposited N often accounted for 88–97% of below-ground N. Mahieu et al. (2007) showed that the ratio of rhizodeposited N: plant N obtained with split-root was 10% higher than that obtained with cotton-wick. Furthermore, the values were higher in the field than in the greenhouse experiments, though the root-to-shoot ratios were markedly lower in the field than in the greenhouse. Consistently with other studies, roots represented less than 5% of the total plant weight in the field (Voisin et al., 2002), since they represented at least 10–20% of the plant weight in the greenhouse pots. In their greenhouse study, Mahieu et al. (2007) found a significant relationship between the amount of N rhizodeposited by a pea plant and the plant-N content that could contribute to explain this difference, since plant-N contents of field peas were higher than those of greenhouse plants.

4 N Rhizodeposition Pathways

4.1 General Considerations

The term rhizodeposition was first used to describe carbon loss from roots (Lynch and Wipps, 1990) but also includes N loss, as most organic compounds lost by roots also contain N (but see Wichern et al., 2008). Less N than C is rhizodeposited, but deposition of both elements cannot be distinguished (Bais et al., 2006) as in both cases, the potential pathways for rhizodeposition are (1) senescence, death and decay of roots and nodules; (2) exudation of soluble compounds; (3) sloughing-off of root border cells, and (4) secretion of mucilage. Quantitative data providing reliable estimation of these pathways are sparse but a recent review concerning carbon rhizodeposition showed that sloughing-off of border cells and secretion of mucilage represent a very small proportion of carbon rhizodeposition (N'guyen, 2003). This proportion must be even smaller for N rhizodeposition, as little N is present in mucilages.

4.2 Senescence of Roots and Nodules

Several studies have demonstrated that death of nodules and roots is a major source of biological fixation of N for the soil (Dubach and Russelle, 1994; Russelle et al., 1994). Its importance is undisputed but reliable quantitative data are sparse, as no methodology is available to clearly distinguish rhizodeposition due to death and decay of below-ground tissues from rhizodeposition due to exudation of soluble compounds. By comparing the accumulation of biologically fixed $^{15}N_2$ in fine roots and nodules of alfalfa and birdsfoot trefoil and with soil surrounding the roots, Dubach and Russelle (1994) have estimated that decomposition of these tissues is the main pathway for N rhizodeposition. Though little quantitative data are available concerning fixed nitrogen in legume rhizospheres, quantification of underground N transfer from legumes to intercropped grasses is now well documented. Because transfer increases with plant age, it is often proposed that N release from senescence of belowground residues of legumes coupled with grass uptake is the dominant factor in N exchange (Høgh-Jensen and Schjoerring, 1997; Moyer-Henry et al., 2006). Release of N through degradation of above-ground tissues is highly dependent on numerous factors such as mycorrhizal fungi, bacteria, root herbivory or defoliation (Ta and Faris, 1988; Johansen and Jensen, 1996; Ayres et al., 2007). Numerous studies have established that N transfer between plants can also occur between young plants, through mycorrhizal networks interconnecting plants or through exudation of N compounds by legumes coupled with uptake by grasses (Paynel et al., 2001; Moyer-Henry et al., 2006).

4.3 Exudation of Soluble Compounds

The N released from roots and nodules as low-molecular-weight substances, such as soluble root exudates, amino acids, hormones and enzymes, is also poorly quantified in soil conditions. Most of the experiments on N exudation have been carried out in the laboratory, in hydroponically grown plants or in sand cultures. Between 3% (Ta et al., 1986) and 4.5% of the fixed N is released by alfalfa to the solution as soluble compounds, while between 10 (Brophy and Heichel, 1989) and 30% (Ofosu-Budu, 1990) of fixed N is released to the nutrient solution. *Robinia pseudoaccacia* L., which is an N_2-fixing tree, has also been observed to release a significant, but minor, proportion of fixed N to the solution, as dissolved organic nitrogen (Uselman et al., 1999).

The main N compound released is generally ammonium, which is the main product of the nitrogenase enzyme, but significant proportions of ureides and amino acids are also recovered in root exudates of alfalfa, soybean and clover (Ta et al., 1986; Brophy and Heichel, 1989; Paynel and Cliquet, l2003). Among amino acids found in root exudates of various species including white clover and alfalfa, glycine and serine have often been recovered in high proportions (Svenningsson et al., 1990; Paynel et al., 2001; Hertenberger and Wanek, 2004; Lesuffleur et al., 2007) despite also constituting a major amino acid in rhizospheric soils (Kielland, 1995; Jones et al., 2005). The reverse is true for other amino acids such as asparagine and glutamine, which are recovered in low proportions in exudates but in high proportions in root extracts, showing that amino acid root exudation is a selective process. Ammonium and amino acids are also recovered in root exudates of non-fixing plants (Paynel and Cliquet, l2003), but use of ^{15}N-labelled amino acids has shown that efflux of glycine and serine from roots of legumes is higher than from roots of grasses (Lesuffleur et al., 2007). Like the other components of rhizodeposition, root exudation is altered by numerous biotic factors, such as mycorrhizal fungi and root herbivores (Murray et al., 1996; Bais et al., 2006) and abiotic factors, such as defoliation and CO_2 enrichment (Ayres et al., 2007; Bazot et al., 2008).

5 Conclusion

In conclusion, biological fixation of N can act as a sustainable source of N and can complement or replace fertiliser inputs. This review highlights that numerous agricultural practices have been developed all around the world to take advantage of the biological reduction of atmospheric N to ammonia realised by some prokariots. N fixation is performed by these prokariots alone or in symbiosis with plants. Legumes form a symbiosis with *Rhizobium* but release a substantial part of the biologically fixed N into the rhizosphere. As a consequence, biological N fixation can act as a sustainable source of N and contribute to decreasing fertiliser inputs. However, the part of this N available for non-fixing crops remains difficult to assess. N rhizodeposition is mainly due to senescence and decay of roots and nodules, and exudation of N compounds by living roots. The main N compounds released by legume roots are ammonium, amino acids and ureides, but a wide range of organic compounds released by plant roots remain to be determined. A significant effort has been made in the last decade to develop tracer methods suitable for quantifying N rhizodeposition in realistic conditions. Long-term studies using the split-root and the cotton-wick techniques have shown that N rhizodeposition increases with plant age and plant N content, but more information is lacking concerning the effects of plant-N partitioning and of root characteristics. Ecological functions of these rhizodeposits are still unknown, but they may constitute a rapidly incorporating source of C and N for soil microorganisms and neighbouring plants. Further investigations combining assessments of C and N rhizodeposition are needed to obtain a better understanding of these fluxes in the rhizosphere of legumes.

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Models of Biological Nitrogen Fixation of Legumes

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Contents

Abstract Leguminous crops have the ability to fix nitrogen (N) biologically from the atmosphere. This can benefit not only the legumes themselves but also any intercropped or subsequent crops, thus reducing or removing the need to apply N fertilizers. Improved quantification of legume biological nitrogen fixation (BNF) will provide better guidance for farmers on managing N to optimise productivity and reduce harmful losses to the environment. There are many techniques available for the direct quantitative measurement of legume BNF in the field and in controlled environments. However, these are time-consuming and therefore expensive, and generate data relevant only to the time and place of measurement. Alternatively, legume BNF can be estimated by either empirical models or dynamic mechanistic simulation models. Comparatively, simulation by a dynamic model is preferable for quantifying legume BNF, because of its capability to simulate the response of N fixation to a wide range of environmental variables and legume growth status. Currently there is no published review of the approaches used to simulate, rather than measure, legume BNF. This review of peer-reviewed literature shows that most simulation models estimate the N fixation rate from a pre-defined potential N fixation rate, adjusted by the response functions of soil temperature, soil/plant water status, soil/plant N concentration, plant carbon (C) supply and crop growth stage. Here, we highlight and compare the methods used to estimate the potential N fixation rate, and the response functions to simulate legume BNF, in nine widely-cited models over the last 30 years. We then assess their relative strengths in simulating legume BNF with varying biotic and abiotic factors, and identify the discrepancies between experimental findings and

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_39,

from Liu et al., Agron. Sustain. Dev. (2010). In press. DOI: 10.1051/agro:2010008

simulations. After this comparison, we identify the areas where there is the potential to improve legume BNF simulation in the future. These include; (1) consideration of photosynthetic C supply, (2) refining the various effects of soil mineral N concentration, (3) characterization and incorporation of excess soil water stress and other factors into models, and (4) incorporation of the effects of grazing, coexistence and competition with intercrops and weeds into models to improve their practical relevance to sustainable agricultural systems. This review clarifies, for the first time, the current progress in legume BNF quantification in simulation models, and provides guidance for their further development, combining fundamental experimental and modelling work.

Keywords Nitrogen fixation • Soil mineral nitrogen• Legume • Simulation • Review

1 Introduction

Increasing demand for agricultural production typically results in greater fertilizer application (Whitehead, 1995; Wood, 1996). Nitrogen (N) applied in fertilizers or manures which is not taken up by crops can be released into the atmosphere as nitrogenous greenhouse gases (Flechard et al., 2007) or leached into ground water (Stout et al., 2000; Trindade et al., 2001), with resulting environmental implications. Rather than relying purely on applications of N fertilizer, alternative N sources are needed to help develop more sustainable farming systems. Legumes have the potential to fulfil this requirement due to their unique ability to fix N biologically from the atmosphere, benefiting not only the legumes themselves but also the intercropped or subsequent crops.

The ability to fix atmospheric N comes from the symbiotic relationship between legumes and rhizobia, bacteria in soil, through which the legume supplies energy and carbon (C) to rhizobia through the products of photosynthesis, and rhizobia provides the legume with N, mainly in the form of ammonium (Howard and Rees, 1996). The symbiosis initiates from the infection of legume roots by *Rhizobium*, forming root nodules where N fixation occurs. The N fixation process needs the nitrogenase enzyme, as a catalyst for the reaction to split the nitrogen molecule and change it into ammonia with energy and electron supply (Postgate, 1982). Garg and Geetanjali (2007) reviewed the processes and signalling involved in symbiotic N fixation in legume nodules at a micro scale.

This kind of symbiosis exists in many types of legumes, including grain legumes, forage legumes, and some leguminous trees. Grain legumes and forage legumes are used to build up soil N fertility in sustainable farming systems (Hossain et al., 1996; Jensen, 1997; McCallum et al., 1999), especially in temperate zones. The benefit to soil N fertility from grain/forage legumes is positively correlated with N fixation (Evans et al. 1989, 2001) assuming that the plant residues are incorporated into soil after crop harvest. Therefore, quantifying legume biological N fixation (BNF) is very important for a better understanding of how to design sustainable farming systems, where subsequent crops benefit from legume N fixation, and where N losses and thus environmental pollution may be reduced.

There are many techniques available for measuring legume BNF in the field and in controlled environments (Goh et al., 1978; Sheehy et al., 1991; Herridge et al., 2008; Carlsson and Huss-Danell, 2008). Most of these methods involve destructive sampling of plants or disturb the original soil structure, except for the acetylene (C_2H_2) reduction assay (Carlsson and Huss-Danell, 2008). However, the C_2H_2 reduction assay can only be used over a short time period (minutes or hours), and legume N fixation rate varies seasonally with growth stage of the plant, so the time of measurement affects precision and reliability (Carlsson and Huss-Danell, 2008). These methods are able to reveal the response of N fixation to varying factors in real situations, but they are limited to the conditions prevailing at the time of measurement and cannot be used to predict N fixation.

Thus, there is a need for methods to predict legume BNF quantitatively to help make decisions about developing and managing sustainable farming schemes. Estimating N fixation from crop yield or empirical models based on historical crop data can be used, but these are restricted to a particular site for the same crop. Modelling is potentially a better tool to understand and quantify legume BNF as it is based on knowledge of the mechanisms involved, climate and management, and can accurately represent variations in legume BNF under different situations. However, models require very detailed information on the environment and plant genetic performance, in addition to historical crop datasets for calibration and validation. This means that models can not be applied to a given site unless this data is available.

A further complication is that the mathematical functions used to simulate legume BNF vary between models. For example, the rate of potential N fixation is estimated either by plant N demand and uptake (Bouniols et al., 1991; Cabelguenne et al., 1999), nodule biomass (Boote et al., 2002; Wu and McGechan, 1999), root biomass (Thornley et al., 1995; Soussana et al., 2002) or aboveground biomass (Sinclair, 1986; Robertson et al., 2002). Also the response of N fixation to soil mineral N concentration in the root zone, which is closely correlated to N fixation and thus to the benefit to soil N fertility from legumes (Evans et al. 1987, 1989), differs between models. N fixation is either very sensitive (Wu and McGechan, 1999; Schwinning and Parsons, 1996) or tolerant (Sharpley and Williams, 1990; Bouniols et al., 1991) to high soil N concentration in different models.

In this paper we explore and compare the modelling methods published for quantifying legume BNF by grain and forage legumes at the plot/field scale. More specifically, we contrast the different schemes and functions used to simulate the response of N fixation to biotic and abiotic factors and analyse their relative strengths and weaknesses. We then identify gaps in the current models and make suggestions to improve the simulation of legume BNF in future models.

2 Factors Influencing Legume Biological Nitrogen Fixation

The complex process of legume BNF is affected by environmental conditions such as temperature, water content, N concentration, root zone pH, plant nutrient status including C and N substrates in roots, and genetic variation in potential N fixation capacity. It is also affected by plant nutritional status such as phosphorus (P) and potassium (K) levels that control nodule growth and nitrogenase activity directly or indirectly (Havelka et al., 1982). In this section we focus only on how legume BNF responds to environmental conditions and plant C and N substrates with a view to improving models.

2.1 Temperature

Generally, soil temperature inhibits legume BNF through its control on nodulation, nodule establishment, and nitrogenase activity when it is either too high or too low (Roughley and Dart, 1970; Roughley, 1970; Whitehead, 1995). Therefore, minimum and maximum soil temperatures, and the range of temperature between these which are favourable for N fixation, could be used to define the response of N fixation to soil temperature.

Soil temperature in the root zone is one of controlling factors for nodulation and nodule establishment. For example, the nodulation of arrowleaf clover (*Trifolium vesiculosum* Savi.) is accelerated at a root temperature of 25°C compared with that growing at both 18°C and 32°C (Schomberg and Weaver, 1992). However, the response of nodule establishment to soil temperature differs between species and varieties. In soybean (*Glycine max* (L.) Merr.), more nodules are produced in the early growth stage at 25°C, while 20°C is optimal for nodule size after nodule generation is completed compared to 15°C and 30°C (Lindemann and Ham, 1979). In contrast, nodule establishment is enhanced with increasing temperature in the range of 10–35°C for white clover (*Trifolium repens* L.) regardless of the varieties and the rhizobia strains (Richardson and Syers, 1985; Whitehead, 1995).

Nitrogenase activity responds slightly differently to soil temperature between species. There are a large number of studies on the response of N fixation to temperature in legume crops, some of which are summarized in Table 1. Minimum temperatures for N fixation differ among species from 2°C to 10°C, and normally tropical and subtropical legumes have higher minimum temperatures than temperate species. Nitrogenase activity is high around 12–35°C and reaches maximum at 20–25°C in most legumes. N fixation in subterranean clover (*Trifolium subterraneum* L.) is very active at a wide range of temperatures, from 5°C to 30°C, but declines dramatically with low temperature and almost stops at 2°C. Generally, 35°C or 40°C is the upper limit of temperature for legume BNF.

Table 1 Response of N fixation to soil temperature (°C) for legume species

Species	Minimum	Optimum range	Maximum	Reference
Alfalfa (*Medicago sativa* L.)	2	20–25		Waughman (1977)
	2	35	40	Dart and Day (1971)
Arrowleaf clover (*Trifolium vesiculosum*)		25		Schomberg and Weaver (1992)
Barrel medic (*Medicago truncatula* Gaertn.)	2	20	35	Dart and Day (1971)
Big-leaved lupin (*Lupinus polyphyllus* Lindl.)	1.5	25		Waughman (1977)
Birdsfoot trefoil (*Lotus corniculatus* L.)	10	25–27	35	Rao (1977)
Common bean (*Phaseolus vulgaris* L.)			35	Piha and Munns (1987)
			40	Michiels et al. (1994)
Common vetch (*Vicia sativa* L.)	2	20	40	Dart and Day (1971)
Cowpea (*Vigna unguiculata* (L.) Walp)	5	40		Dart and Day (1971)
Faba bean (*Vicia faba* L.)	2	20–30	40	Dart and Day (1971)
	5	20		Waughman (1977)
Field/garden pea (*Pisum sativum* L.)	5	15–25		Halliday (1975)
	0.5	25		Waughman (1977)
		20–26		Lie (1971)
Guar (*Cyamopsis tetragonoloba* Guar)			37–40	Pate (1961)
Narrowleaf lupin (*Lupinus angustifolius* L.)	10	20–30		Halliday (1975)
Peanut (*Arachis hypogaea* L.)			40	Kishinevsky et al. (1992)
Purple vetch (*Vicia atropurpurea* Desf.)		24		Pate (1961)
Red clover (*Trifolium pratense* L.)	2	25	35	Dart and Day (1971)
		12–26		Small and Joffe (1968)
Soybean (*Glycine max* L.)		27		Kuo and Boersma (1971)
		20–25		Lindemann and Ham (1979)
	5	20–35	40	Dart and Day (1971)
Subterranean clover (*Trifolium subterraneum* L.)	2	5–30		Dart and Day (1971)
	5	12–32		Gibson (1971)
		15		Roughley and Dart (1969)
White clover (*Trifolium repens* L.)	5	13–26		Halliday and Pate (1976)
		26		Small and Joffe (1968)
		21		Masterson and Murphy (1976)
	9			Frame and Newbould (1986)

2.2 Soil Water Status

In a similar manner to soil temperature, soil water content in the root zone controls N fixation through nodule establishment and nodule activity, plus gas permeability (Sprent, 1976; Weisz et al., 1985; Weisz and Sinclair, 1987; Sinclair et al., 1987). Soil water deficit inhibits N fixation (Whitehead, 1995; Goh and Bruce, 2005), and the inhibition is reinforced as drought stress becomes more intense (Albrecht et al., 1984). N fixation by peanut (*Arachis hypogaea* L.) grown in soil at two-thirds of field water capacity is reduced by 18–40% compared to that in well-watered soil, and by 44–69% when the soil water is one-third of field capacity (Pimratch et al., 2008). In addition, waterlogging can seriously reduce N fixation through depression of the establishment and activity of nodules (Havelka et al., 1982).

It is not easy to quantify the relationship between N fixation and soil water status precisely, due to the limitations of experimental controls, stress periods and plant recovery (Engin and Sprent, 1973; Ledgard and Steele, 1992). Pimratch et al. (2008) tried to determine N fixation under drought stress with multiple-linear regression but the correlation coefficients between the reduction of N fixation under drought compared to under field capacity and the soil water deficit was not statistically significant. A sigmoid function has been used to describe the response of N fixation by common bean (*Phaseolus vulgaris* L.), black gram (*Vigna mungo* (L.)

Hepper) and cowpea (*Vigna unguiculata* (L.) Walp) to soil water stress, showing a sharper decline in N fixation as soil water stress becomes more severe (Serraj and Sinclair, 1998; Sinclair et al., 1987).

2.3 N Concentration in the Root Zone

It has been widely reported that soil mineral N in the root zone inhibits legume nodulation (Abdel Wahab et al., 1996; Herridge et al., 1984), nodule establishment (Imsande, 1986) and nitrogenase activity (Purcell and Sinclair, 1990; Eaglesham, 1989) as it costs less energy for legumes to take up N from soil than fix N biologically from the atmosphere (Cannell and Thornley, 2000; Phillips, 1980; Ryle et al., 1979; Wood, 1996).

Normally, the severity of inhibition of N fixation by soil mineral N increases with soil mineral N content (Macduff et al., 1996; Lamb et al., 1995; Waterer and Vessey, 1993a; Chalifour and Nelson, 1987). It has been reported that a certain concentration of mineral N in the root zone, defined as "starter N", stimulates nodule establishment and N fixation compared to non-mineral N in some circumstances. And the concentrations of "starter N" that stimulate legume BNF vary widely with cultivar and growth conditions but are normally less than 4mM for ammonium (NH_4^+) and less than 2 mM for nitrate (NO_3^-) (Bethlenfalvay et al., 1978; Schomberg and Weaver, 1992; Gulden and Vessey, 1997; Gan et al., 2004). However, the time of external N application in relation to legume growth stage affects nodule growth and N fixation, and the later N is applied, the less nodule growth and N fixation is inhibited in field pea (*Pisum sativum* L.) (Jensen, 1986; Waterer and Vessey, 1993b). This is probably due to nodules being well-established in the early stages before N is applied. Moreover, the inhibition of N fixation by NO_3^- was more severe than that of NH_4^+ in white clover, field pea and soybean (Svenning et al., 1996; Bollman and Vessey, 2006; Gan et al., 2004) even though high amounts of either NH_4^+ or NO_3^- retarded N fixation.

2.4 Carbon Demand for Fixation

Photosynthate partitioned to roots supports nodule growth, provides energy for N fixation, maintains a functional population of rhizobia, and allows the synthesis of amino compounds produced from N fixation (Minchin and Pate, 1973; Layzell et al., 1979; King et al., 1986). Even though it is difficult to distinguish the proportion of CO_2 generated by N fixation from that generated by respiration for nodule growth and maintenance (Warembourg and Roumet, 1989), the correlation between the rate of CO_2 produced from either nodulated roots or nodules and N fixation rate may be used to evaluate C consumption by N fixation (Lawrie and Wheeler, 1973; Mahon, 1977a, b; Warembourg and Roumet, 1989).

The C cost per unit fixed N (gC per g N fixed) varies widely with species, growth stage and environmental conditions, and ranges from 1.4 to 8.5 (Minchin and Pate, 1973; Phillips, 1980; Minchin et al., 1981; Sheehy, 1987; Schulze et al., 1999). For example, it is reported that the C cost is 1.54 gC g N fixed^{-1} in cowpea and 3.64 gC g N fixed^{-1} in white lupin (*Lupinus albus* L.) from nodules during early vegetative stage (Layzell et al., 1979; Layzell et al., 1981) while it is 6.3–6.8 gC g N fixed^{-1}for soybean, cowpea and white clover from nodulated roots at periods of intense N fixation (Ryle et al., 1979). The C cost determined by CO_2 released from nodulated roots is generally higher than that from nodules as the former includes root respiration. The C cost of N fixation also varies with growth stages (Ryle et al., 1979; Twary and Heichel, 1991), but it is a matter of debate that the C cost increases (Warembourg and Roumet, 1989; Voisin et al., 2003) or decreases (Adgo and Schulze, 2002) with the course of legume life cycle. In addition, the strain of *Rhizobium* may affect the C cost significantly. For example, the C cost in alfalfa (*Medicago sativa* L.) nodules formed by strain P207 is an average 9.4 molC molN^{-1} which is 59% higher than that of nodules formed by strain I02F51, 5.9 molC molN^{-1} (Twary and Heichel, 1991).

2.5 Seasonal Regulation of Legume Biological Nitrogen Fixation

The rate of legume BNF changes with physiological growth stages. It is low in the early growth stages while nodules are establishing (Lawrie and Wheeler, 1973) and reaches a maximum value between early flowering and early seed-filling, depending on the species and growing conditions (Lawn and Brun, 1974; Klucas, 1974; Nelson et al., 1984; Jensen, 1987). After the peak, N fixation decreases dramatically or even ceases during seed-filling (Herridge and Pate, 1977; Beverly and Jarrell, 1984; Sinclair et al., 1987), due to nodule senescence (Lawrie and Wheeler, 1973) and poor C supply as a result of the strong demand for seed dry matter accumulation (Herridge and Pate, 1977; Voisin et al., 2003, 2007).

3 Quantification of Legume Biological Nitrogen Fixation

Legume BNF may be quantified by direct measurement, estimation based on yield or with empirical models, or simulation by crop models. The methods used to measure N fixation directly so far, such as the acetylene reduction/hydrogen increment assay, N difference, ^{15}N-labelling and ureide, have been thoroughly reviewed (Herridge et al., 2008; Carlsson and Huss-Danell, 2008). The review of these methods is beyond our focus, and in this paper we describe and compare only the methods to estimate and simulate N fixation.

3.1 Estimation With Empirical Models

A static estimation of N fixation during the whole growing season may use either economic yield or above-ground dry matter. The equation is:

$$N_{fix} = \alpha \cdot DM \cdot f_{leg} \cdot N_{con} \cdot \%Ndfa \cdot (1 + R_{root}) \qquad (1)$$

where DM is the yield or aboveground dry matter, f_{leg} is the proportion of legume if it is intercropped, N_{con} is the N concentration in the legume, $\%Ndfa$ is the proportion of total plant N derived from N_2 fixation, and R_{root} is the ratio of the fixed N belowground to the fixed N aboveground. Values for the parameters f_{leg}, N_{con} and R_{root} for different species at a range of sites are summarized in Table 2. α is a parameter that has different definitions according to author. For example, α may represent the decline in $\%Ndfa$ under high soil mineral N conditions, in terms of net mineral N input (g N m^{-2}), and is calculated as:

$$\alpha = 1 - \beta \cdot N_{net.inorg} \qquad (2)$$

where β evaluates the sensitivity of legume BNF to soil mineral N (Korsaeth and Eltun, 2000). This is set to 0.028 for white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), 0.043 for grey peas (*Pisum arvense* L.) and common vetch (*Vicia sativa* L.). Alternatively, α is used as a modifier to compensate for the proportion of fixed N transferred to a companion crop, consumed by grazing animals, or lost by immobilization (Høgh-Jensen et al., 2004).

The second method to estimate N fixation is by empirical models based on the correlation of fixed N in the final yield against variables, such as harvested dry matter or the proportion of legume in mixed leys. A linear equation has been fitted to the measured data of mixed white clover and grass swards at different sites from four countries. This showed a significant correlation between fixed N and the extra dry matter of mixed leys, compared with corresponding pure grass either for cut swards or grazed swards (Watson and Goss, 1997). Kristensen et al. (1995) found fixed N at harvest increased linearly with clover dry matter content in mixed swards through statistically analyzing the experimental data from different sites with distinct soil types and irrigation schemes. The equation for the estimation of N fixation (kg N ha^{-1}) is summarized as,

$$N_{fix} = c + d \cdot Leg \qquad (3)$$

where Leg is the extra dry matter increment (kg ha^{-1}) in white clover mixed leys compared with a pure grass ley in Watson's estimation, and clover dry matter content (%clover) in clover mixed leys for Kristensen's study (1995), respectively; and c and d are parameters the values of which are summarized in Table 3.

The first method described above is apparently a direct estimation of N fixation, and the parameter values can be easily measured on site or estimated from the literature. It does not strictly require an adequate dataset for multiple years to determine the parameters, so it is easy to use. However, when determining the parameter values, data from years of abnormal weather should be avoided, and the properties of the soil should be relatively stable year-on-year. As these equations are independent from environmental factors such as soil properties and weather conditions, they are only applicable and accurate for similar sites and average weather conditions. In addition, the parameter values need to be adjusted if the equations are used for different sites or legumes.

Table 2 Parameter values used to estimate N fixation in equation (1)

Species	Condition	N_{con} (%)	%$Ndfa$ (%)	R_{root}	Reference
White clover	Stock camp	4.9	61	–	Ledgard et al. (1987)
White clover	Gentle slope	4.9	82	–	
Subterranean clover	Gentle slope	4.9	82	–	
Subterranean clover	Steep slope	4.9	82	–	
Slender birds-foot trefoil	Steep slope	4.9	82	–	
Lucerne	Cut 1–2 years	2.7	74	0.25	Høgh-Jensen et al. (2004)
Red clover	Cut 1–2 years	3.0	74	0.25	
White clover	Intercropping with grass, cut 1–2 years	4.3	95	0.25	
White clover	Intercropping with grass, grazed 1–2 years	4.3	80	0.25	
Read clover	Intercropping with grass, cut 1–2 years	3.0	95	0.25	
Red clover	Intercropping with grass, grazed 1–2 years	3.0	80	0.25	
White clover	Intercropping with grass, cut >2 years	4.3	95	0.25	
White clover	Intercropping with grass, grazed >2 years	4.3	75	0.25	
White clover	1st cut	3.94	92.3	For all cuts, 0.27 (year 1 and 2), 0 (year 3)	Korsaeth and Eltun (2000)
	2nd cut	3.49	92.3		
Red clover	1st cut	3.14	92.3		
	2nd cut	2.91	92.3		
Grey peas		2.80	65.4	0.045	
Common vetch		3.00	65.4	0.27	

Table 3 Values of parameters, c and d, in linear empirical models expressed in equation (3)

Condition	c	d	Model reference
Cut swards	6.8	0.067	Watson and Goss (1997)
Grazed swards	–168.1	0.067	
1–2 cropping years	18	4.47	Kristensen et al. (1995)
3–5 cropping years	19	2.77	

In contrast, the second method is based on statistical correlation and assumes that N fixation has a strong linear relationship to the variables. It is more flexible to use and can be applied to one specific site or multiple sites with different soil types, depending on how the empirical relationship is developed and which sites the data were obtained from. This approach has a higher data requirement compared to the first method, and the data should be representative and adequate to guarantee the correlation and determine the parameter values. However, as with the first method, these approaches are restricted to specific sites because the equation is not able to represent the interaction between plant and environment mechanistically.

3.2 Mechanistic Simulation of Legume Biological Nitrogen Fixation as a Subroutine in Crop Models

The simulation of legume BNF in soybean developed by Duffy et al. (1975), probing into the biophysiochemical transformations of N in tile-drained soil, might be the earliest mechanistic model involving leguminous N fixation. The rate of N fixation by soybean in the model depends on the root growth rate, which is derived from the sigmoid relationship with the number of days after planting.

Table 4 Simulation models that include legume BNF, and the factors considered in each model.* indicates model named here after the first author's name. f_T, f_W, f_N, f_C and f_{gro} are the factor of soil temperature, soil/plant water, soil/plant nitrogen, plant carbon and plant growth stage, respectively, used in equations (4, 5)

	Factors						
Model	f_T	f_W	f_N	f_C	f_{gro}	Simulated legume specie	Reference
Sinclair Model*		✓				soybean	Sinclair (1986)
		✓			✓	soybean, cowpea, black gram	Sinclair et al. (1987)
EPIC		✓	✓		✓	soybean	Sharpley and Williams (1990); Bouniols et al. (1991); Cabelguenne et al. (1999)
Hurley Pasture Model	✓	✓	✓	✓		white clover	Thornley (1998); Thornley and Cannell (2000); Thornley (2001)
			✓	✓		field pea	Eckersten et al. (2006)
Schwinning Model*			✓			white clover	Schwinning and Parsons (1996); Schmid et al. (2001)
CROPGRO	✓	✓		✓	✓	soybean, peanut, drybean, velvet bean, faba bean, cowpea	Boote et al. (1998); Sau et al. (1999); Hartkamp et al. (2002); Boote et al. (2002, 2008)
SOILN	✓	✓	✓			white clover	Wu and McGechan (1999)
APSIM		✓	✓		✓	soybean, chickpea, peanut, mungbean, lucerne	Herridge et al. (2001); Robertson et al. (2002)
Soussana Model*			✓			white clover	Soussana et al. (2002)
STICS	✓	✓	✓		✓	field pea and other legumes	Brisson et al. (2009); Corre-Hellou et al. (2007, 2009)

In more recent simulation models of BNF in legumes, the most popular method to estimate the rate of legume BNF is a potential or maximum fixation rate modified by the influence of environmental factors. The potential fixation rate is estimated based on either a demand-uptake mechanism or on the dry matter of plant tissues, and is varied with plant growth stages. The environmental factors normally include soil temperature, soil or plant water content, soil mineral N or substrate N concentration in plant tissues and substrate C concentration in the plant. Other factors, such as soil pH, salinity and the supply of other nutrients, have not been included in models to date.

In this paper we review the most-used recent simulation models in which a legume N fixation function has been implemented (Table 4). As crop models may be used under different circumstances, the estimation of N fixation may have various versions within the same model. The general expression of the calculation in the majority of the reviewed models can be written as:

$$N_{fix} = N_{fixpot}\ f_T\ f_W\ f_N\ f_C\ f_{gro} \tag{4}$$

while in the EPIC and STICS models it is:

$$N_{fix} = N_{fixpot}\ f_T\ \min(f_W, f_N)\ f_{gro} \tag{5}$$

where, N_{fixpot} is the potential N fixation rate (g N fixed day^{-1}), f_T is the influence function of soil temperature, f_W is a soil water deficit or flooding function, f_N is the function of soil mineral N or root substrate N concentration, f_C is the function of substrate C concentration in plant or root, f_{gro} is the influence factor of growth stage and min is the mathematical function to take a minimum value between f_W and f_N. There is an extra function, f_a, representing the limitation by anoxia in the STICS model.

3.2.1 Potential N Fixation Rate

There are two definitions on a potential legume BNF rate used in the models based either on the difference between N demand and uptake by a legume, or on the N fixation capacity of legume nodules.

The EPIC and APSIM models use variations of the first definition to estimate potential N fixation rate. The EPIC model assumes that the total plant N demand is equal to the potential N fixation (Bouniols et al., 1991; Cabelguenne et al., 1999). APSIM defines critical N concentrations for plant tissues and uses these to estimate N demand by maintaining non-stressed N levels in plant tissues and supporting the N demand of new tissues. This N demand is met by either N uptake from soil and/or N fixation. The former has a higher priority because the process is less energy consuming than N fixation (Macduff et al., 1996); N fixation is only calculated if N uptake can not meet the plant N demand. Thus the potential N fixation is assumed to be the difference between plant N demand and N uptake (Herridge et al., 2001; Robertson et al., 2002).

The second definition is based on the strong relationship between N fixation and either nodule size/biomass (Weisz et al., 1985; Voisin et al., 2003) or root biomass (Voisin et al., 2007). As the biomass of both nodules and roots are difficult to measure in the field, some studies have used above-ground biomass to replace nodule/root biomass, based on the relationship between these two variables (Denison et al., 1985; Bell et al., 1994; Yu et al., 2002). The potential fixation rates used in the models are shown in Table 5.

3.2.2 Impact of Soil Temperature

The impact of soil temperature on N fixation rate is assumed to follow a four-threshold-temperature function in most of the reviewed models:

$$f_T = \begin{cases} 0 & (T < T_{min} \; or \; T > T_{max}) \\ \frac{T - T_{min}}{T_{optL} - T_{min}} & (T_{min} \leqslant T \leqslant T_{optL}) \\ 1 & (T_{optL} \leqslant T \leqslant T_{optH}) \\ \frac{T_{max} - T}{T_{max} - T_{optH}} & (T_{optH} < T < T_{max}) \end{cases} \tag{6}$$

where T is soil temperature (°C), T_{min} is the minimum temperature below which N fixation ceases, T_{optL} and T_{optH} define the optimal temperature range within

Table 5 Potential fixed N rate used in the models. Value in APSIM varies with growth stage

Model	Species	Maximum specific N fixation rate	Unit	Based on	Reference
Sinclair Model	soybean	0.55–0.7×10^{-3}	g N gshoot DM^{-1} d^{-1}	shoot biomass	Sinclair (1986); Sinclair et al. (1987)
	cowpea	0.7–0.8 ×10^{-3}	g N gshoot DM^{-1} d^{-1}	shoot biomass	
	black gram	0.7–0.75×10^{-3}	g N gshoot DM^{-1} d^{-1}	shoot biomass	
APSIM	legumes	1.0–6.0×10^{-3}	g N gshoot DM^{-1} d^{-1}	shoot biomass	(APSIM source code: http://apsrunet.apsim.info/svn/development/trunk/apsim/)
	soybean, chick pea, mungbean, peanut, lucerne	N demand-uptake	g N d^{-1}	N demand after N uptake	Herridge et al. (2001); Robertson et al. (2002)
EPIC	soybean	total N demand	g N d^{-1}	total N demand	Bouniols et al. (1991); Cabelguenne et al. (1999)
Hurley Pasture Model	clover	0.175	g N gnodule DM^{-1} d^{-1}	nodule biomass	Brugge and Thornley (1984)
	whiteclover	0.05–1.0×10^{-3}	g N groot DM^{-1} d^{-1}	root biomass	Thornley, 1998; Thornley and Cannell (2000); Thornley (2001)
	pea	0.012–0.027	g N groot DM^{-1} d^{-1}	root biomass	Eckersten et al. (2006)
CROPGRO	faba bean	0.05	g N gnodule DM^{-1} d^{-1}	nodule biomass	Boote et al. (2002)
	soybean	0.045	g N gnodule DM^{-1} d^{-1}	nodule biomass	
SOILN	white clover	0.1106	g N gnodule DM^{-1} d^{-1}	nodule biomass	Wu and McGechan (1999)
Soussana Model	white clover	0.04	g N groot $DM^{-1}d^{-1}$	root biomass	Soussana et al. (2002)
STICS	legumes	–	g N gshoot DM^{-1}	aboveground growth rate	Brisson et al. (2009)
	field pea	0.028	g N gshoot DM^{-1}	aboveground growth rate	Corre-Hellou et al. (2007); Corre-Hellou et al. (2009)

Table 6 Values of threshold temperatures (°C) used in three models that use equation (6) to simulate the effect of temperature on legume BNF

Model	Species	T_{min}	T_{optL}	T_{optH}	T_{max}	Reference
CROPGRO	soybean	5	20	35	44	Boote et al. (2008)
	cowpea	5	20	35	44	
	velvet bean	5	23	35	44	
	dry bean	4	19	35	44	
	peanut	7	23	34	44	
	faba bean	1	16	25	40	
SOILN	white clover	9	13	26	30	Wu and McGechan (1999)
STICS	legumes	0	15	25	35	Brisson et al. (2009)

which the response function to soil temperature is the unit, and T_{max} is the maximum temperature above which fixation stops. The values of these four temperatures vary among models and are shown in Table 6.

A slightly different function is used in the Hurley Pasture model to simulate the influence of temperature on N fixation and most plant rate responses of white clover (Thornley, 1998):

$$f_T = T^2(45 - T) \times 10^{-4}. \tag{7}$$

There are variations in the response functions adopted in the reviewed models. For example, the functions for white clover in Hurley Pasture and SOILN differ in curve shapes and threshold temperatures (Fig. 1). There is a much wider range of temperature for N fixation in Hurley Pasture than SOILN, perhaps because the function in the Hurley Pasture model is not specially assigned to the N fixation module, but is used to simulate most plant processes. The function reaches unit at 20°C for both models and the maximum value of the function occurs at 30°C in Hurley Pasture, while the function is zero in SOILN at the same temperature. The value of the function in Hurley Pasture is higher than SOILN when the soil temperature is below 10°C, but this reverses when soil temperature is in the range 10 to 20°C. Over 20°C there is a large difference between Hurley Pasture and SOILN. This may be because Hurley Pasture was developed in southern Britain, where the temperature is often between 4 and 16°C (Thornley and Cannell, 2000) and thus it might not perform well at much higher temperatures.

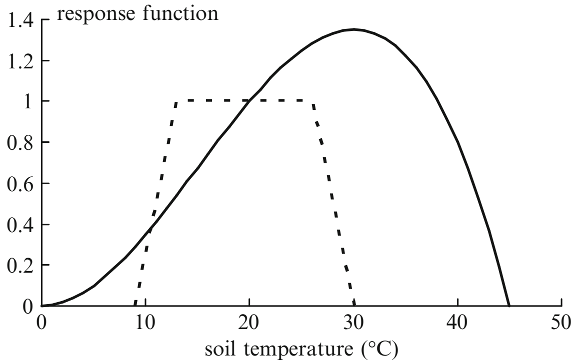

Fig. 1 The response of N fixation rate in white clover to temperature in the Hurley Pasture Model (solid line) and SOILN (dotted line)

3.2.3 Impact of Soil Water Status

Soil water stress in the form of either a deficit or excess in the root zone could inhibit nodule nitrogenase activity. The effect of water deficit is considered in some models, but only few models take account for the influence of excess water. An exponential or linear equation derived from experimental data to represent the response of legume BNF rate to soil water deficit is incorporated into most of the reviewed models.

In STICS, the soil is divided into depth layers, and the proportion of these soil layers where water contents are above permanent wilting point is used as the water deficit factor.

In Sinclair's model, an empirical sigmoid relationship between relative nodule nitrogenase activity, expressed as C_2H_2, and transpirable soil water was developed based on experimental data (Sinclair, 1986; Sinclair et al., 1987):

$$f_W = -1 + \frac{2}{1 + e^{(-m * f_{TSW} + n)}} \tag{8}$$

where f_{TSW} is the fraction of transpirable soil water, and the total transpirable soil water content is defined as the difference between field capacity and the soil water content when the transpiration rate from drought-stressed plants decreases to less than 10% of that from well-watered plants (Sinclair, 1986); m and n are constants which defines the sensitivity of legume BNF to low soil water content. Values of m and n are 6 and 0 for soybean, 9 and 0.03 for both cowpea and black gram, respectively.

A linear function is incorporated into APSIM, EPIC (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999) and SOILN (Wu and McGechan, 1999):

$$f_w = \begin{cases} 0 & (W_f \leqslant W_a) \\ \varphi_1 + \varphi_2 \cdot W_f & (W_a < W_f < W_b) \\ 1 & (W_f \geqslant W_b) \end{cases} \tag{9}$$

where W_f is the relative available soil water, expressed as the ratio of available soil water content to that at field capacity, ϕ_1 and ϕ_2 are coefficients, and W_b is a critical value of W_f above which N fixation is not inhibited by soil water content and W_a is the threshold below which N fixation is totally restricted by soil water deficit. The values for the parameters are shown in Table 7.

Although a linear expression similar to equation (9) is used in CROPGRO to simulate the impact of water stress on N fixation, the variable W_f is defined as the ratio of root water uptake to transpiration demand (Boote et al., 2008). Furthermore, the value of W_f on the current day is kept to compare with the average of W_f for the last eight days, to account for the prolonged impact of any previous severe drought on N fixation. The final value of W_f is equal to the lesser of these two values.

The upper soil layer above 30 cm depth is prone to be dry or wet when drought or irrigation occurs, and this causes a lag between water potential in the root nodules and that in the soil (Sprent, 1972; Albrecht et al., 1984). Therefore, water potential within roots could be a more stable indicator to quantify the relationship between water stress and N fixation. In the Hurley Pasture model, it is assumed that N fixation is controlled through chemical activity in roots, which is further influenced by root water potential and soil temperature. Thus the effect of water on N fixation is combined together with temperature (Thornley, 1998):

$$f_W = e^{20*\left[\frac{18*\varphi_{rt}}{8314*(T_s+273.15)}\right]} \tag{10}$$

where ϕ_{rt} is root water potential (J kg^{-1}) and T_s is soil water temperature (°C).

Compared with water deficit, the influence of excessive water on the simulation of N fixation has received very little attention in published studies. In Sinclair's model, a simple assumption is adopted that N fixation is set to zero once flooding happens (Sinclair, 1986; Sinclair et al., 1987). In STICS, the restraint of legume BNF by excessive water is considered as a stress from anoxia, which is calculated as the proportion of soil layers that are in anaerobic conditions in the nodulation zone (Brisson et al., 2009). The evaluation of N fixation inhibition by water excess is incorporated into CROPGRO (Boote et al., 2008) by computing the fraction of pore space filled with water. When pore space is fully filled with water, N fixation is inhibited, but the validation of this rule to date is insufficient.

3.2.4 Effect of Soil Mineral N or Internal Substrate N

A natural logarithmic function to represent the effect of soil mineral N content on N fixation by white clover is incorporated in SOILN model:

$$f_N = \begin{cases} 1 - 0.0784 \ln N_s & (N_s \geqslant 1) \\ 1 & (N_s < 1) \end{cases} \tag{11}$$

where N_s is soil mineral N concentration (mg N m^{-3}).

As N uptake and N fixation are the main N source for legumes, the influence of soil mineral N on legume BNF rate could be presented indirectly by its influence on N uptake from soil. In Schwinning's model, such a scheme is used:

$$f_N = \varepsilon \times (1.0 - f_{Nup}) = \varepsilon \times \left(1.0 - f_{\max} \frac{1}{1 + K_N/N_s}\right) \tag{12}$$

where ε is the efficiency of legume BNF and a value of 0.6 was used for white clover, f_{max} is a maximal

Table 7 Parameter values in the response function to soil water deficit

Model	APSIM											EPIC	SOILN
Parameter value	soybean	peanut	navy bean	mung bean	lupin	lucerne	faba bean	cow pea	chick pea	butterfly pea	field pea	soybean	White clover
ϕ1	–0.33	0	0	0	0	0	0	0	0	0	0	–0.82	0.216
ϕ2	1.67	2.5	2	2	2	1.25	2	2	2	2	2	1.82	0.789
Wb	0.8	0.4	0.5	0.5	0.5	0.8	0.5	0.5	0.5	0.5	0.5	1	1
Wa	0.2	0	0	0	0	0	0	0	0	0	0	0.45	–

fraction of N derived from soil mineral N uptake, which is assumed to be 0.85 for white clover to make sure that N fixation still retains even in high soil mineral N concentrations in the root zone; K_N is the nitrate content (g N m^{-2}) at which an N uptake rate reaches half its maximal rate and N_S is the actual soil nitrate content (g N m^{-2}).

Even though the schemes in SOILN and Schwinning's model are different and f_N in Schwinning's model is never greater than 0.6 due to the limitation by legume BNF efficiency, they describe a similar trend of N fixation response to soil mineral N by white clover. The values of f_N from these two functions are very close while soil mineral N is higher than 0.05 g N m^{-2} (Fig. 2).

The impact of soil mineral N is assessed as a linear function when soil nitrate concentration (N_{sNitra}) is between 10 and 30 g N m^{-3} within 0.3 m top soil in EPIC (Sharpley and Williams, 1990; Cabelguenne et al., 1999):

$$f_N = \begin{cases} 1 & (N_{sNitra} \leqslant 10) \\ 1.5 - 0.05 N_{sNitra} & (10 < N_{sNitra} < 30)\,. \end{cases} \tag{13}$$

A similar function is incorporated in STICS as in EPIC, with a different value of N_{sNitra}. Moreover, in STICS, nodulation progress is also inhibited by high soil nitrate concentration, which is represented by a reduction of potential N fixation rate. Once the soil nitrate concentration in the nodulation zone is greater than a critical value, N_{fixpot} equals a baseline value; otherwise, N_{fixpot} is kept at the normal value (Brisson et al., 2009).

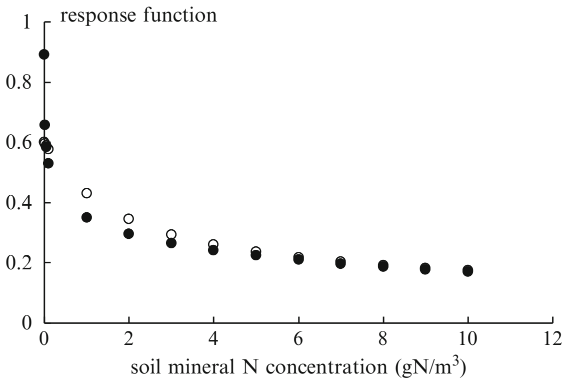

Fig. 2 N fixation response to soil mineral N concentration by white clover in the Schwinning Model (closed circle) and SOILN (open circle), assuming the nodulation zone is 40 cm deep

Both the Hurley Pasture Model and Soussana's model use plant substrate N concentration to simulate the influence of N nutritional status on N fixation rate:

$$f_N = \frac{1}{1 + N_{inter}/K_r} \tag{14}$$

where N_{inter} (g N g^{-1}r.wt) is root substrate N concentration in the Hurley Pasture Model or plant substrate N concentration in Soussanna's model, respectively; and K_r is a coefficient inhibiting N fixation at high internal N concentration and it is set to 0.02 for white clover and 0.01 for field pea in the Hurley Pasture Model (Thornley, 1998, 2001; Eckersten et al., 2006), 0.004 for both a normal cultivar and a low-soil-N-uptake breeding cultivar, and 0.0004 for a low-N-fixation breeding cultivar in Soussana's model for white clover (Soussana et al., 2002).

3.2.5 Influence of Plant Substrate C or C Supply

Biological N fixation has a high energy demand and the rate of legume BNF depends on the C supply, which is the energy source for N fixation. Experimental observations demonstrate that the enhancement of photosynthetic C allocated to roots which is available for N fixation and nodule establishment increases nodule numbers and N fixation rate (Voisin et al., 2003; Haase et al., 2007; Kirizii et al., 2007). However, among the reviewed models only the Hurley Pasture and CROPGRO models implemented this mechanism.

In the Hurley Pasture Model, a Michaelis-Menten function is used to demonstrate the effect of root substrate C on the N fixation of white clover and field pea:

$$f_c = \frac{1}{1 + K_C/C_r} \tag{15}$$

where C_r is legume root C substrate concentration (gC g^{-1} r.wt), and K_c is a Michaelis-Menten constant, set to be 0.01 for white clover (Thornley, 2001) and 0.035 for field peas (Eckersten et al., 2006).

In CROPGRO, photosynthetic carbohydrate supports not only the energy consumption of N fixation but is also the substrate supply for nodule biomass ac-

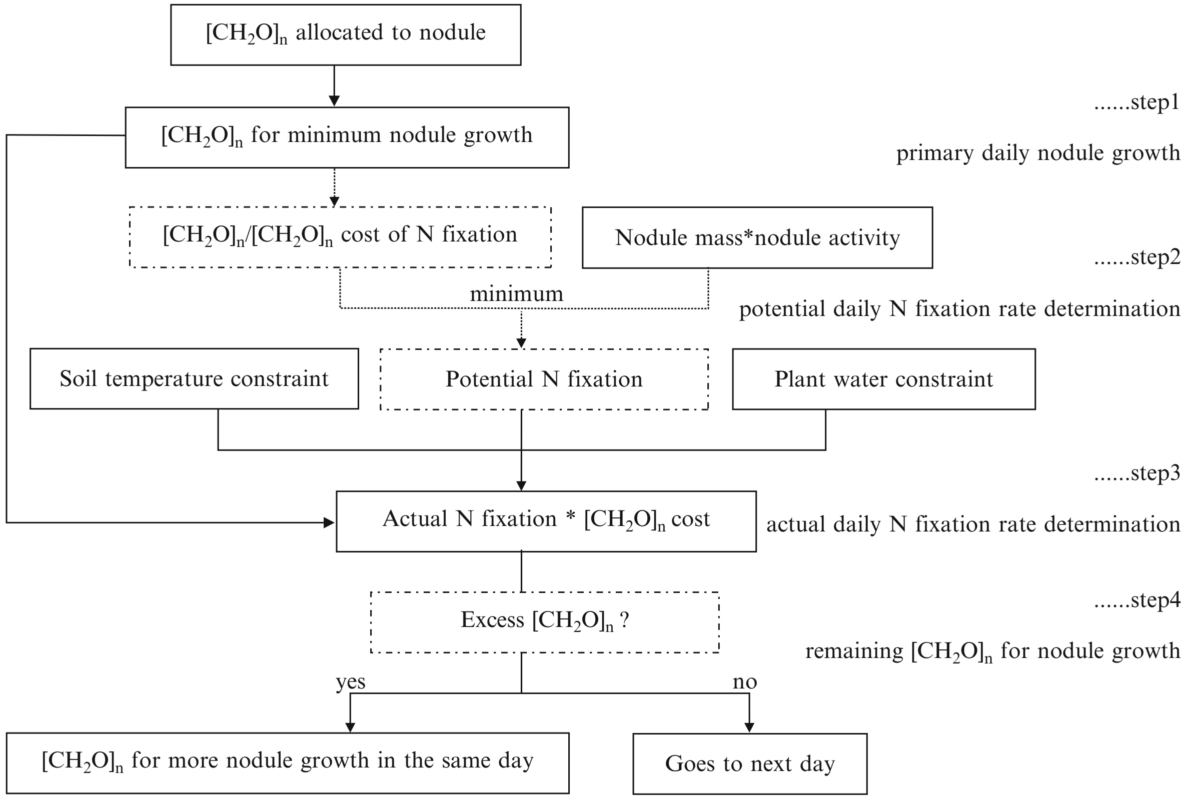

Fig. 3 The representation of daily carbohydrate routes in nodules used in CROPGRO (Boote et al., 2008). $[CH_2O]_n$ in dotted line means that the amount of $[CH_2O]_n$ here is only used for calculation, neither for fixation nor nodule growth

cumulation. Carbohydrate allocated to nodulated roots is divided into three parts with priorities: requirement for minimum nodule growth, the cost for N fixation and the requirement for nodule normal growth (Fig. 3). First, it needs to guarantee the minimum daily nodule growth (step 1). Then the carbohydrate left over after the reduction for the minimum nodule growth is assumed to be potentially available for N fixation to determine potential N fixation rate (step 2), and in this step carbohydrate is not truly used to fix N until the actual N fixation rate is determined (step 3). If there is any carbohydrate left after the third step, the remainder will be used to produce more nodules (step 4) (Boote et al., 2008).

3.2.6 Changes in N Fixation with Plant Growth Stage

The quantitative effect of plant growth stage on legume BNF rate is considered in very few models and in general the process is stopped forcibly after the legume attains a certain growth stage. For example, N fixation ceases at the beginning of seed growth for cowpea and black gram, but continues until the end of seed-filling for soybean in Sinclair's model (Sinclair et al., 1987), whereas it stops at physiological maturity in CROPGRO (Boote et al., 2008).

A more specific function, similar to the temperature response function, is incorporated into EPIC and STICS to simulate the seasonal change of N fixation (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999):

$$f_{gro} = \begin{cases} 0 & (g < g_{min} \ or \ g > g_{max}) \\ \frac{g - g_{min}}{g_{optL} - g_{min}} & (g_{min} \leqslant g \leqslant g_{optL}) \\ 1 & (g_{optL} \leqslant g \leqslant g_{optH}) \\ \frac{g_{max} - g}{g_{max} - g_{optH}} & (g_{optH} < g < g_{max}) \end{cases} \tag{16}$$

where g_{min} is a period before which no fixation happens because of inadequate nodulation, expressed as the percentage of total growing period required; g_{optL} and g_{optH} are the beginning and end time within which legume BNF rate is not limited by growth stage; and g_{max} is the time after which N fixation ceases because of nodule senescence. The values of the parameters for soybean are set to 10%, 30%, 60%, and 80% of the life cycle by Bouniols et al. (1991), and 15%, 30%, 55% and 75% by Cabelguenne et al. (1999). In the STICS model, g_{min} and g_{max} are the times of nodule initiation and senescence respectively, and g_{optL} and g_{optH} correspond to 27% and 80% of nodule life (Brisson et al., 2009).

3.3 Summary of Approaches

In addition to direct field measurements, estimates of legume BNF can be based on harvested yield or be derived from simple empirical models. However, these approaches generate data that is strictly valid only for the prevailing conditions at the time and place of measurement, with limited potential for use in wider-scale predictions. Simulation of legume BNF by models that incorporate the kinetics of N fixation may be the best approach as they may help us to understand the nature of the detailed relationships between N fixation and environmental and plant factors. Thus they permit more accurate simulation of legume BNF under a much broader range of farming systems and environmental conditions than other approaches.

Our review has found that the method used to simulate legume BNF most frequently in recent publications is to modify potential N fixation rate by factors such as soil temperature, soil/plant water, soil/plant N, plant C and plant growth stage. However, which of these factors are included and the functions used to relate them to the potential N fixation rate differ between models. Despite this range of approaches, the simulation of legume BNF in recent models may be conveniently summarized as:

(i) Potential N fixation rate may be estimated based on plant N demand and N uptake from the soil, in which case a critical plant N concentration needs to be defined appropriately for each species. Alternatively, if the potential N fixation rate is estimated from nodule/root/aboveground dry matter, then the maximum specific N fixation rate has to be estimated experimentally, as this value varies widely between species and sites.

(ii) The influence of soil temperature on legume BNF is commonly represented by a four-threshold-temperature function, or a sigmoidal cubic function in the Hurley Pasture Model (Thornley, 1998). The values of these four threshold-temperatures vary with species and cultivar.

(iii) The relationship between legume BNF rate and soil water deficit, alternatively represented by low plant water status in some models, is described by either exponential or linear equations. The choice of equations has only a small effect on the output, unless some species are more sensitive than others to water deficit and this is likely as species will have been bred for different environment. However, the effect of excessive water on N fixation is over-simplified or absent in all models and this factor needs more attention.

(iv) The sensitivity of legume BNF to a factor representing general N availability, e.g. soil mineral N or plant N content, is the function that differs most between different models. In the EPIC model (Sharpley and Williams, 1990; Cabelguenne et al., 1999), legume BNF is unaffected until soil mineral N in the root zone is higher than 10 g N m^{-3} whereas in SOILN (Wu and McGechan, 1999) and Schwinning's model (1996) legume BNF is highly sensitive to even small amounts of soil mineral N. This latter pattern is also seen in the Hurley Pasture Model (Thornley, 2001) and Soussana's model (2002), which use root and plant N concentrations respectively instead of soil mineral N concentration.

(v) Factors that increase plant C status have a positive effect on legume BNF. The relationships are sometimes represented by Michaelis-Menten functions, or are included in more complex simulations together with nodule growth and the synthesis of the products of legume BNF.

(vi) The change in legume BNF with legume growth stage follows a function that contains four threshold values in most of the models. However, in APSIM, the same effect is achieved by using different values of potential specific N fixation rate for each growth stage.

4 Discussion

There has been much progress in simulating N fixation by forage and grain legumes since the first simple model by Duffy et al. (1975). However, the analysis presented above has revealed areas of the simulation schemes that are in need of refinement, and identified some potentially important factors omitted from all current models. These areas, and suggestions for their improvement, are discussed below.

It is preferable to define the key parameter of potential N fixation rate based on nodule mass rather than on the plant N deficit after soil N uptake. This is because normally legume BNF is not completely inhibited by soil mineral N and some species and cultivars continue to fix N even when soil mineral N is relatively high (Lamb et al., 1995; Blumenthal and Russelle, 1996). However, not all the models have the potential to estimate nodule biomass accurately. In these cases root or shoot biomass is used instead of nodule biomass to estimate potential N fixation rate, and this is based on the strong relationships between nodule size and plant size (Denison et al., 1985; Sinclair, 1986), and between nodule biomass and root biomass (Macduff et al., 1996). On this basis a constant ratio of nodule biomass to root or shoot biomass is assumed, and linear functions are used to calculate the potential N fixation rates in these models. In practice, however, this ratio varies with legume growth stage (Schiltz et al., 2005; Bollman and Vessey, 2006) and is affected by other environmental factors, such as soil mineral N (Voisin et al., 2002; Bollman and Vessey, 2006). Thus the constant ratio of nodule biomass to root or shoot biomass assumed in some models does not represent the real situation and would be better defined as a temporal factor that changes with environmental conditions. The importance of this is shown by the fact that even when the nodule biomass is available and is used to estimate potential legume BNF, the relationship between legume BNF rate and nodule mass varies with growth stage (Voisin et al., 2003).

The above section has shown how measurements of plant or nodule biomass may be used to modify the maximum potential legume BNF to provide an estimate of legume BNF in the field. However, a potentially more accurate approach is to use the amount and allocation pattern of photosynthetically-fixed C in the plant. Carbon is required for nodule growth, as an energy source for legume BNF and for the synthesis of N compounds produced from legume BNF (Minchin and Pate, 1973; Voisin et al., 2003), and many studies have shown a strong correlation between legume BNF and the C respired by nodules, nodulated roots (Mahon, 1977a, b; Ryle et al., 1979), or photosynthetic allocation (Lawrie and Wheeler, 1973; Voisin et al., 2003, 2007). Of all the models considered here, C sink is closely related to nodule growth and legume BNF only in CROPGRO (Boote et al., 2008). Thus improved experimental evidence of C allocation and usage by nodules and incorporation of these processes more widely into models would do much to improve estimates of potential legume BNF.

As mentioned above, soil mineral N in the rhizosphere inhibits legume root nodulation and legume BNF, and this inhibition is more severe under higher N concentrations, although moderate levels of soil mineral N stimulate nodulation and N fixation in some circumstances. It is difficult to give the critical value of soil N concentration below which nodulation and N fixation are stimulated (Munns, 1970; Streeter, 1988; Waterer et al., 1992; Waterer and Vessey, 1993b), and the issue about “starter N” is still controversial (Bethlenfalvay and Phillips, 1978; Bethlenfalvay and Phillips, 1978; Waterer and Vessey, 1993b; Gulden and Vessey, 1997). Taken together, these factors explain why this stimulating effect of soil mineral N is not incorporated into any of the current models of legume BNF, and suggest that much further experimental work may be needed before it is possible to do so. The lack of inclusion of this process is a probable cause of the large discrepancy between modelled and actual legume BNF in some circumstances.

A further process that is absent from the current models of legume BNF is that they cannot distinguish the different inhibitory effects of soil nitrate and ammonium in the rhizosphere. Experimentally it has been shown that legume BNF is less sensitive to ammonium than nitrate (Svenning et al., 1996; Bollman and Vessey, 2006; Gan et al., 2004), but all models use soil nitrate or total mineral N concentration.

Our analysis of the currently-used models of legume BNF has revealed that the inhibition of legume BNF by soil mineral N concentration might be overestimated in the model that uses nodule biomass to determine the potential N fixation rate, N_{fixpot}. Considering equation (4), N_{fixpot} is the potential N fixation rate, calculated as the amount of fixed N per unit of nodule biomass multiplied by nodule biomass. Thus if nodule biomass is reduced by high soil mineral N concentration, N_{fixpot} will also be reduced. However, this model also uses f_N, the factor that relates legume BNF to soil mineral N concentration. This factor is derived experimentally from measurements of legume BNF under different levels of soil mineral N concentration, and by default this includes any inhibitory effect of soil mineral N concentration on legume BNF. Thus in STICS (Brisson et al., 2009), the effect of soil mineral N concentration on nodule biomass is effectively incorporated twice. We suggest that to avoid this problem, future models could remove the effect of nodule biomass on f_N by basing estimates of the latter on measurements taken from plants with well developed nodules. The effect of high soil mineral N concentration on nodule biomass should be included as it is currently, by its effect on N_{fixpot}.

In both experimental work and models of legume BNF, the effect of the water status of either soil or plant on legume BNF has concentrated on cases of water deficit rather than flooding. Despite many studies on the effect of soil water deficit on legume BNF, the nature of the relationship between N fixation and water soil status is poorly characterised. A linear or exponential function has been widely used in modelling studies to date except in the Hurley Pasture Model. CROPGRO includes an eight-day moving average of a water factor, which is used to simulate the prolonged effect of drought on N fixation. This is consistent with the conclusion that the inhibition of N fixation by drought in peanut and cowpea is not alleviated immediately once the drought releases (Venkateswarlu et al., 1990). Some studies have found that drought stress on N fixation is caused more by the inhibition of photosynthesis than by drought directly (Huang et al., 1975), which highlights the importance of the link between N fixation and photosynthesis. However, some studies have shown that N fixation is more sensitive than photosynthesis to drought (Sprent, 1976) and N fixation may decline before transpiration or photosynthesis decreases (Sinclair et al., 1987; Sall and Sinclair, 1991; Purcell et al., 1997).

Stress from excessive water is omitted or oversimplified in all models, even though the proportion of water in pore spaces has been suggested as an approach to evaluate the restriction on nodule nitrogenase activity by excessive water in CROPGRO. More studies are needed on the effect of excess water on legume BNF in the future, both experimentally and in simulations.

In summary, the difficulty of simulating legume BNF is because of the large variance in N fixation between sites and species, and over time. Biological N fixation is a highly complex process which integrates plant and soil processes in the macro-environment with the micro-environmental processes of rhizobial bacteria in nodules (Nelson and Child, 1981; Bolaños et al., 1994; Rice et al., 2000). It is difficult to represent these two completely different but closely related processes in one model. Also, there is currently inadequate information to quantify reliably the relationship of legume BNF with those factors such as soil water and plant C. This is because legume BNF is not only sensitive to temperature, water, N and C cycling, which have been incorporated into models, but also to soil pH (Rechcigl et al., 1987; Peoples et al., 1995; Ibekwe et al., 1997; Rice et al., 2000; Le Roux et al., 2008), O_2 permeability (Cowan, 1978; Weisz and Sinclair, 1987; Faurie and Soussana, 1993; Serraj et al., 2001), salinity (Serraj and Drevon, 1998) and other nutrition regimes (Lynd et al., 1984; Collins et al., 1986; Bolaños et al., 1994; Chaudhary et al., 2008), which are currently absent from models despite frequently being cited as important factors.

Furthermore, in terms of the practical uses and benefits of N fixed by legumes, realistic field scenarios should be considered. In temperate agricultural systems, mixed pastures of legumes and grasses are common; intercrops of forage and grain legumes with cereal crops for animal feed are increasing in importance, especially in low-input and organic farming systems (Hauggaard-Nielsen et al., 2001, 2003; Andersen et al., 2004; Corre-Hellou et al., 2006). In these systems the interactions, both competitive and facilitative, between the components alter legume BNF in mixtures compared to monocultures (Malézieux et al., 2009). Intercropping enhances the proportion of legume N derived from fixation due to the higher competition for soil N. However, the total legume BNF is often reduced as a consequence of its dependence on not only soil N but also legume growth. In addition to deliberate intercropping, weeds have a similar influence on legume BNF. This may have a big influence on yield and N accumulation of the main crops in pure legume stands if the weeds are relatively competitive (Hauggaard-Nielsen et al., 2001, 2006). Schwinning and Parsons (1996) and Thornley (2001) incorporate the interaction between legumes and grasses in their models, which have been reviewed by Malézieux's (2009). Therefore, these simulation schemes of interspecies competition are not reviewed here as we have focused on legumes only.

Apart from intercropping, other field managements such as grazing by livestock, especially in forage legume grasslands, affects N fixation through its effects on both legume growth and soil mineral N cycling due to urine and dung inputs. Thornley and Cannell (2000) simulated mineral N availability in grazed grassland with legume BNF through the effect on leaf area by grazing in the Hurley Pasture Model. Schwinning and Parsons (1996) implement not only the effect of grazing on leaf cover but also the urine deposits from animals into their model. However, their simulation scheme of N fixation is simpler than in other models as it considers only the direct influence of soil mineral N on BNF. Many of these dynamic and competitive aspects with grazing and other crops should be better incorporated into future models that aim to have real agricultural relevance.

5 Conclusion

This review critically interprets the methods used to quantify legume BNF by the most commonly used experimental and modelling approaches. In particular, it highlights and compares the functions used to simulate legume BNF by different models and assesses their relative strengths in predicting N fixation with varying biotic and abiotic factors. We found that:

(i) As a base to estimate legume BNF, all models use the potential N fixation rate although they differ in how they estimate this. Different functions are incorporated into each of the models to estimate how the maximum potential N fixation rate is restrained by soil temperature, soil water, soil/plant N concentration, plant C status and legume growth stages.

(ii) Estimations of potential N fixation rate based on above ground biomass or root biomass are not as reliable as those based on nodule biomass (although see (iii) below). C supply from photosynthesis is recommended as the factor best able to explain the potential N fixation rate.

(iii) The stimulating effect on legume BNF at relatively low levels of soil mineral N should be distinguished from the inhibition of legume BNF by soil mineral N. The simulated inhibition of legume BNF by soil mineral N might be exaggerated if potential N fixation is modified by functions that include nodule biomass and the effect of soil mineral N on potential N fixation (f_N).

(iv) More experimental work is needed to characterise the effect of both soil water deficit and excess soil water on legume BNF. The responses of legume BNF to other factors currently absent from all models, such as soil pH and O_2 permeability, need to be included and reinforced with adequate experimental work.

(v) Intercropping of legumes, either with grain crops or in grasslands, as well as the presence of grazing livestock, affect legume BNF in the fields. Models of legume BNF should take better account of these important practical uses of legumes.

Acknowledgements Y. Liu thanks the China Scholarship Council for financial support. SAC receives funding from the Rural and Environment Research and Analysis Directorate (RERAD) of the Scottish Government. The authors thank Drs M. Robertson and P. Devoil for the source code of the APSIM model, and N. Brisson and M. Launay for details of the STICS model.

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Arbuscular Mycorrhizal Networks: Process and Functions

Neera Garg and Shikha Chandel

Contents

Abstract An unprecedented, rapid change in environmental conditions is being observed, which invariably overrules the adaptive capacity of land plants. These environmental changes mainly originate from anthropogenic activities, which have aggravated air and soil pollution, acid precipitation, soil degradation, salinity, contamination of natural and agro-ecosystems with heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), global climate change, etc. The restoration of degraded natural habitats using sustainable, low-input cropping systems with the aim of maximizing yields of crop plants is the need of the hour. Thus, incorporation of the natural roles of beneficial microorganisms in maintaining soil fertility and plant productivity is gaining importance and may be an important approach. Symbiotic association of the majority of crop plants with arbuscular mycorrhizal (AM) fungi plays a central role in many microbiological and ecological processes. In mycorrhizal associations, the fungal partner assists its plant host in phosphorus (P) and nitrogen (N) uptake and also some of the relatively immobile trace elements such as zinc (Zn), copper (Cu) and iron (Fe). AM fungi also benefit plants by increasing water uptake, plant resistance and biocontrol of phytopathogens, adaptation to a variety of environmental stresses such as drought, heat, salinity, heavy metal contamination, production of growth hormones and certain enzymes, and even in the uptake of radioactive elements. The establishment of symbiotic association usually involves mutual recognition and a high degree of coordination at the morphological and physiological level, which requires a continuous cellular and molecular dialogue between both the partners. This has led to the identification of the genes, signal transduction pathways

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_40,

from Garg and Chandel, Agron. Sustain. Dev. 30 (2010) 581–599. DOI: 10.1051/agro:2009054

and the chemical structures of components relevant to symbiosis; however, scientific knowledge on the physiology and function of these fungi is still limited. This review unfolds our current knowledge on signals and mechanisms in the development of AM symbiosis; the molecular basis of nutrient exchange between AM fungi and host plants; and the role of AM fungi in water uptake, disease protection, alleviation of various abiotic soil stresses and increasing grain production.

Keywords Arbuscular mycorrhiza • Environmental stresses • Phytopathogens • Sustainable agriculture

1 Introduction

There is a pressing need to strike a balance between food production for the ever-increasing world population and conserving biodiversity, as well as supply of organic or low-input products for the markets (Dodd, 2000). Earlier, soil was treated as a 'resource base' with its function of support subordinate to the production of food and fiber, and on the scale of priorities, the soil had always taken second place (Bethlenfalvay and Schüepp, 1994). Recently, scientists have recognized the importance of the soil not only as an agricultural resource base (Stewart et al., 1991), but as a complex, living and fragile system that must be protected (Reagnold et al., 1990) and managed for its own sake (Pierce and Lal, 1991) to guarantee its long-term stability and productivity (Aryal and Xu, 2001).

Increased environmental awareness has progressively led to a shift from conventional intensive management to low-input, sustainable crop production agroecosystems. In low-input cropping systems the natural activities of microorganisms contribute to the biocontrol of plant pathogens and improved supply of nutrients, thus maintaining crop health and production. Symbiotic mycorrhizal fungi, such as arbuscular mycorrhizal (AM) fungi, form a key component of the microbial populations influencing plant growth and soil productivity (Johansson et al., 2004). The multifunctional nature of AM fungi include weathering, dissolution and cycling of mineral nutrients (Finlay and Rosling, 2006; Wallander, 2006; Helgason and Fitter, 2009), nutrient mobilization from organic substrates (Finlay, 2008), carbon cycling (Johnson et al., 2002), effects on plant communities and ecosystems, and mediation of plant responses to various environmental stresses, such as soil salinity, heavy metal toxicity, drought and heat stress, soil acidification, and plant pathogens (Colpaert, 2008; Finlay et al., 2008), as well as a range of possible interactions with groups of other soil microbes (Finlay, 2008). The natural roles of these microorganisms may have been marginalized in intensive agriculture, since microbial communities in conventional farming systems have been modified due to tillage (Sturz et al., 1997; McGonigle and Miller, 1996) and high inputs of inorganic fertilizers, herbicides and pesticides (Gianinazzi and Schüepp, 1994; Gianinazzi et al., 2002). A better understanding of the microbial interactions is therefore crucial for the development of sustainable management of soil fertility and crop production.

The vast majority of plant species in terrestrial ecosystems form symbioses with rhizosphere microbes to take up essential nutrients (Bonfante and Genre, 2008; Parniske, 2008; Helgason and Fitter, 2009). A number of these microbes inhabit the rhizosphere, including mycorrhizal fungi, nitrogen-fixing bacteria and other plant-growth-promoting rhizobacteria (van der Putten et al., 2007). The endosymbiosis formed between roots of more than 80 % of land plant species and arbuscular mycorrhizal (AM) fungi is ubiquitous and the most widespread symbiotic association in the plant kingdom (Redecker et al., 2000; Helgason and Fitter, 2009). The AM fungi were formerly included in the order Glomales in the Zygomycota (Redecker et al., 2000; Bonfante and Genre, 2008), but they have recently been moved to a newly ascribed phylum, Glomeromycota (Garg et al., 2006; Parniske, 2008; Smith and Read, 2008). Arbuscular mycorrhizal fungi develop extensive, below-ground extraradical hyphae fundamental for the uptake of inorganic phosphate and other immobile nutrients from the soil and their translocation to the host plant (Giovannetti et al., 2006; Smith and Read, 2008). Fungal penetration and establishment in the host roots involve a complex sequence of events and intracellular modifications (Bonfante-Fasolo and Perotto, 1992). In contrast, the nitrogen-fixing root nodule symbiosis is almost completely restricted to the legumes (Zhu et al., 2006). The symbiotic relationships between legumes and rhizobacteria involve extensive signaling between the two organisms (Smit et al., 2005). These microbes can stimulate plant growth and reproduction by providing their hosts with services such as increased access to limiting nutrients (phosphorus and nitrogen) and enhanced uptake of water. In exchange, the plants provide these microbes with carbon (Parniske, 2008; Smith and Read, 2008). An important characteristic of legumes is that they utilize more nitrogen and phosphorus from the environment with the help of nodulation bacteria and mycorrhizal fungi living in their roots (Smith and Read, 2008). Thus, dual application of AM fungi and *Rhizobium* acts as biological fertilization in legumes. Moreover, there are ameliorative synergistic effects of this dual application (Abd-Alla et al., 2000).

2 A Journey Through Mycorrhizal Symbiosis

The term 'mycorrhiza' originates from the Greek *mycos*, meaning 'fungus', and *rhiza* meaning 'root', and was first used in 1885 (Frank, 1885) to describe the intimate association between biotrophic mycorrhizal fungi and plant roots. Approximately 80 % of land plant species that have been studied form the mycorrhizal symbiosis (and 92 % of plant families), which exist everywhere, from tiny home-gardens to large ecosystems (Wang and Qiu, 2006; Helgason and Fitter, 2009). Six types of mycorhizas; arbuscular, arbutoid, ecto-, ericoid, monoptropoid and orchid, are categorized by their distinct morphological characteristics (Wang and Qiu, 2006; Garg et al., 2006). Of them, arbuscular mycorrhiza (AM) is the most common and predominant type (Fig. 1). Arbuscules, specific 'little-tree-shaped' fungal structures, serve as the main sites of nutrient exchange between the plant and the fungus (He and Nara, 2007). They are branched, microscopic haustorial structures of the fungal symbiont that form within living cortical cells of the root (Manchanda and Garg, 2007). This structure is common to all associations of this type of mycorrhiza (Franken et al., 2007). AM fungi are soil inhabitants with a presumed origin at least 460 million years ago (Redecker et al., 2000; Bonfante and Genre, 2008). Perhaps due to this ancient association with plants, AM fungi have lost their ability to live and complete their life cycle in the absence of a green partner (Requena et al., 2007). The Glomeromycota consists of approximately 150 isolates which colonize a wide range of both mono- and dicotyledonous plant species (Paszkowski, 2006).

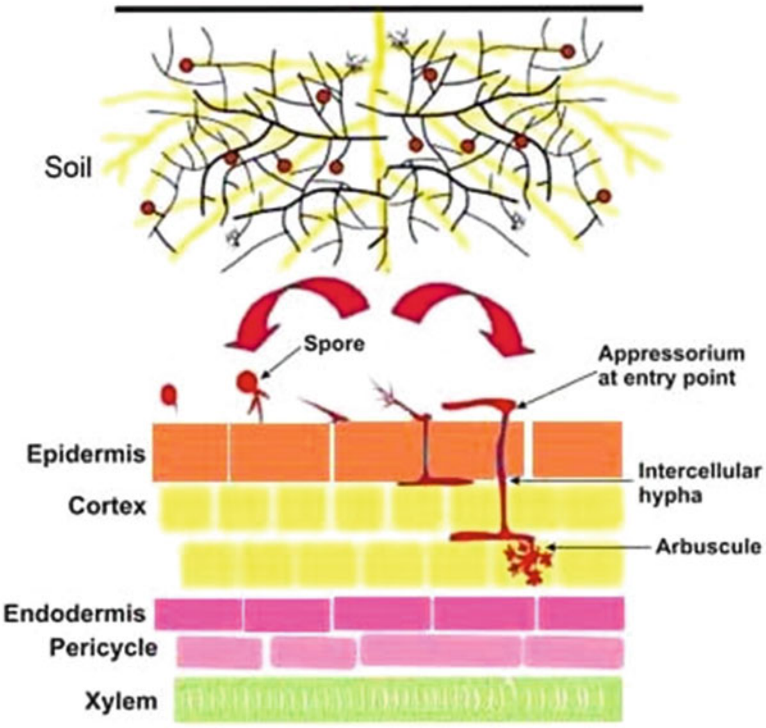

Fig. 1 Sequence of events leading to the formation of arbuscular mycorrhizal (AM) symbiosis (schematic representation)

These AM fungi communities influence a number of important ecosystem processes, including plant productivity, plant diversity and soil structure (Van der Heijden et al., 1998). So, not only do the activities of AM fungi have multiple functions that enhance plant performance, but they also play crucial roles in the development of soil properties and the health of the entire ecosystem.

2.1 Arbuscular Mycorrhizal Development

The establishment of AM symbiosis can be envisaged as a programed sequence of phenotypic changes, corresponding to distinct recognition events which lead the two partners, host plant and fungal symbiont, to a high degree of morphological and physiological integration.

2.1.1 Early Stages of Fungal Asymbiotic Growth

Arbuscular mycorrhizal fungi are obligate biotrophs, unable to complete their life cycle during asymbiosis (Bonfante and Bianciotto, 1995). AM fungal spores are the only plant-independent phase of the mycobiont. They are round-shaped structures with a thick cell wall and average diameters between 50 and 100 μm. They contain a very large number of nuclei, up to 2000 per spore (Bécard and Pfeffer, 1993). After germination, hyphae are always coenocytic. Studies on two AM fungal species have shown that these are haploids with an unusually high genetic variation (Hijri and Sanders, 2004; Hosny et al., 1997). Assessments of the genome size of these fungi have shown extreme variations between different species ranging from about 16.5 Mb in *Glomus intraradices* (Hijri and Sanders, 2004) up to 1058.4 Mb in *Scutellospora gregaria* (Hosny et al., 1998). AM spores germinate under appropriate water and temperature conditions and hyphae grow for about 2–3 weeks. Several nuclei from the spore move into the extending mycelium and some of them undergo mitosis (Requena et al., 2000). During this time the fungal colonies extend a few centimeters, showing a characteristic growth pattern with marked apical dominance and infrequent hyphal branching. In the absence of a host root, growth ceases after about 2–4 weeks and hyphal septation from the apex occurs (Mosse, 1988). The apical septation is accompanied by extensive vacuolization and retraction of protoplasm towards the spore (Logi et al., 1998). During this asymbiotic phase, the fungus lives mainly on its triacylglyceride reserves. This phase of growth in the absence of signals from the plant is what is known as the asymbiotic stage (Requena et al., 2007). Thus, in the asymbiotic stage, spores germinate and AM fungi show limited hyphal development in the absence of a host plant.

2.1.2 Arbuscular Mycorrhizal Life Cycle

The establishment of the AM symbiosis begins with the colonization of a compatible root by the hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots (Requena et al., 1996). After attachment of a hypha to the root surface by means of an appressorium, the fungus penetrates into the cortex and forms distinct morphologically specialized structures: inter- and intracellular hyphae, coils and arbuscules. Arbuscules are specialized hyphae, similar to haustoria from the plant pathogenic fungi, formed as intercalary structures between the coil hyphae, and are the site of mineral nutrient transfer to the plant and potentially the site of carbon acquisition by the fungus (Requena et al., 2007; Pumplin and Harrison, 2009). After host colonization, the fungal mycelium grows out of the root exploring the soil in search of mineral nutrients, and it can colonize other susceptible roots. The fungal life cycle is completed after formation of asexual chlamydospores on the external mycelium. Distinct morphological stages can therefore be identified during the life cycle of arbuscular mycorrhizal fungi (Requena and Breuninger, 2004). This clearly shows that the host plant plays a key role in orchestrating the AM infection process (Eckardt, 2005).

The sequence of steps leading to an AM symbiosis is largely conserved among different combinations of fungal and plant species. Overall, these developmental processes require molecular communication between the AM fungus and the plant, including exchange and perception of signals by the symbiotic partners (Bucher, 2007). Thus, the complex morphological and physiological alterations of both symbiotic partners accompanied by the recognition process suggest that AM symbiosis is the result of multifaceted, fine-tuned signaling events (Paszkowski, 2006).

2.2 Plant Signal and Fungal Perception

This phase of mycorrhizal fungi can be divided into three steps.

2.2.1 Pre-symbiotic Phase

Fungal Responses to Plant-Derived Signals

For both symbionts, the period before physical contact (appressorium formation) involves recognition and attraction of appropriate partners and other events promoting an alliance. There is increasing evidence showing that the fungus and plant start to recognize each other long before the first colonization structures on the root epidermis appear (Requena et al., 2007). Spores of AM fungi persist in the soil and germinate spontaneously, independently of plant-derived signals. However, root exudates and volatiles may promote or suppress spore germination, indicating the existence of the presence of spore 'receptors' responsive to alterations in the chemical composition of the environment (Bécard et al., 2004; Harrison, 2005). It has been known for a long time that germinating hyphae from spores respond to the presence of roots in their vicinity. In the vicinity of a host root, fungal morphology shifts towards enhanced hyphal growth and extensive hyphal branching (Giovannetti et al., 1993b; Buee et al., 2000). Although no directional growth has been observed towards the root, several experiments showed that exudates from the host root elicit growth stimulation in contrast to non-host root exudates (Giovannetti et al., 1993a,b, 1996). These observations suggest that the fungus senses a host-derived signal; ('branching factor'), leading to intensified hyphal ramification that is likely to increase the probability of contact with a host root. Hence, distinction between host and non-host occurs to a certain degree at this early point in the interaction (Paszkowski, 2006).

In many plant-microbe interactions, the dialogue between the two symbionts is initiated by the presence of plant phenolic substances such as flavonoids. Interestingly, there are many reports showing that, indeed, flavonoids (exogenously applied to spores) exert a positive effect on hyphal growth during asymbiosis (Akiyama et al., 2002). However, flavonoids might not be essential for the plant-fungal recognition since a study using maize mutant plants impaired in flavonoid production showed that they were able to form mycorrhizal symbiosis similarly to wild-type plants (Bécard et al., 1995). A major step forward in deciphering the molecular cross-talk in the AM symbiosis was the identification of a branching factor present in root exudates as a strigolactone (5-deoxy-strigol) (Akiyama et al., 2005). Strigolactones have been isolated from a wide range of mono- and dicotyledonous plants and were previously found to stimulate seed germination of parasitic weeds such as *Striga* and *Orobranche* (Bouwmeester et al., 2003). Strigolactones were previously described as sesquiterpenes (Akiyama et al., 2005); however, the use of carotenoid mutants of maize and inhibitors of isoprenoid pathways in maize, sorghum and cowpea showed that strigolactones are derived from the carotenoid biosynthetic pathway (Matusova et al., 2005). Nevertheless, the results do indicate that processed carotenoid derivatives are involved at multiple stages in the development of the AM symbiosis, possibly by stimulating intraradical fungal branching (Paszkowski, 2006). It is likely that other signals, such as thigmotrophic signals from the plant surface or secondary metabolites produced in plants after perception of the fungus, are required for appressorium formation and symbiosis progression (Requena et al., 2007). These chemical signals exuded by the plant and the thigmotrophic signals from the rhizodermis are possibly recognized by receptor proteins associated with the fungal plasma membrane (Requena et al., 2007). Requena et al. (2002) used suppressive subtractive hybridization (SSH) to create a subtractive cDNA library from *Glomus mosseae* enriched in genes induced during the asymbiotic phase. With this approach, a novel gene (GmGin1) encoding a two-domain protein with a putative role in signaling was identified. Expression analyses showed that GmGin1 was downregulated upon entry into symbiosis, suggesting it could play a role at the plant recognition stage (Requena et al., 2002). GmGin1 could be a sensor for plant signals and is located at the cell membrane. It undergoes splicing in response to signals from the plant. These interesting results show that the chemical communication with the plant symbiont not only modifies fungal gene expression but it is also able to induce post-transcriptional modification of fungal proteins (Requena et al., 2007). Transcript profiling of AM fungi during appressorium formation showed that plant contact induces the activation of genes from different categories, including several components related to Ca^{2+}-signaling, including a putative P-type Ca^{2+}-ATPase, a calmodulin, a leucine zipper EF-protein, and a Ca^{2+}-induced Ras inactivator (CAPRI) (Breuninger and Requena, 2004). This suggests the involvement of Ca^{2+} as a second messenger in the transmission of plant-derived signals, leading to appressorium formation in the AM symbiosis (Requena et al., 2007). Thus, upon detection of a host root there is vigorous investment in the production of fungal hyphae, which can then rapidly make contact with essential carbon sources. A major breakthrough in the molecular interactions between both the partners was the identification of the host branching factor 5-deoxy-strigol, which induces the so-called presymbiotic stage characterized by continued hyphal growth, increased physiological activity and profuse branching of hyphae, and the gene, GmGin1, which plays a role in the development of appressoria during AM symbiosis in the plants.

Plant Responses to Fungus-Derived Signals

The plant responds to the microbial profile of the rhizosphere in different ways depending upon the type of organism present. Detection of pathogen-derived elicitors triggers plant signaling cascades that lead to a defense response (Glazebrook, 2005). On the other hand, plant defense responses are either not mounted at all or mounted only transiently before being suppressed during AM symbiosis (Harrison, 2005). Extensive forward genetic approaches were used to dissect the components of the signal perception and transduction pathways in the AM symbiosis in legume species. These approaches led to the identification of several symbiosis genes, the corresponding plant mutants of which are generally unable to support infection by AM fungi. Yet the nature and function of mycorrhization (Myc) factors are still an enigma. Myc factors are likely to be soluble, fungus-derived compounds that trigger expression of mycorrhizal-responsive genes (Kosuta et al., 2003) and structural changes in host roots (Olah et al., 2005).

GUS reporter expression [*EARLY NODULATION11 (ENOD11)*-promoter: β-glucuronidase], which is responsive to both AM fungi and a rhizobial Nod-factor, before contact was monitored in root sections adjacent to intensely branching hyphae. The intensity and distribution of GUS expression indicated the detection by the plant of a compound released by the fungus (Kosuta et al., 2003). Interestingly, when contact and penetration were permitted, GUS expression was restricted to infected and associated cells (Chabaud et al., 2002; Genre et al., 2005), indicating the induction of a suppressor activity in non-colonized neighboring cells (Parniske, 2004). When contact is made and a precise area of penetration and colonization is established, gene expression is restricted to cells in direct contact with the penetrating fungus. Similarly, during preinfection and infection stages in the interaction of *Medicago truncatula* with nitrogen-fixing *Rhizobia*, GUS expression was found in the rhizodermis of the larger root section before contact and upon subsequent bacterial entrance into the root tissue became confined to the area of infection, namely the infection site and the invaded nodule (Journet et al., 2001). This resemblance in expression patterns between the two types of root symbiosis suggests that the responses are a part of a rather general symbiont ‘anticipation’ program (Paszkowski, 2006). To conclude, the infected cells in the plant roots upon perceiving Myc factors trigger the expression of SYM genes (*ENOD11*-promoter GUS reporter gene), common to both bacterial and fungal root endosymbioses, providing a glimpse of conservation and specialization of signaling cascades essential for nodulation and mycorrhiza development.

2.2.2 Early Symbiotic Phase

Appressorium Development

The onset of the symbiosis is marked morphologically by the formation of appressoria, the cell-to-cell contact between the fungus and plant and the site of fungal ingress into the host root. The formation of appressoria is one of the first morphological signs that recognition between the plant and the fungus has occurred (Garcio-Garrido and Ocampo, 2002). The development of appressoria can be considered to be the result of successful presymbiotic recognition events when fungal and plant partners are committed to an interaction (Giovannetti et al., 1993a). Structurally, appressoria differ from hyphae by being flattened, elliptical hyphal tips that adhere by unknown means to the surface of host rhizodermal cells. This morphological switch is reflected by changes in fungal gene transcription (Breuninger and Requena, 2004). While physical and chemical rhizodermal cell wall features are required and are sufficient to elicit appressorium formation, penetration needs the support of intact cells whose coordinated, matching response accommodates the fungus (Paszkowski, 2006). Therefore, the discovery that the plant cell actively prepares the intracellular environment for AM fungal hyphae elicited its role during the infection process, and emphasized the significance and indispensable participation of plant processes in the coordinated invasion (Genre et al., 2005, 2008).

Arbuscular Mycorrhizal (AM) Fungi Penetration

Actual penetration of the host by AM fungi is by means of a penetration hyphae or infection peg from the appressorium that penetrates either by force or by production of cell wall-dissolving enzymes (Linderman, 1994). Specific plant-regulated processes associated with penetration have been delineated. In *Lotus japonius*, for example, an epidermal cleft opens between two adjacent rhizodermal cells through which the fungus enters. From here, invasion of the rhizodermal cells is initiated and the fungus trespasses through the underlying exodermal cells (Parniske, 2004). In addition to entering through a rhizodermal cleft, the fungus can also traverse rhizodermal cells directly, as shown in detail for *Gigaspora gigantea* penetrating roots of *Medicago truncatula* (Genre et al., 2005). Hence, infection only occurs after preparatory activities in the plant cell. These cytological studies illustrate nicely the extensive complementary contribution made by host cell activity to fungal penetration. This emphasizes the significance and indispensable participation of plant processes in the coordinated invasion (Genre et al., 2005, 2008).

The infection process by arbuscular mycorrhizal fungi is also characterized by a low and regulated production of cell wall-degrading enzymes by the fungus. The production of exo- and endoglucanases, cellulases, xyloglucanases and pectolytic enzymes including polygalacturonase has been demonstrated in various investigations (Garcia-Garrido and Ocampo, 2002). In conclusion, AM fungi seem to colonize the root tissues of host plant by means of a combination of mechanical and enzymatic mechanisms and the coordinated participation of host cell machinery, which aids in the easier penetration of the mycorrhizal fungi into the root cortex (Bonfante and Perotto, 1992).

2.2.3 Mature Symbiotic Phase: Process and Signaling

Arbuscule Development

There are two main morphological types (*Paris* and *Arum*) of AM symbioses with structurally different interfaces. *Paris*-type AM are characterized by intracellular fungal coils that grow directly from cell to cell with little or no intercellular phase, whereas in *Arum*-type AM, a highly branched intracellular tree-like fungal structure known as the arbuscule is formed, subtended by intercellular hyphae (Dickson et al., 2007). Arbuscules are the key feature of the AM symbiosis as they represent an extreme form of intimacy and compatibility and are thought to be the site of nutrient transfer from the fungus to the host plant (Hughes et al., 2008). Following colonization of the host cell by the arbuscule, the architecture of the host cell undergoes remarkable changes. Despite the intense activity of both the partners leading towards arbusculated cells, arbuscules collapse after 2–4 days, leaving an intact cortical cell that is then able to host another arbuscule (Paszkowski, 2006; Pumplin and Harrison, 2009).

It is not known what triggers fungal entrance into the host cell but the perception of a radical sugar gradient between the vascular tissue and the outer cell layers may be involved in induction of arbuscule formation (Blee and Anderson, 1998). Alternatively, the initiation of arbuscule collapse may be caused by endogenous fungal signaling or coordinated signaling cross-talk. Development of arbuscules is at least partially under the control of the host genetic program. In addition, proteins of the SYM pathway are either required for arbuscule development, such as *LjCASTOR*, *LjSYM15* and likely *LjSYm6*, or contribute to arbuscule formation, such as *LjPOLLUX*, *LjNup 133* and *LjSYM* 24 (Kistner et al., 2005). Global gene expression profiling has been undertaken to identify AM-regulated genes (Guimil et al., 2005, Hojnec et al., 2005). In many cases gene expression was monitored at the stage of a mature symbiosis and long lists of induced and suppressed genes have been created. The list of genes probably includes those essential for signaling during the initiation and formation of arbuscules (Paszkowski, 2006). Hence, formation of arbuscules within host cells is associated with dramatic morphological and physiological alterations in both the symbiotic partners. The molecular basis underlying arbuscule formation has elucidated the role of numerous genes that encode proteins, directly or indirectly involved in a signal transduction network that is required for the development of intracellular accommodation structures for symbiotic fungi by the host cell (Parniske, 2008).

The Symbiotic Interface and Nutrient Transfer

In root symbiosis, the symbiosome is the cellular environment hosting the microbial partner where the mutual exchange of nutrients and metabolites occurs. In AM, this is the cortical cell lumen harboring hyphal coils or arbuscules surrounded by the perihyphal or periarbuscular plasma membrane, respectively. The periarbuscular membrane, continuous with the plasma membrane of the cortical cell, is a key interface in the symbiosis; however, little is known of its lipid or protein composition or the mechanisms of its development (Pumplin and Harrison, 2009). Both the microbe- and the plant-derived symbiosome membranes tightly regulate the exchange of compounds, which is generally facilitated by membrane-integral transport proteins such as phosphate transport and the P-type H^+-ATPase (Bucher, 2007). Recently, live-cell imaging with fluorescently-tagged proteins has revealed the spatial and temporal information about the protein composition of the periarbuscular membrane. The study indicates that the periarbuscular membrane is composed of at least two distinct domains; an 'arbuscule branch domain' that contains the symbiosis-specific phosphate transporter, MtPT4, and an 'arbuscule trunk domain', that contains the blue copper-binding protein, MtBcp1 (Pumplin and Harrison, 2009).

Extensive transcript profiling has revealed numerous genes that are reported to be upregulated or repressed in mycorrhizae (Liu et al., 2003; Guimil et al., 2005; Hojnec et al., 2005; Kistner et al., 2005). Experimental evidence also exists for cell-specific localization of either transcripts or promoter activity of several genes involved in arbuscule function (i.e. P nutrition) and development. These include genes encoding P-type H^+-ATPases (Krajinski et al., 2002) and P_i transporter genes from tomato and potato (Nagy et al., 2005), *Medicago truncatula* (Harrison et al., 2002), *Lotus japonicus* (Maeda et al., 2006), and the cereals barley, wheat and maize (Glassop et al., 2005). *Medicago truncatula* serine carboxypeptidase (*MtSCP1*), a gene sharing identity with Ser carboxypeptidase II proteins from barley, wheat and *Arabidopsis thaliana*, and *MtCell*, a gene coding a membrane-anchored endo-1, 4-β-D-glucanase-like protein, have been shown to be upregulated in the root cortex upon colonization of *M. truncatula* with *Glomus versiforme* (Liu et al., 2003). A mycorrhiza-specific class III chitinase gene is similarly regulated in cells containing developing or mature arbuscules (Bonanomi et al., 2001). The availability of expressed sequence tags (EST) of the model legume *M. truncatula*, and AM fungus, *Glomus intraradices*, permits identification of genes required for development of symbiotic interfaces. Candidate genes have been characterized and genes encoding one plant arabinogalactan protein (AGP) and three AGP-like (AGL) proteins have been identified. AGL proteins encoded by two AGL genes from *G. intraradices* (GiAGLs) represent a new class of AGPs not found in non-AM plants (Schultz and Harrison, 2008). Interestingly, several genes have been identified that are induced both in mycorrhized cortical cells and during rhizobial root colonization in legume species; for example, the early nodulin genes *ENOD2*, *ENOD40* (van Rhijn et al., 1997) and *ENOD11* (Chabaud et al., 2002), the *Vicia faba* leghemoglobin gene *VFLb29* (Vieweg et al., 2004), and the gene calcium-binding protein 1 (*Cbp1*), which encodes a protein sharing similarities with calcium-binding proteins (Kistner et al., 2005). This supports the model outlined for the common symbiotic pathway in AM and rhizobium-induced nodule development using signal transduction based on conserved mechanisms (Paszkowski, 2006; Bucher, 2007). Therefore, the transporters that mediate metabolite exchange at the interface between the plant and the fungus are of biotechnological interest, and some candidate genes encoding mycorrhiza-specific plant phosphate transporters have been cloned and sequenced (Parniske, 2008). These findings provide good support for the hypothesis that in infection processes in the symbioses between plant roots and two different microorganisms, i.e. AM fungi and rhizobial bacteria, similar signal perception and transduction cascades initiate mycorrhization and nodulation (Parniske, 2004; Manchanda and Garg, 2007).

3 Mycorrhizae and Their Diverse Roles

Mycorrhizae are the rule in nature, not the exceptions. In this association the fungus takes over the role of the plant's root hair and acts as an extension of the root system (Muchovej, 2004). The beneficial effects of AM fungi result from one or several of the mechanisms. With mycorrhizal colonization in the roots, there is increased absorption surface area, greater soil area exposed, greater longevity of absorbing roots, better utilization of low-availability nutrients and better retention of soluble nutrients, thus reducing reaction with soil colloids or leaching losses (Muchovej, 2004; Selvaraj and Chelleppan, 2006). AM increase establishment, nodulation and atmospheric nitrogen fixation capacity in legumes (Turk et al., 2008). Mycorrhizae influence the colonization of roots by other microorganisms, and reduce the susceptibility of roots to soil-borne pathogens such as nematodes or phytopathogenic fungi (Selvaraj and Chelleppan, 2006). AM also modify soil-plant-water relations, thus promoting better adaptation of plants to adverse conditions, such as drought, salinity or heat stress (Fig. 2). At elevated heavy metal concentrations in soils, mycorrhizal fungi have been shown to detoxify the environment for plant growth (Muchovej, 2004). The real significance of mycorrhizal fungi is that they connect the primary producers of ecosystems, plants, to the heterogeneously distributed nutrients required for their growth, enabling the flow of energy-rich compounds required for nutrient mobilization whilst simultaneously providing conduits for the translocation of mobilized products back to their hosts. Hence, understanding of the ecology and functioning of the AM symbiosis in the natural or agricultural ecosystem is essential for the improvement of plant growth and productivity.

3.1 Mycorrhizal Symbiosis and Mineral Nutrition

Obligately depending on plant photosynthates as energy sources, the extensive mycelial systems (the vegetative parts of the fungus) effectively explore soil substrates and acquire soil inorganic nutrients including the major macro-nutrients N, P and K and some micronutrients, Cu, Fe and Zn, with some capacity for acquiring organic nitrogen and phosphorus. These soil-derived nutrients are not only essential for AM development but are also partly transferred to the host plant (Smith and Read, 1997; Leake et al., 2004).

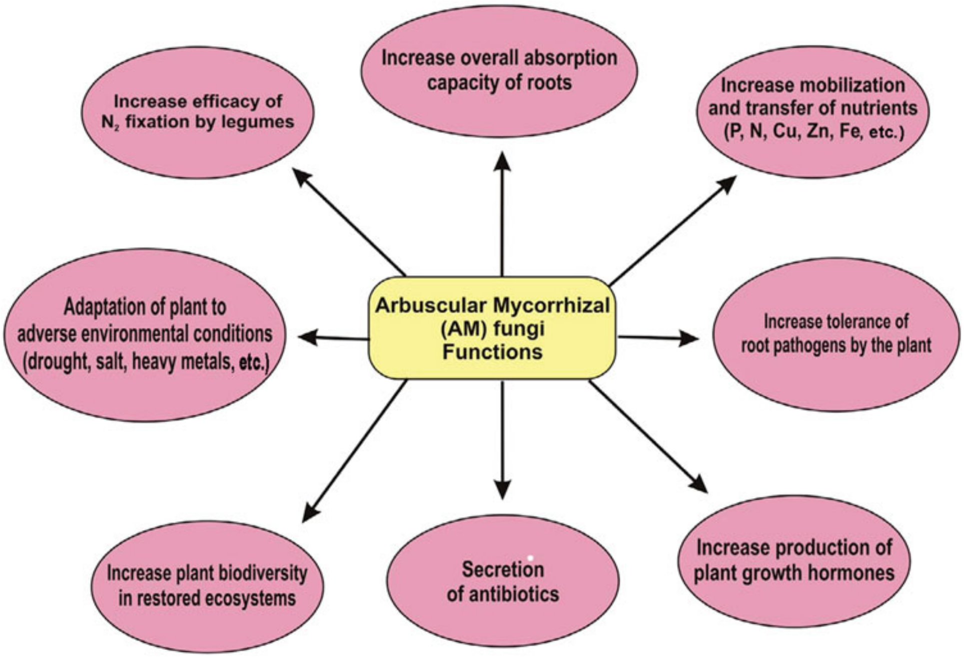

Fig. 2 Functional diversity of arbuscular mycorrhizal (AM) symbiosis in terrestrial ecosystems: An overview

3.1.1 Phosphate Uptake Assisted by the AM Symbiosis

The major role of AM fungi is to supply infected plant roots with phosphorus, which is an extremely immobile element in soils (Bucher, 2007). Even if phosphorus is added to soil in soluble form it soon becomes immobilized as organic phosphorus, calcium phosphates or other fixed forms (Wetterauer and Killorn, 1996). AM fungi are known to be effective in increasing nutrient uptake, particularly phosphorus and biomass accumulation of many crops in low phosphorus soil (Osonubi et al., 1991).

The Route of Symbiotic P_i Uptake

AM fungi improve plant acquisition of phosphate (P_i). It was recently shown that, depending on the particular plant-fungus combination, symbiotic phosphate uptake may partially participate or even dominate over all P_i acquisition (Smith et al., 2003). Strongly reduced mobility of P_i in the soil and rapid P_i uptake into the root lead to the development of a P_i depletion zone around the root hair cylinder and a rapid decline in P_i acquisition over time (Marschner, 1995; Roose and Fowler, 2004). The extraradical mycelium of AM fungus grows far beyond the depletion zone, reaching a new pool of soluble phosphate (Smith and Read, 1997). Whereas in non-mycorrhizal roots the extension of the P_i depletion zone is closely related to root hair length (Marschner and Dell, 1994), in mycorrhizal roots the depletion zone of P_i greatly exceeds the root hair cylinder. This indicates that P_i, which is not directly available to the plant, is delivered by the fungal hyphae. Thus, the presence of the P_i depletion zone in the rhizosphere is a major factor contributing to the advantage of plants forming mycorrhizal associations. Strictly speaking, a mycorrhized plant does not constitute a rhizosphere but rather a 'mycorrhizosphere', composed of the rhizosphere and the hydrosphere. In this symbiotic system, the fungus bridges the mycorrhizosphere and P_i is transported, in the form of polyphosphates, from the AM fungus soil interface to the intraradical symbiotic interface (Bucher, 2007).

The proposed metabolic route of symbiotic P_i acquisition starts with the assimilation of inorganic P_i at the hyphal-soil interface by fungal high-affinity transporters (Harrison and van Buuren, 1995; Maldonado-Mendoza et al., 2001; Benedetto et al., 2005). Inside the fungus, inorganic P_i is translocated in the form of polyphosphate from fungal structures outside of the root to those inside (Solaiman et al., 1999; Ohtomo and Saito, 2005). Before release into the periarbuscular interface, phosphate becomes depolymerized into inorganic P_i (Ohtomo and Saito, 2005). P_i is acquired from the interface by plant-encoded phosphate transporters. Such transporters have been identified from several plant backgrounds and were shown to be transcriptionally induced during the development of the AM symbiosis (Harrison et al., 2002; Paszkowski et al., 2002; Glassop et al., 2005; Nagy et al., 2005; Bucher, 2007; Javot et al., 2007; Pumplin and Harrison, 2009). Further, it has been demonstrated that the mycorrhizal P_i uptake pathway could dominate P_i supply to plants irrespective of whether colonized plants exhibited improved growth and total P uptake (Smith et al., 2003, 2004). The fact that the AM fungal contribution to plant P_i uptake is greater in a root-hairless mutant than its wild type indicates that fine tuning of both uptake pathways is required to meet the needs of the plants for this important nutrient (Jakobsen et al., 2005). To conclude, activation of the 'mycorrhizal' uptake pathway is therefore characterized by the induction of mycorrhiza-specific phosphate transporters and (partial) downregulation of the 'direct' uptake pathway phosphate transporters. Given that most phosphorus can be taken up via the 'mycorrhizal' uptake pathway, it can be hypothesized that mycorrhiza-upregulated plant phosphate transporters play a pivotal role in plant productivity and fitness in most natural and agricultural ecosystems.

Pht1 Genes Involved in the AM Symbiosis

Legumes such as *M. truncatula* and *L. japonicus* (Young et al., 2003) and solanaceous plants establish mutualistic AM symbiosis under natural conditions (Barker et al., 1998) and can thus be used as experimental systems for molecular-genetic work in mycorrhizae. Interestingly, a H^+-ATPase gene exhibited arbuscule-specific expression in mycorrhizal tissue of *M. truncatula* (Krajinski et al., 2002), and a H^+-ATPase protein was localized in the plant membrane around arbuscule hyphae in a tobacco mycorrhizal plant, which clarified the existence of nutrient transport activities at the interface between the two symbiotic organisms (Gianinazzi-Pearson et al., 2000). The identification of the potato P_i transporter gene *StPT3*, which is expressed in cortical cells colonized by AM fungi, represented a starting point for a detailed analysis of P_i transport at the AM symbiotic interface in solanaceous species. *StPT3* has been clearly identified as a high-affinity transporter. To date, one mycorrhizal-induced P_i transporter coding gene has been identified from *M. truncatula* (*MtPT4*; Harrison et al., 2002; Javot et al., 2007), three have been found in solanaceous species (*StPT3/LePT3*; *StPT4/LePT4* and *StPT5/LePT5*; Karandashov and Bucher, 2005; Nagy et al., 2005), two in rice (*OsPT11* and *OsPT13*; Paszkowski et al., 2002; Guimil et al., 2005) and one in maize, barley and wheat (Glassop et al., 2005).

Despite the large number of mycorrhizal-inducible P_i transporters identified to date, the functional genomics of P_i transport at the symbiotic interface between AM fungi and host plants is not well understood. To date, the most intriguing study on the functional role of mycorrhizal-specific P_i transporters originates from work on *StPT3*-like *LjPT3* from *Lotus japonicus* (Maeda et al., 2006). Knockdown of the *LjPT3* gene resulted in reduced growth of plants carrying transformed roots which were colonized by a mycorrhizal fungus, reduced allocation of radiotracer P_i in the shoot, and decreased fungal colonization of mycorrhizae (Bucher, 2007). The identification of mycorrhiza – specific P_i transporters indicate the presence of a mycorrhiza-specific P_i uptake system in vascular plants. Moreover, it is tempting to speculate that mycorrhizal P_i transporters are involved in self/non-self recognition in mycorrhizae.

3.1.2 Nitrogen Transfer at the Mycorrhizal Interface

As well as benefiting plants by aiding phosphorus uptake from soil (Harrison and van Buuren, 1995), AM fungi can take up and transfer significant amounts of inorganic nitrogen (NH_4^+ or NO_3^-) to their host plants (He et al., 2003). The availability of nitrogen frequently limits plant growth, and depending on soil conditions, nitrogen transfer by mycorrhizal fungi can represent a significant route of uptake by the plant (He et al., 2003). AM fungi have been strongly implicated in the transfer of nitrogen from one plant to another (He et al., 2003), can increase the utilization of different forms of nitrogen by plants (Hodge et al., 2001) and have been shown to take up nitrogen directly and transfer it to host roots (He et al., 2003). Experimental observations have indicated that arginine is usually the principal nitrogenous product accumulated during periods of ammonium feeding at the uptake site, providing support for the importance of this amino acid in N transfer between fungal and plant cells.

The extraradical hyphae of AM fungi are able to take up and assimilate ammonium (NH_4^+) (Johansen et al., 1992, 1993, 1996), nitrate (NO_3^-) (Bago et al., 1996; Johansen et al., 1996) and amino acids (Hawkins et al., 2000; Hodge et al., 2001) from their surroundings and translocate N from diverse sources to the plant (Hawkins et al., 2000; Azcón et al., 2001; Vazquez et al., 2001). Assimilation of NH_4^+ is a principal means of N absorption in AM fungal systems (Hawkins et al., 2000; Toussaint et al., 2004). N uptake and incorporation into amino acids via the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle has been found in AM fungi (Smith et al., 1985). Stable isotope labeling has now suggested that inorganic nitrogen is taken up by the extraradical mycelium, incorporated into amino acids, translocated from extra- to intraradical fungal structures as arginine and then transported as ammonium to the plant (Govindarajulu et al., 2005; Jin et al., 2005). Further support for this uptake route comes from the finding that transcript abundance of key enzymes of nitrogen assimilation and arginine breakdown preferentially accumulate in extra- and intraradical mycelia, respectively (Govindarajulu et al., 2005). A decline in the levels of the major amino acids present together with a decrease in the activity of fungal enzymes involved in the nitrogen assimilation during the mycorrhizal colonization process is seen (Blaudez et al., 1998). The extrusion of ammonia from fungal cells follows other pathways than those mediated by Amt proteins (ammonia transporter), either by passive efflux of the deprotonated form or by protein-mediated mechanisms. Thus, fungal cells are able to maintain a low cytoplasmic ammonia concentration, thus retaining a constant assimilatory capacity and in turn allow for sustained export into the plant root cells (Chalot et al., 2006). Therefore, the operation of a metabolic route assists the AM fungi in N uptake, transport and assimilation.

Various models have been proposed by Jin et al. (2005), Govindarajulu et al. (2005) and Chalot et al. (2006) which involve a number of steps for direct transfer of ammonia from fungal to plant cells (i) AM fungi take up inorganic N (NH_4^+, NO_3^-); (ii) absorbed N is mostly incorporated and stored in arginine; (iii) AM fungi assimilate the N through GS/GOGAT, asparagine synthase and the urea cycle; (iv) stored arginine can be co-transported with PolyP intact to the intraradical mycelium form the extraradical mycelium of AM fungi, and arginine is also bi-directionally transported within the extraradical mycelium; and (v) N released from transported arginine is transferred to the host as NH_4^+ and can be incorporated into other free amino acids in mycorrhizal roots, while carbon (C) not transferred to the host is recycled back to the extraradical mycelium. In analogy to the path of symbiotic P_i uptake, the arbuscule may be the site of symbiotic nitrogen uptake involving plant encoded nitrogen transporters located within the periarbuscular plant membrane (Paszkowski, 2006). Apart from the role of AM fungi in facilitating the uptake of inorganic N by plants, AM fungi also give their host plant the ability to use organic matter as a source of N (Hodge et al., 2001). A key role of the AM symbiosis in linking the process of N mineralization to plant N demand in soil, where the AM symbiosis regulates the recycling of plant residue into living plant biomass, thus impacting the structure of the soil microbial community, has been verified (Leigh et al., 2009; Nayyar et al., 2008). The mechanisms involved in the fungal delivery of N are a matter of considerable interest because, depending on N availability and mobility and given the near-ubiquity of the AM symbiosis, these processes may represent a significant nutritional benefit to the plant. The existence of this pathway and the high flux of nitrogen through it indicate that the arbuscular mycorrhizal symbiosis can effectively transfer large amounts of nitrogen from the soil to plant roots (Jackson et al., 2008).

3.2 Alleviation of Salt Stress by Arbuscular Mycorrhizal (AM) Fungi

Soil salinization is an ever-increasing threat to the cultivation of crop plants around the globe, with this problem being serious in arid and semi-arid areas. The arbuscular mycorrhizal (AM) symbiosis is a mutually beneficial interaction and is an important integral component of the natural ecosystem. AM fungi occur naturally in saline environments (Garcia and Mendoza, 2007) and have been shown to increase plant yield in saline soils. In saline and sodic soils, drainage is poor and salt accumulates on the surface of the soil, thus adversely affecting plant growth. Salinity affects the formation and function of mycorrhizal symbiosis (Giri et al., 2003; Juniper and Abbott, 2006). However, several studies have demonstrated that inoculation with mycorrhizal fungi improves growth and productivity of plants under a variety of salt stress conditions (Giri and Mukerji, 2004; Giri et al., 2007). Recently, many researchers reported that AM fungi could enhance the ability of plants to cope with salt stress (Yano-Melo et al., 2003; Rabie, 2005; Cho et al., 2006; Ghazi and Al-Karaki, 2006; Sannazzaro et al., 2006) by improving uptake of plant nutrients such as P, N, Zn, Cu and Fe (Cantrell and Lindermann, 2001; Asghari et al., 2005; Ghazi and Al-Karaki, 2006). The improvement in the plant P status has been suggested as the most important strategy of salinity stress tolerance in AM colonized plants (Giri et al., 2003). However, other studies have shown that salt stress tolerance of AM plants is not always related to improved P status (Feng et al., 2002), and other physiological processes help in AM plant growth improvement under such conditions.

AM fungi can play an important role in increasing the carbon dioxide exchange rate, transpiration and stomatal conductance (Feng et al., 2002), improving ion imbalance (Zandavalli et al., 2004; Giri et al., 2007), protecting enzyme activity (Giri and Mukerji, 2004; Rabie and Almadini, 2005), facilitating water uptake (Feng et al., 2002; Sheng et al., 2008; Colla et al., 2008), and favorably adjusting the osmotic balance and composition of carbohydrates (Ruiz-Lozano, 2003).

Thus, these microorganisms play an important role in soil productivity and plant nutrition under a variety of salt stress conditions. Owing to the importance of AM fungi under salt stress conditions, they have been considered as bio-ameliorators of saline soils. Mycorrhizal colonization can improve the physiological performance of stressed plants, leading to higher yields and quality. The induction of resistance is provided through a discriminated absorption of the ions present in the circulating solution, through better balance of mineral nutrient uptake, by influencing the hormonal balance of the host plant or by increasing water uptake.

3.3 Plant Water Relationship

Although most of the work done with AM fungi has concentrated on their effects in plant mineral nutrition, there is also increasing interest in drought resistance of mycorrhizal plants (Allen and Boosalis, 1983). AM fungi are important in sustainable agriculture because they improve plant water relations and thus increase the drought resistance of host plants (Allen and Allen, 1986; Nelsen, 1987). Improved plant water status and changes in water relations have been attributed to a wide variety of mechanisms, including some mechanisms not directly related to phosphorus nutrition or water uptake (Davies et al., 1992). The abilities of specific fungus-plant associations to tolerate drought are of great interest (Ruiz-Lozano et al., 1995). AM fungi infection has been reported to increase nutrient uptake in water-stressed plants (Busse and Ellis, 1985), enabling plants to use water more efficiently and to increase root hydraulic conductivity (Graham and Syversen, 1984). Root water uptake depends on root hydraulic conductance, which is ultimately governed by aquaporins (Luu and Maurel, 2005). Aquaporins are membrane intrinsic proteins that form a pore in all cell membranes of living organisms, facilitating the passive water flow through membranes following an osmotic gradient (Kruse et al., 2006). Plasma membrane intrinsic proteins (PIPs) regulate all water transport through plant tissues. The plants over-expressing or lacking one or more PIP genes have more or less root water uptake capacity, respectively (Aharon et al., 2003; Javot et al., 2003). In fact, AM plants are frequently more tolerant to drought and salt stresses than non-AM plants (Rosendahl and Rosendahl, 1991; Khalvati et al., 2005; Al-Karaki, 2006; Porcel et al., 2006; Aroca et al., 2007). AM plants are able to take up more water from the soil than non-AM plants under water-deficient conditions (Marulanda et al., 2003; Khalvati et al., 2005). However, this capacity depends on the fungal species, *Glomus intraradices* being one of the most efficient AM fungi in enhancing plant water uptake from the soil among six fungi tested (Marulanda et al., 2003).

Aroca et al. (2007) evaluated how AM symbiosis influences root hydraulic properties, aquaporin expression and abundance in roots of *Phaseolus vulgaris* L. plants under drought, cold or salinity conditions. They reported that colonization of *P. vulgaris* roots by the AM fungus *Glomus intraradices* prevented leaf dehydration caused by drought and salinity treatments as revealed by the higher relative water content (RWC) of AM leaves compared with non-AM leaves. These results confirm the beneficial effect of AM fungi in host plant water status under these two stresses (Rosendahl and Rosendahl, 1991; Porcel et al., 2006). The protection of mycorrhizal plants against water stress is related to the effects that the endophytes have on increasing leaf conductance and transpiration as well as P and K uptake. Potassium plays a key role in plant water stress and has been found to be the cationic solute which is responsible for stomatal movement in response to changes in bulk leaf water status (Ruiz-Lozano et al., 1995). Thus, AM symbiosis regulates root hydraulic properties and enhances root hydraulic conductance tolerance to drought, cold and salinity stresses (Aroca et al., 2007). Amelioration of drought stress by different AM fungal species can be ascribed to specific physiological (CO_2 fixation, transpiration, water-use efficiency) and nutritional (P and K) mechanisms according to the fungus involved in the symbiotic association. Suitably adapted AM fungal isolates are potentially important for maintaining and restoring the plant-soil equilibrium in sustainable agriculture situations (Ruiz-Lozano et al., 1995). The role played by AM fungi in alleviating water stress of plants has been investigated and it appears that drought resistance is enhanced. The precise mechanisms underpinning this are still in doubt but it could be an indirect effect of the extraradical mycelium improving nutrient absorption. The alleviation of water stress is not only limited to arid or semi-arid zones of the planet, but also where short-term droughts occur; an increased reliance on AM fungi for nutrient uptake can be frequently detected.

3.4 Protection of Host Roots from Pathogens

Multitrophic interactions are powerful forces shaping the structure of living communities. Plants encounter a great diversity of organisms in their environment: some of these interactions are beneficial, e.g. symbiotic fungi and insect pollinators, while some are detrimental, e.g. herbivore insects and pathogenic microorganisms. These interactions between below-ground and above-ground organisms are receiving increasing attention because they may influence plant defenses against biotic and abiotic stresses (van Dam et al., 2000). AM symbiosis is mutualistic interaction between plant roots and soil fungi that is considered beneficial to the plant because of the increased uptake of phosphorus and other scarcely mobile nutrients by mycorrhizal plants. Several investigations have demonstrated their positive impact on nutrient uptake and growth of banana (Jaizme-Vega and Azcón, 1995; Yano-Melo et al., 1999), improved resistance to abiotic stresses (Rufyikiri et al., 2000; Ruiz-Lozano, 2003) and biotic stresses caused by nematodes (Jaizme-Vega et al., 1997; Elsen et al., 2001) and *Fusarium oxysporum* f. sp *cubense*, the causal agent of panama disease (Jaizme-Vega et al., 1998).

Most studies on the interactions between AM fungi and plant parasitic nematodes reported that root colonization by AM fungi increases tolerance of the host to *Meloidogyne* species, such as that of tomato and white cover to *Meloidogyne hapla* (Cooper and Grandison, 1986); peanut to *M. arenaria* (Carling et al., 1996); banana to *M. incognita* (Jazme et al., 1997) and *Prunus* root stocks to *M. javanica* (Calvet et al., 2001). Colonization by AM fungi induces resistance or tolerance to a variety of pathogens in tomato and in other plants (Trotta et al., 1996; Lingua et al., 2002). These changes are mediated by a variety of mechanisms, including the upregulation and downregulation of specific genes (Tahiri-Alaoui and Antoniw, 1996), that result in localized and systemic responses by the plant. These responses include the synthesis of new isoforms of chitinases and glucanases and the thickening of the cell walls (Azcón-Aguilar et al., 2002; Pozo et al., 2002), that may affect herbivore colonization. Declerck et al. (2002) carried out investigations on the interaction between four AM fungi, *Glomus* sp., *Glomus proliferum*, *G. intraradices* and *G. versiforme*, and the root-rot fungus *Cylindrocladium spathiphylli*. Pre-inoculation of plants with AM fungi attenuated the detrimental effect of the pathogen. These results corroborate previous studies involving other plants grown in the presence of root pathogenic fungi, as these plants were less affected when colonized with AM fungi (Jaizme-Vega et al., 1998; Abdalla and Abdel-Fattah, 2000). The development of root-rot symptoms on bananas was considerably affected by the presence of AM fungi. The lower disease severity observed in mycorrhizal banana plants was associated with improved growth of the plants. Therefore, AM fungal symbiosis mainly affected the host-pathogen relationship by improving P nutrition, leading to greater resistance to root-infecting fungi. More recently, the effects of different species of AM fungi on parasitism rates have been reported (Gange et al., 2003) but this study did not demonstrate a direct link between AM and attraction of insect parasitoids.

Guerrieri et al. (2004) tested the hypothesis that an AM symbiosis makes tomato plants significantly more attractive towards the aphid parasitoid, *Aphidius ervi* (Haliday), which is well known for its efficiency against the potato aphid pest. The positive effect of mycorrhizal colonization on the attraction of the parasitic wasp, *A. ervi*, to its host plants and a negative effect on insect pest populations observed in this study have important implications for understanding of insect population dynamics and predicting the plant defense mechanisms. Castillo et al. (2006) investigated the effects of single and joint inoculation of olive planting stocks cvs. Arbequina and Picual with the AM fungi, *G. intraradices*, *G. mosseae* or *G. viscosum*, and the root knot nematodes *M. incognita* and *M. javanica* on plant performance and nematode infection. They concluded that prior inoculation of olive plants with AM fungi contributed to improved health status and vigor and reduced the severity of root galling by root-knot nematode. In conclusion, these results demonstrate the positive impact of AM fungi on P nutrition and plant growth under root pathogen pressure. However, this does not imply that better P nutrition alone accounts for increased resistance against phytopathogens. In the past decade, some authors have suggested that other mechanisms could be involved: proposed mechanisms include activation of the plant defense system and disease resistance (Benhamou et al., 1994; St. Arnaud et al., 1994); direct or indirect competition in the rhizosphere (St. Arnaud et al., 1994); biochemical changes in the plant and anatomical changes in the roots (Benhamou et al., 1994; Hooker et al., 1994); and competition for host resources in root tissues (Hooker et al., 1994). Hence, AM fungi have been shown to increase resistance to root-infecting pathogenic fungi and root-invading nematodes. The reasons for increased resistance to pathogens are not well understood, but a well-established AM infection is a prerequisite for protection. This is an intriguing area of research and bioprotection may be the primary role for AM fungi in some natural ecosystems rather than nutrient acquisition. The current emphasis on low input-based agrotechnology for crop production systems has stimulated the use of AM fungi as bioprotectors, phytostimulators or biofertilizers against plant diseases caused by soil-borne pathogens.

3.5 Mycorrhizoremediation

Biosphere pollution by heavy metals and nucleotides has been accelerated dramatically during the last few decades due to mining, smelting, manufacturing, treatment of agricultural soils with agro-chemicals and soil sludge, etc. (He et al., 2005). Heavy metals such as lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg), being added to our soils through industrial, agricultural and domestic effluents, persist in soils and can either be adsorbed in soil particles or leached into groundwater. Phytoremediation (such as phytoextraction, phytostabilization and rhizofiltration) of soils contaminated by heavy metals has been widely accepted as a cost-effective and environmentally friendly clean-up technology. However, the progress in this field is hindered by lack of understanding of complex interactions in the rhizosphere and plant-based mechanisms which allow metal translocation and accumulation in plants (Yu et al., 2004). Complex interactions between roots, microorganisms and fauna in the rhizosphere have a fundamental effect on metal uptake and plant growth. Some AM fungi are adapted to adverse conditions so they can benefit plants under a variety of environmental stresses. AM fungi are involved in plant interactions with soil toxic metals, either by alleviating metal toxicity to the host or by accentuating it (Meharg, 2003; Pawlowska and Charvat, 2002). Despite the significant role that AM fungi play in plant interactions with soil toxic metals and the ubiquity of AM fungi in the soil environment, only recently has progress been made towards understanding the cellular mechanisms utilized by AM fungi to metabolize heavy metals and alleviate their cytotoxicity (Lanfranco et al., 2002). Numerous studies have revealed that AM fungi confer upon plants tolerance against heavy metal stress (Davies et al., 2001; Levyal et al., 2002; Liao et al., 2003). Plant associations with AM fungi are suggested as a potential biological solution to ameliorate plant resistance to metal toxicity and restore fertility of soils polluted by heavy metals such as Cd (Vivas et al., 2005). A well-developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by better nutrient acquisition (P, N, Zn, Cu, Fe, etc.), water relations, pathogenic resistance, phytohormone production, contribution to soil aggregation, amelioration of soil structure, and thus improved success of all kinds of bioremediation (Jeffries et al., 2003; Gaur and Adholeya, 2004). Immobilization of metals in the fungal biomass is proposed as a mechanism by which these fungi may increase plant tolerance to heavy metals. Mycorrhizal roots may act as a barrier against metal transport, reducing transfer and enhancing root/shoot Cd ratios (Andrade and Silveria, 2008). This effect is attributed to metal adsorption on hyphal walls, since chitin has an important metal-binding capacity (Joner et al., 2000; Bi et al., 2003; Christie et al., 2004). Recently, it has been suggested that glomalin, a glycoprotein produced by AM fungi, may have a metal chelating function, diminishing metal availability for plants (Zhu, 2001; Gonzalez-Chavez et al., 2004; Khan et al., 2006; Saleh and Saleh, 2006). Another possible mechanism of metal tolerance includes dilution of metal concentration in plant tissues due to the promotion of plant growth by AM fungi (Andrade and Silveria, 2008), uptake exclusion by precipitation or chelation in the rhizosphere (Kaldorf et al., 1999), and P-mediated effects on the host plant (Wang et al., 2005). The AM fungi associated with metal-tolerant plants may contribute to the accumulation of heavy metals in roots in a non-toxic form inside hyphal cell walls or complexed into phosphate materials inside the cells (Galli et al., 1995). However, the effect of AM fungi on the uptake of metals by plants is not yet totally clear, with reports finding increased uptake of metals (Tonin et al., 2001; Liao et al., 2003; Whitefield et al., 2004; Citterio et al., 2005), decreased uptake (Weissenhorn et al., 1995, Chen et al., 2003) or no uptake effects (Trotta et al., 2006; Wu et al., 2001). Factors such as the metal element and its availability, plant species, fungal species or strain and differences in mycorrhizal and non-mycorrhizal plant size and P content in experiments may account for different results (Levyal and Joner, 2000).

AM fungi, therefore, play a significant ecological role in the phytostabilization of potentially toxic trace element-polluted soils by sequestration and, in turn, help mycorrhizal plants to survive in polluted soils. They can alter plant productivity, because AM fungi can act as biofertilizers, bioprotectants or biodegraders (Xavier and Boyetchko, 2002). Their potential role in phytoremediation of heavy metal-contaminated soils and water is also becoming evident (Khan, 2001; Jamal et al., 2002; Hayes et al., 2003). Thus, the benefits of mycorrhizae may be associated with metal tolerance and also with metal plant nutrition. Therefore, in degraded and contaminated soils, that are often poor in nutrients and with low water-holding capacities, mycorrhizae formation would be of great importance. In conclusion, a biotechnological goal is to use a combined inoculation of selected rhizosphere microorganisms to minimize toxic effects of pollutants and to maximize plant growth and nutrition (Saleh and Saleh, 2006). The ecological complexity and diversity of plant-microbe-soil combinations and the role that AM play in phytorestoration of heavy metal-contaminated soils, i.e. mycorrhizoremediation, thus need to be taken into consideration to restore the fertility of heavy metal soil (Khan et al., 2006). Ecosystem restoration of heavy metal-contaminated soil practices need to incorporate microbial biotechnology research and development. Since AM fungi are reported to be present on the roots of plants growing on heavy metal-contaminated soils, isolation of indigenous stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in disturbed ecosystems.

3.6 Miscellaneous Roles of Arbuscular Mycorrhizal (AM) Fungi

Roots colonized by AM fungi are often thicker and carry fewer root hairs. Such changes in morphology are expected to be under phytohormonal control (Selvaraj, 1998). Abscisic acid (ABA) was found to be considerably enhanced in both roots and shoots of AM plants as compared with non-mycorrhizal control (Danneberg et al., 1992). Also, an increase in Indole Acetic Acid (IAA), gibberellin and cytokinin level was observed in *G. fasciculatum*-inoculated *P. juliflora* recorded by Selvaraj (1998), showed the influence of the AM fungi, *G. fasciculatum*, on increased level of growth hormones. Barea and Azcon-Aguilar (1982) also noted that in axenic experiments, mycorrhizal fungi produced auxin-, gibberellin- and cytokinin-like substances and stimulated plant growth.

The mycorrhizal plants also showed increased production of certain enzymes. Increased peroxidase is one of the most widespread biochemical activities in diseased and injured plant tissues. Spanu and Bonfante-Fasolo (1998) measured the cell wall-bound peroxidase in *Allium porrum* during root growth and development of *G. versiforme*. Pacovsky et al. (1990) studied peroxidase activity in *Phaseolus vulgaris* infected by *G. etunicatum* and found that peroxidase activity increased in the mycorrhizal plants. Phosphatases of the mycorrhizae are both specifically induced in the presence of *Glomus* spores and are sensitive to the level of phosphate in the environment (Pacovsky et al., 1991). Selvaraj (1998) found that due to inoculation of AM fungi, *G. fasciculatum* acid phosphatase activity was increased in leaves and roots of *P. juliflora*. Tisserant et al. (1993) observed that histochemical tests revealed the presence of alkaline phosphatase in *Glomus*-infected roots of *A. porrum* and *Platamus acerifolia.* The presence of AM specific alkaline phosphatase activity in *A. cepa* and *P. accindentalis* plants inoculated with *G. mosseae* has been reported (Gianinazzi-Pearson and Gianinazzi, 1976). Mycorrhizae also enable plants to survive in disturbed and endangered ecosystems, as those are polluted by radioactive elements. In an atomic power station, products of nuclear fission reactions such as cesium, ^{137}Cs, and strontium, ^{90}Sr, are regularly released into the environment as a result of weapons testing, nuclear power production and nuclear fuel reprocessing (Haury and Schikarski, 1997). Plants absorb the Cs and Sr less efficiently than their nutrient analogues potassium and calcium, respectively (Zhu and Smolders, 2000). Both Cs and Sr are taken up from soil solution by plants as K^+ and Ca^{2+}, as they are similar to those cations in chemical properties (White and Bradley, 2000). AM fungi are beneficial for the uptake of nutrients and also for plants to survive, even from a disturbed soil after radionuclide deposition (Ahiabor and Hirata, 1994). Selvaraj et al. (2004) studied the effect of AM spores, *G. fasciculatum*, on two plants, *Phyllanthus niruri* and *Ecliptica alba*, and revealed that AM fungi was more beneficial for plant growth. It was noticed that in spite of growth disturbance, AM fungi-inoculated plants were efficient in tolerating the endangered ecosystems. The two experimental host plants showed more uptake of K^+ and Ca^{2+} in the roots of AM-inoculated plants as compared with the uninoculated plants. Thus, through the help of AM fungi and the soil's nature to hold the radionuclide to prevent the expression of radioactivity, chances are greater for the vegetation to survive in the disturbed ecosystems in a better way. There is very strong circumstantial evidence therefore that AM fungi would enhance uptake and recycling of radionuclides, particularly ^{137}Cs and strontium, ^{90}Sr (Selvaraj et al., 2004). Thus, plants receive support from AM fungi, with the help of its symbiotic association, in the aspect of uptake of phosphorus and other nutrients, enhancement of growth hormones, increase in protein content, increased tolerance to drought and heavy metals, increase in salinity tolerance and resistance to root-borne pathogens. Also, AM fungi can help in uptake of radionuclides in a disturbed ecosystem with radioactive elements (Selvaraj and Chelleppan, 2006).

So, not only do the activities of AM fungi have multiple functions that enhance plant performance and productivity, but they also play crucial roles in the development of soil properties and the health of the entire ecosystem. Thus, with important nutrient uptake functions of AM fungi being appropriately managed, mycorrhizae can potentially offer a more effective and sustainable element biofortification to curb human malnutrition.

4 Conclusion

The increasing consumer demands for organic or sustainably-produced food requires the incorporation of natural roles of microorganisms, such as arbuscular mycorrhizal (AM) fungi. It is clearly revealed from the preceding discussion that mycorrhizal symbiosis plays fundamental roles in shaping terrestrial ecosystems and must be considered as an essential factor for soil fertility and for promoting plant health and productivity. Over the past few years, a novel and unexpected developmental capacity of plant cells has been discovered that is essential for the intracellular uptake of AM fungi. Recent progress in the molecular and genetic analysis of mycorrhizal development has provided novel insights into the evolution of this symbiosis. Elucidation of molecular events associated with signaling and nutrient acquisition processes has moved rapidly forward. Several genes (GmGin1, *Lj POLLUX*, *Lj Nup 133, Lj SYM 24,* AM-specific Pht1-encoding genes and plant encoded nitrogen transporters) and signaling cascades have been identified which play a role in the establishment of mycorrhizal symbiosis. Collectively, these fungal symbionts confer tolerance to drought, heat, salinity, osmotic stress, disease and herbivory, and thus promote growth and nutrient acquisition. AM symbiosis occurs in almost all habitats, including disturbed soils contaminated with heavy metals, and plays an important role in metal tolerance. Thus, we can conclude that some plants are unable to endure habitat-imposed abiotic and biotic stresses in the absence of fungal endophytes. The use of stress-tolerant mycorrhizal fungi may be a promising strategy to develop tools for soil reclamation and amelioration.

Despite the identification of genes, proteins and enzymes involved in fungal and plant perception, and P and N transport and assimilation, very little is known about how these elements are transferred to the hosts. Thus, the understanding of molecular mechanisms and signaling pathways coupled to AM symbiosis needs further refinement and there is a need to unravel the complexity of the biology of AM symbiosis.

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Efficient N Management Using Winter Oilseed Rape

Klaus Sieling and Henning Kage

Contents

Abstract During the last decades the acreage of winter oilseed rape has been increased considerably in Europe. Rapeseed can take up a large amount of nitrogen before winter (>100 kg N/ha) and thus prevent nitrate leaching and pollution. Winter wheat is often grown subsequently, using oilseed rape as a favorable preceding crop. However, under wheat large nitrogen losses via leaching are frequently observed in humid climates during winter, mainly due to high amounts of soil mineral N available in fall and the small N uptake in fall of wheat as a subsequent crop. The low N offtake by the seeds results in a lower N-use efficiency and increases the N surpluses (>90 kg N/ha) compared with winter wheat (c. 40 kg N/ha). In addition, a large soil N pool increases the risk of N_2O emission, with its impact on climate change. In our review we discuss several options to increase nitrogen-use efficiency in oilseed rape-based cropping systems ranging from optimizing N fertilization practices to options arising from adopted tillage practices and crop rotation. N application in fall normally increases dry matter accumulation and N uptake before winter. However, because of its limited yield effects in most situations, fall N supply also boosts N surpluses. N fertilization in spring exceeding the need of the crop for optimal seed yield increases the risk of N leaching and decreases the farmer's net revenue. Considering the amount of N taken up by the canopy before the first spring application improves the determination of the optimal spring N supply. Measuring canopy N in fall gave the best results. At the cropping system level, time and intensity of soil tillage after the harvest of oilseed rape has concurrent goals of controlling volunteer rape, and achieving a successful establishment of the following crop, but avoiding an increased N mineralization. Changing

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_41,

from Sieling and Kage, Agron. Sustain. Dev. 30 (2010) 271–279. DOI: 10.1051/agro:2009036

the crop rotation by growing catch crops which prevent N from leaching is very effective in reducing N losses from the system by >40%. However, the economic losses from growing a usually less profitable spring crop probably limit the acceptance by farmers. Despite the problems addressed above, looking at the whole cropping system, oilseed rape is indispensable because of its beneficial effects on yield levels and nitrogen-use efficiency of following cereals, especially wheat, because alternative crops are often not realistic alternatives.

Keywords Oilseed rape • *Brassica napus* • N fertilization • N leaching • Seed yield • N balance

1 Introduction

The ‘Nitrate Directive’ (Directive 91/676/EEC) released in 1991 by the European Union aims to reduce water pollution caused or induced by nitrates from agricultural sources and prevent such pollution (European Union, 1991). In addition, the ‘Water Framework Directive’ (Directive 2000/60/EC) expands the scope of water protection to all waters and sets clear objectives that a ‘good ecological and chemical status’ must be achieved for all European water bodies by 2015 (European Union, 2000). The Water Frame Directive demands the prevention of deterioration in status and a progressive reduction of pollution. Concerning agricultural production, the impact of nitrogen (N) fertilization on the nitrate concentration of the water bodies becomes a crucial point in this context. Agriculture is the major contributor to nitrate contamination of groundwater (Fraters et al., 1998). For example, the German implementation of the Nitrate Directive (‘Düngeverordnung’) became effective in 2007 in a revised form and defines for the first time, among other regulations, legal thresholds for the N balance, calculated from N fertilization minus N offtake by the harvest products (Düngeverordnung, 2006). The surplus must not exceed a threshold of 90 kg N/ha on a 3-year average in the years 2006–2008, declining to 60 kg N/ha in 2009–2011 in order to reduce the environmental impact of N fertilization. Purely arable cropping systems with low amounts of organic N inputs are often regarded as low risk in terms of N leaching. The calculations of Henke et al. (2007), however, revealed that ex post optimized N levels of an oilseed rape – winter wheat – winter barley rotation were near the threshold of 60 kg N/ha, and a current survey of N balances in regions with high percentages of oilseed rape in the crop rotation showed that the N surpluses were often clearly above the acceptable level (Kelm et al., 2007).

N balances on a field scale or in larger areas are often used to estimate the leaching risk (Doluschnitz et al., 1992; Lord et al., 2002; Jansons et al., 2003; Sacco et al., 2003). However, there is much evidence that, in the short term, the link between fertilizer use (except excessive amounts) and nitrate in water is not very direct (Fig. 1) (Sieling and Kage, 2006). The N surplus in itself may not be sufficient to quantitatively determine the amount of N loss via various pathways, because of the complex interactions with other environmental parameters. For example, the large reserve of organic N in soils and vegetation will inevitably contribute nitrate to leaching once the land is tilled (Macdonald et al., 1989; Sylvester-Bradley and Chambers, 1992). N leaching depends on the amount of water percolating through the soil, mainly affected by winter rainfall (Webb et al., 2000), and the N concentration in the leachate. The latter is closely related to the mineral N pool in the soil at the beginning of and/or during the leaching period (Goss et al., 1991). Most of the N leached from arable soils originates from inorganic N present in late summer, fall or early winter when soils start to drain and plant

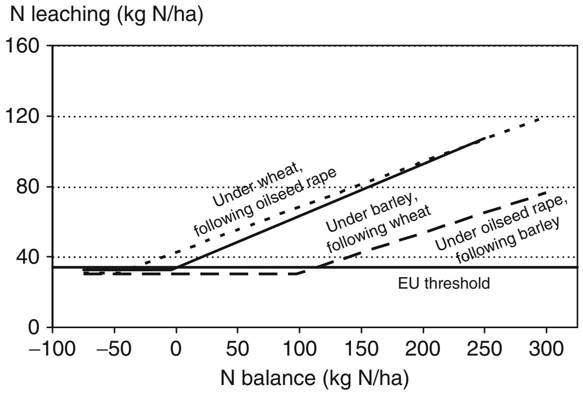

Fig. 1 Relationship between N balance (kg N/ha) and annual N leaching (kg N/ha) during the subsequent leaching period in different preceding crop – crop combinations (1991/92–1999/2000). The EU drinking water threshold (50 ppm nitrate) is given for 300 mm percolation (Sieling and Kage, 2006)

demand is low or non-existent. Generally, N leaching positively correlates with the N supply. Mineral N application has been shown to significantly increase N losses only for N rates exceeding the ex post determined economic optimum (e.g. Engels and Kuhlmann, 1993; Davies and Sylvester-Bradley, 1995; Goulding et al., 2000). Results from the Broadbalk Wheat Experiment in Rothamsted (United Kingdom) indicated that in most years only a little fertilizer-derived N remained in inorganic form at harvest from applications of up to 192 kg N/ha (Glendining et al., 1996). In the short term, therefore, reduction of N fertilization below the optimum had only small effects on fall soil mineral N and, in consequence, on N losses over winter (Macdonald et al., 1989; Lickfett, 1993; Zerulla et al., 1993; Shepherd and Sylvester-Bradley, 1996). More serious problems may arise from the use of organic manures and slurries, which are often applied to the stubbles after harvest on arable land, when no plant uptake occurs. Compared with mineral N fertilizers, crops utilize slurry N poorly, due to overwinter losses by leaching or denitrification (Smith and Chambers, 1992; Sieling, 2005).

On the other hand, however, the simple N balance is often the single parameter that can be estimated at the field, farm and regional level. It can give useful risk indications of specific farming practices, especially in the wider environment and if integrated over longer time periods (Öborn et al., 2003). Even if N fertilization meets plant N requirement in time and rate, long-term application of inorganic N fertilizer and, to a greater extent, of organic manures, may induce the building up of soil organic matter due to increased amounts and higher N concentration of crop residues being returned to the soil at harvest (Glendining et al., 1996). Up to certain levels this may be seen as a positive effect on soil fertility and the overall carbon balance, but it should also be clear that in consequence, N mineralization of soil organic N in the fallow periods, and consequently N leaching, may increase.

Farmers are asked to reduce the impact of nitrogen on the environment. Increased nitrogen supply to crops may increase yield but decreases N-use efficiency (Kuhlmann and Engels, 1989; Sieling and Hanus, 1997). If it has not been volatilized or denitrified, the N amount not utilized by the crop can accumulate in the soil and, in consequence, escalates the risk of leaching with corresponding environmental consequences.

Especially in rotations including winter oilseed rape N leaching is often a great problem (Fig. 1). Indeed, oilseed rape can be a suitable crop to conserve nitrogen throughout the winter, because of its large fall N uptake of 40–60 kg N/ha (Barraclough, 1989; Aufhammer et al., 1994). Due to its early development, oilseed rape often can utilize only a small proportion of the nitrogen mineralized in spring, especially under weather conditions with slow temperature increase in spring (Sieling et al., 1998a). Additional large amounts of easily mineralizable crop residue (petals and leaves) return to the soil after flowering. The harvest index (0.35) and the N-use efficiency of oilseed rape are low compared with cereals (Aufhammer et al., 1994; Shepherd and Sylvester-Bradley, 1996; Malagoli et al., 2005; Rathke et al., 2005; Sieling et al., 1998a, b, 1999; Sieling and Kage, 2006; Henke et al., 2007; Berry, 2009). Moreover, oilseed rape leaves the soil in a favorable structure, leading to an increased N release. All these facts result in large N amounts remaining in the soil (Shepherd and Sylvester-Bradley, 1996; Beaudoin, 2005). In most situations the subsequent crop following rape will be winter wheat (Sieling, 2005). Even sown early, N uptake of wheat before winter normally does not exceed 20 kg N/ha, which is only a small portion of the soil mineral N usually present after oilseed rape. In consequence, most of these N residues cannot be completely taken up by the subsequent wheat crop, and increase the risk of N leaching into the groundwater during the following percolation period (Goss et al., 1993; Sieling and Kage, 2006). Having a crop rotation where each crop is always following the same preceding crop, a differentiation between the preceding crop and the direct crop effects on N leaching is not possible.

Several approaches to reduce N leaching have been discussed, e.g. changes in crop rotation, growing of catch crops, reducing soil tillage in fall and reducing N fertilization (Allison et al., 1998; Kuhlmann and Engels, 1989; Lindén and Walgren, 1993; Rinnofner et al., 2008; Shepherd and Lord, 1996; Smith et al., 1990). However, acceptance by farmers often remains low, mainly due to economic losses.

The aim of this review was to investigate the necessity and the extent of some management measures carried out during (N fertilization in fall, N fertilization in spring) or following (soil tillage after harvest, growing catch crops) the rapeseed crop in order to improve the N-use efficiency and to reduce the environmental impact of oilseed rape production.

2 N Fertilization in Fall

Applying fall nitrogen to oilseed rape is common practice in NW Europe. In the last few years farmers have changed the crop rotation, replacing barley by wheat. In consequence, the sowing date is often delayed. In addition, they increasingly pass on plowing during seedbed preparation to reduce energy costs. To compensate for this worsening of establishment conditions, they additionally apply about 30–50 kg N/ha in fall, often directly upon the stubble of the preceding crop, to ensure crop N supply and adequate crop growth before winter.

In general it has to be distinguished if the rapeseed crop is actually able to take up fall-applied N before winter. If low temperature limits crop growth due to delayed sowing dates, applied N increases the soil mineral N ($NO_3{-}N$ plus $NH_4{-}N$) pool and, in consequence, the potential of N leaching during the subsequent percolation period (Table 1, sown in the 1st decade of September) (Sieling and Kage, 2007). In contrast, Engström et al. (2009) reported that application of 30 or 60 kg N/ha, at sowing, to winter oilseed rape did not affect the N leaching or seed yield. If fall N, especially in early-sown oilseed rape (Dejoux et al., 2003), leads to a better growth and enhances N accumulation before winter, it is a moot question whether the seed yield increases as well. Ogilvy and Bastiman (1992) reported that, although plots receiving nitrogen in the seedbed or at the two leaf stage appeared more vigorous before winter compared with unfertilized plants, neither the number of plants established, the survival over winter nor the seed yield appeared to be affected by this treatment (see also Table 1, sown in the 1st decade of September). Sieling and Kage (2007) observed after a fall N supply of 40 kg N/ha a yield increase of about 0.2 t/ha which was related to an additional N offtake by the seeds of about 4 kg N/ha. In maximum only 10% of the applied N amount was removed from the system, whereas 36 kg N/ha remained in the soil and charged the N balance (Table 2). Colnenne et al. (2002) and Flénet et al. (2009) observed in their experiments severe N deficiencies in fall, described in terms of the nitrogen nutrition index, together with a reduction in shoot biomass, tap root

Table 1 Effect of fall-applied N and sowing date on N uptake before winter, soil mineral N before winter and seed yield of oilseed rape in 2005/06 (Henke, 2008)

Sowing date Fall	Middle of August		Beginning of September	
N (kg N/ha)	0	40	0	40
N uptake before winter (kg N/ha)	95	131	65	74
Soil mineral N content before winter (kg N/ha)	19	23	34	63
Seed yield (t/ha)	4.69	4.75	4.80	4.86

Table 2 Effect of 40 kg N/ha applied in fall on above-ground dry matter before winter, N uptake before winter, seed yield and simple N balance of the fall-applied fertilizer N of oilseed rape (2003/04–2005/06, cv. Talent, minimum tillage following wheat)

	Above-ground dry matter (g/m^2)	N uptake (kg N/ha)	Seed yield (t/ha)	N balance (kg N/ha)
Unfertilized control	48.2^{b‡}	18.2^{b}	4.59^{b}	–
40 kg N/ha on the wheat stubble	68.1^{ab}	24.7^{b}	4.71^{ab}	+36
40 kg N/ha directly after drilling	86.6^{a}	36.3^{a}	4.69^{ab}	+37
40 kg N/ha in the 2-4 leaf stage of oilseed rape	87.0^{a}	36.7^{a}	4.81^{a}	+33

‡ Within a column, means followed by the same letter are not significantly different at $P < 0.05$.

biomass, leaf area index and radiation-use efficiency compared with well supplied treatments. However, despite severe fall N deficiencies, no difference in seed yield was apparent. The authors assumed that all the time allowed enough growth in fall to ensure sufficient regrowth in spring. However, in other experiments fall nitrogen gave a small yield response where the preceding cereal straw was baled or incorporated instead of burning (Chalmers, 1989; Chalmers and Darby, 1992).

Due to its ability to take up substantial quantities of nitrogen before winter (Barraclough, 1989), oilseed rape is regarded as a suitable crop to utilize fall slurry and to prevent slurry nitrogen from leaching during the percolation period. Detailed analysis revealed the higher leaching potential of fall slurry (up to 4.3 times) and, to a smaller extent, of spring slurry (up to 1.7 times) compared with mineral N fertilizers. The larger losses from slurry could be caused by its slower rate of N mineralization and the release of N when the crop cannot use it (Sieling et al., 1998a; Sieling and Kage, 2006). Applying slurry (80 kg N_{total}/ha) in spring during crop growth instead of fall allows one to reduce the additional mineral N fertilization by 40 kg N/ha (Fig. 2). However, further details about the effect of slurry applied in fall and/or in spring on seed yield are scarce.

N fertilization of oilseed rape in fall seems to be necessary only if N really limits crop growth, for example on poor soils or in the case of minimum tillage if the seeds germinate within a straw layer. If farmers assume that the rapeseed crop needs N in fall, N fertilizer should be applied to the crop itself and not before sowing (Sieling and Kage, 2007). On the other hand, the effect of a late N shortage in fall, e.g. on light soils, on yield potential of an early-sown and well-established rapeseed crop remains unclear.

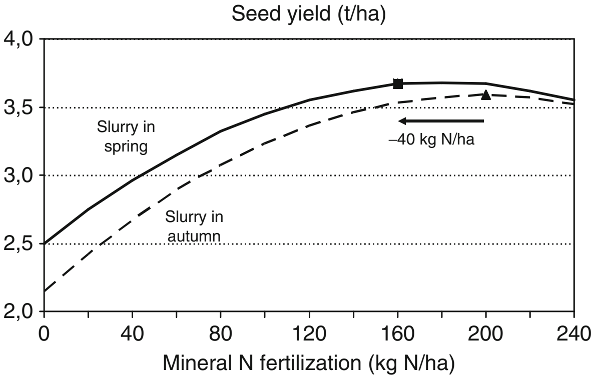

Fig. 2 Effects of slurry application in fall and spring on seed yield of oilseed rape

3 N Fertilization in Spring

An exact estimation of fertilizer N demand has become increasingly important to minimize the environmental impact, but also to achieve high seed yields and maximum economic returns. For this purpose, a better understanding of the course of N uptake and the yield formation is essential. Seed yield of oilseed rape consists of the number of plants/m^2, the number of pods per plant, the number of seeds per pod and the single seed weight (Grosse et al., 1992; Diepenbrock, 2000; Rathke et al., 2006). The onset of branches and buds starts even in late fall, whereas the final number of pods per plant and seeds per pod are determined within 1–2 weeks after flowering. Fall-sown oilseed rape normally receives nitrogen in spring as a split application at the beginning of growth and at stem elongation. This means that N fertilizers are normally applied before yield formation occurs. In contrast to wheat, it is not possible to directly support yield formation. In addition, some results showed that nitrate uptake increases from stem elongation to the beginning of flowering, whereas nitrate uptake during pod filling was low (Merrien et al., 1988; Jensen et al., 1997; Rossato et al., 2001). Other authors reported that N uptake remained high even after the start of pod filling (Schjoerring et al., 1995; Hocking et al., 1997; Malagoli et al., 2005). Spink (2009) pointed out that late N is required to maintain pod filling. However, most of the N supply in the pods was achieved mainly by N mobilization from vegetative parts (stem, leaves, taproot) (Malagoli et al., 2005).

The spring nitrogen requirement mainly depends on expected yield level and soil type (Chalmers and Darby, 1992). Zhao et al. (1993) suggested a maximum yield response to a N rate around 200 kg N/ha, which was in good agreement with the results of Bilsborrow et al. (1993), who obtained >85% of the maximum recorded yield with an application of 150 kg N/ha. Over the years 1989/90 to 1991/92, Aniol (1993) tested N amounts from 0 to 320 kg N/ha, varying the timing and distribution pattern. Using a multiple regression model he estimated a yield maximum of 5.04 t/ha in plots receiving 308 kg N/ha. Comparing similar amounts of N, distributions with a larger amount at the earlier stages (beginning of growth) increased the seed yield more than high rates at later stages. Split application ensured that high yield performance and optimum yield stability were achieved simultaneously (Boelcke et al., 1991). In contrast, Darby and Hewitt (1990) observed only small effects of timing and distribution of fertilizer N on seed yield. Super-optimal N can cause yield losses and increases the potential for leaching loss during the following winter (Islam and Evans, 1994; Shepherd and Sylvester-Bradley 1996; Sieling et al., 1997; Engström et al., 2009).

N uptake by the seeds ranged from 69 kg/N ha in an unfertilized control to 175 kg/N ha in plots which had received 320 kg N/ha (Aniol, 1993), thus leaving large amounts of fertilizer N in the system. Crop recovery of labeled fertilizer applied to oilseed rape in spring varied considerably with the soil and year, ranging from 31 to 70% (Chalmers and Darby, 1992; Jensen et al., 1997; Macdonald et al., 1997; Sieling et al., 1998a; Beims, 2005). Approximately half of the fertilizer-derived nitrogen was contained in the seed, and, where recovery of labeled nitrogen by the crop was poor, up to 39% of labeled fertilizer remained in the soil.

In Germany N fertilizers are commonly applied according to growth stage (Rathke et al., 2006), taking soil mineral N content at the start of spring growth into account by the ‘Nmin method’ developed by Wehrmann and Scharpf (1979). In general, high canopy N is considered in the calculation of N fertilization rates by subtracting a fixed value of 20 kg N/ha, which hardly accounts for the actual canopy development, and corresponding N amounts taken up by the canopy before fertilization. In contrast, balance-sheet methods regarding the amounts of mineralization of soil-borne N and the amount of N in rapeseed canopies at the end of fall and the end of winter have been developed in France (Reau et al., 1994; Makowski et al., 2005), resulting in an optimized N fertilization of oilseed rape with on average lower doses of N fertilizer (Hébinger, 2009).

In a series of field trials consisting of seven sites and two years, Henke et al. (2009) tried to verify this approach under different climatic conditions in order to derive site-specific N fertilization rates. First, several methods which can easily be used by the farmers for estimating canopy N were tested. The use of digital photos giving information on the leaf area index, and in consequence on the above-ground biomass and N uptake, was limited to canopies with a leaf area index <1. Also counting the number of leaves was not suitable for estimating the amount of N in the aerial biomass, whereas weighing above-ground fresh matter, as done in France, gave good results. Estimating canopy N by weighing fresh matter of a defined area with several replications is based on the assumption that aerial dry matter content and N concentrations vary only a little at early growth stages. Reau et al. (1994) found a nearly constant N concentration of 4.5% N (in dry matter) at early growth stages of oilseed rape. Colnenne et al. (1998) described a critical N dilution curve for winter oilseed rape with a constant N concentration of 4.48% at early growing stages. N concentration decreased when dry matter (t/ha) exceeded 1 t dry matter/ha following the equation $N_{conc} = 4.48\ W^{-0.25}$, where N_{conc} is the N concentration in the aerial dry matter (% N) and W the shoot dry matter (t/ha). The N concentration of 4.5% found by Henke (2008) in aerial dry matter confirmed the results of Reau et al. (1994) and Colnenne et al. (1998). The measured dry matter contents in fall averaged 10%, leading to conversion factors of 45 in fall (kg N/ha in the canopy = 45 * kg fresh matter/m^2) (Henke, 2008).

In a second step, economically optimal N fertilizer amounts were estimated from quadratic N response curves and related to the soil mineral N content in spring, and N in the rapeseed canopy in fall and/or in spring. The site caused a large variation; however, no interactions with the parameters were significant, meaning that the effects were similar at all sites. Soil mineral N in spring showed no correlation with the optimal N fertilizer amounts, since oilseed rape can take up large amounts of N before winter and therefore often depletes the mineral N pool in the soil. Even canopy N in spring only slightly correlated with the optimal N fertilizer amounts, since leaf losses, and consequently N losses, over winter highly influence canopy N at the beginning of spring growth (Dejoux et al., 2003). Based on 2 years, measuring canopy N in fall gave the best results. The regression (Fig. 3) showed a significant negative relationship between canopy N in fall and the optimal N fertilizer amounts. The slope of this regression was –0.7, indicating that these N amounts should be partly considered when calculating N fertilization rates (Henke et al., 2009).

Experimental results on the impact of canopy N in fall or at the beginning of spring growth on the optimal

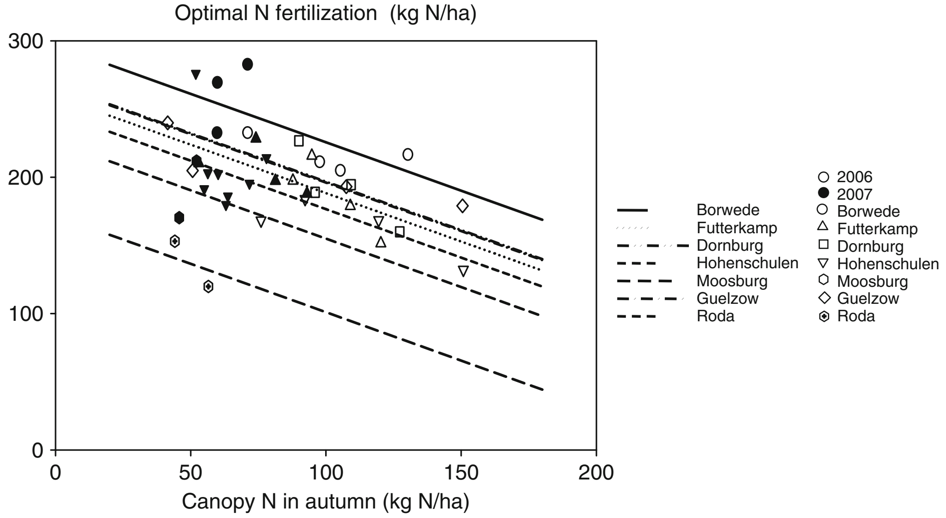

Fig. 3 Regression of the optimal N fertilization on canopy N in fall for seven experimental sites in Germany in two years (Henke et al., 2009)

N fertilizer amounts are scarce. Gabrielle et al. (1998) and Dejoux et al. (1999a, b) reported N losses caused by leaf losses due to frost over winter. Dejoux et al. (1999a) found a recovery of the N losses over winter by the rapeseed crop in spring of about 40% under the climatic conditions of their experimental site located in the Paris Basin (France). This recovery could be explained by the synchronization of lost leaves' decomposition and N uptake by the rapeseed crop in spring (Dejoux et al., 2003). If leaf losses occur over winter, N from dropped leaves is quickly mineralized in spring. Dejoux et al. (1999a) measured a decomposition fraction of added ^{15}N-labeled leaves of 0.94 before harvest. However, Dejoux et al. (2003) also found a fraction of gaseous N losses of 0.4 of N applied with frozen leaves estimated by unrecovered ^{15}N. These results indicate that N losses over winter are likely to be recovered in spring to some extent, and consequently N fertilization rates can be reduced. This recovery potential is totally neglected when canopy N in spring is used as an indicator for N fertilization rates in spring. Additionally, Dejoux et al. (2003) reported that the differences between canopies which varied largely in fall decreased during winter. Consequently, N fertilization rates would be overestimated if canopy N in spring was taken into account when deriving N fertilization rates. For these reasons canopy N in fall seems to be a more favorable indicator compared with canopy N in spring for the derivation of N fertilization rates. Besides the recovery of N derived from fallen leaves in spring, N uptake of unfertilized oilseed rape canopies in fall could be an indicator for the N mineralization potential of the location. In the case of insignificant leaf losses over winter, a well-developed rapeseed canopy showed a higher leaf area index at the beginning of spring growth than a poor canopy, which, in consequence, allows for reduced N fertilization rates (Mendham et al., 1981).

The results of Henke et al. (2009) clearly demonstrate that canopy N in fall is a suitable indicator for optimizing N fertilization rates. The implementation of this approach into practical use by farmers is based on an average rapeseed canopy with 50 kg N/ha accumulated in fall (1.1 kg FM/m^2) which will be fertilized in spring according to the official recommendation (e.g. 200 kg N/ha). Each kg N/ha exceeding the threshold of 50 kg N/ha reduces the N fertilization in spring by 0.7 kg N/ha. However, it is not possible to give absolute recommendations. In addition, the causes of the large site-to-site variations remain to be clarified. On the other hand, besides a reduction of N fertilization if canopy N is high, the study does not indicate that N fertilizer rates need to be increased if canopy N in fall is very poor because the crop is often not able to utilize the additionally applied N for its yield formation.

In the future the algorithm developed by Henke et al. (2009) allows optimizing N fertilization of oilseed rape site-specifically. Results of Müller et al.

(2008) and Behrens and Diepenbrock (2006) showed that N uptake of oilseed rape in fall can be estimated on a field scale using vegetation indices derived from hyperspectral reflection measurements. Based on these data, an application map for the N fertilization in spring can be generated which takes the within-field variation into account according to the approach presented by Henke et al. (2009). In France, a system based on satellite observations called 'Farmstar-colza' is currently being tested to draw a map of the fields with nitrogen supply advice, to practice modular application on the crops and to increase the precision of the crop N absorption assessment (Lagarde and Champolivier, 2006; Hébinger, 2009).

4 N Dynamic After Oilseed Rape

In contrast to the topics discussed above, soil tillage following oilseed rape does not affect the N balance of the crop, but strongly influences the N dynamics in the soil. As shown above, rapeseed demands high levels of nitrogen fertilizer, often exceeding 200 kg N/ha to achieve maximum yields. However, N offtake by the seed and N harvest index, ranging between 0.6 and 0.7, are low compared with cereals (Shepherd and Sylvester-Bradley, 1996; Sieling et al., 1999; Malagoli et al., 2005). Although rapeseed residues lead to N immobilization after incorporation into the soil (Jensen et al., 1997; Justes et al., 1999; Trinsoutrot et al., 2000), soil mineral N content increases regularly during fall (Lickfett, 1993). In consequence, large amounts of nitrate are likely to be leached with drainage water during the subsequent percolation period (Sieling et al., 1999; Sieling and Kage, 2006).

Increasing amounts of N fertilization to oilseed rape strongly raised soil mineral N content at the harvest of oilseed rape and during the subsequent fall. Several studies resulted in a remarkably higher soil mineral N content, sometimes exceeding 100 kg soil mineral N/ha at oilseed rape harvest following a two-straight-line function if high amounts of N fertilizer were used (Shepherd and Sylvester-Bradley, 1996; Sieling et al., 1999; Beaudoin et al., 2005; Makowski et al., 2005). Di and Cameron (2002) reported a threshold level of N fertilization above 200 kg N/ha which increased N leaching in arable cropping. Sieling and Kage (2006) stated a positive correlation between a simple N balance, calculated from N fertilization minus N offtake by the seed, and N leaching, and between mineral N fertilization and N leaching. This positive correlation between excessive N fertilization and N leaching is also confirmed by the scenario calculations made by Henke et al. (2008). Amounts of N fertilizer above 200 kg N/ha led to a strong increase in N leaching, whereas amounts below 200 kg N/ha differed slightly. These results show the importance of more precise calculations of N fertilizer doses considering soil and oilseed rape canopy properties (see Chap. 3).

According to Di and Cameron (2002), the prevention of soil mineral N accumulation in the soil after harvest is the key to reducing N leaching. There are several well-known agronomic measures to reduce mineralization of soil organic N, and in consequence, nitrate leaching after harvest. First of all, reducing tillage depth and delaying tillage after harvest diminish soil disturbance (Goss et al., 1993), and consequently soil N release, since intensive tillage operations after harvest stimulate net mineralization of soil-borne N due to soil disturbance (Lickfett, 1993). Using a combined approach of field trials and modeling, Henke et al. (2008) observed that minimum tillage combined with a short period of growing volunteer rapeseed as a catch crop before sowing of winter wheat can decrease N leaching.

Due to the tillage operations, however, not only the soil is mixed but also oilseed rape residues are incorporated into the soil. The C/N ratio in oilseed rape residues under a regular fertilization regime of about 200 kg N/ha was about 50 to 60. Justes et al. (1999) and Mary et al. (1999) report N immobilization of about 20 kg N/ha after rapeseed residue incorporation ($C/N = 54$, N fertilization 270 kg N/ha). Jensen et al. (1997) observed a decrease in soil mineral N of 18 and 25 kg N/ha after incorporation of 4 or 8 t oilseed rape residues/ha, respectively. In addition, Trinsoutrot et al. (2000) stated that the N amounts mineralized from rapeseed residues and then returned to the subsequent crop are relatively small. The studies showed that straw incorporation is an indispensable part of proper N management after harvest to prevent N leaching (Justes et al., 1999; Trinsoutrot et al., 2000; Beaudoin et al., 2005).

Secondly, changes in the crop rotation such as the introduction of catch crops and spring crops can decrease soil mineral N content during fall and therefore reduce the risk of nitrate leaching after oilseed rape. However, growing catch crops combined with a spring crop is less profitable than cropping winter wheat, which is commonly grown after oilseed rape, and therefore compensatory payments would be necessary, especially in water protection areas. Justes et al. (1999) reported a significant reduction of the soil mineral N content and nitrate leaching by a radish cover crop or volunteer rapeseed compared with a bare soil. Henke et al. (2008) suggested Phacelia as a catch crop, whereas Lickfett (1993) recommended volunteer oilseed rape to grow over winter without any tillage operation, thus reducing mineralization of soil-borne N, and consequently N leaching. However, additionally growing volunteer rapeseed is characterized by two disadvantages. Firstly, due to a lower drainage rate, the nitrate concentration in the percolation water can rise remarkably and exceed the threshold for drinking water of the European Union to a higher extent than the other treatments (Henke et al., 2008). Secondly, growing volunteer rapeseed could be critical because a high density of oilseed rape in the rotation can promote pathogens and pests (Christen, 2006).

5 Conclusion

Concerning N leaching, oilseed rape is an ambivalent crop. In fall, oilseed rape can take up large amounts of N and prevent it from leaching, whereas after harvest, due to a low N-use efficiency, N surplus and, in consequence, N losses via leaching are often high, especially if wheat follows. In order to minimize the environmental impact, farmers have to reconsider critically N fertilization of oilseed rape and to skip practices that have only small effects on yield, e.g. N fertilization in fall. In addition, N already taken up by the crop before winter should be taken into account when estimating spring N fertilization. Each kg fall canopy N exceeding 50 kg N/ha reduces spring N fertilization by 0.7 kg N/ha under German conditions. However, under the currently high prices for oilseed rape, some management measures might make sense from the economic point of view, being an important aspect for the farmers, although they do not decrease the N surpluses. Apart from the discussion of the N balance, appropriate management after oilseed rape harvest can reduce N leaching, if minimum tillage (or even no-till) is used and/or catch crops are grown.

Despite the problems addressed above, it should be pointed out that oilseed rape is indispensable as a favorable preceding crop for cereals, especially wheat. Growing legumes will raise similar problems as in oilseed rape, whereas the area of sugar beets is limited. Increasing the percentage of wheat within the rotation, up to monoculture, is also not sustainable, because wheat yield decreases while the amount of fertilizer N will increase to achieve optimal yields.

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Improving Nitrogen Fertilization in Rice by Site-Specific N Management*

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Contents

Abstract Excessive nitrogen (N) application to rice (*Oryza sativa* L.) crop in China causes environmental pollution, increases the cost of rice farming, reduces grain yield and contributes to global warming. Scientists from the International Rice Research Institute have collaborated with partners in China to improve rice N fertilization through site-specific N management (SSNM) in China since 1997. Field experiments and demonstration trials were conducted initially in Zhejiang province and gradually expanded to Guangdong, Hunan, Jiangsu, Hubei and Heilongjiang provinces. On average, SSNM reduced N fertilizer by 32% and increased grain yield by 5% compared with farmers' N practices. The yield increase was associated with the reduction in insect and disease damage and improved lodging resistance of rice crop under the optimal N inputs. The main reason for poor fertilizer N use efficiency of rice crop in China is that most rice farmers apply too much N fertilizer, especially at the early vegetative stage. We observed about 50% higher indigenous N supply capacity in irrigated rice fields in China than in other major rice-growing countries. Furthermore, yield response of rice crop to N fertilizer application is low in China, around 1.5 t ha^{-1} on average. However, these factors were not considered by rice researchers and extension technicians in determining the N fertilizer rate for recommendation to rice farmers in China. After a decade of research on SSNM in China and other Asian rice-growing countries, we believe SSNM is a matured technology for improving both fertilizer N use efficiency and grain yield of rice crop. Our challenges are to further simplify the procedure of SSNM and to convince policy-makers of the effectiveness of this technology in order to facilitate a wider adoption of SSNM among rice farmers in China.

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[*] Article from the International Workshop on Sustainable Nutrient Management: Technology and Policy organized by Prof. Fusuo Zhang and Dr. Yuxin Miao, May 28–21, 2008, Shijiazhuang, Hebei Province, China.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_42,

from Peng et al., Agron. Sustain. Dev. 30 (2010) 649–656. DOI: 10.1051/agro:2010002

Keywords Site-Specific nitrogen management • Nitrogen use efficiency • Grain yield • Nitrogen response • Rice • China

1 Introduction

Increase in fertilizer nutrient input, especially N fertilizer, has contributed significantly to the improvement of crop yields in the world (Cassman et al., 2003). Development of semi-dwarf rice (*Oryza sativa* L.) varieties in the 1960s assured the achievement of high grain yield under increased N fertilizer rates because of their lodging resistance at high N inputs (Yoshida, 1972). To maximize grain yield, farmers often apply a higher amount of N fertilizer than the minimum required for maximum crop growth (Lemaire and Gastal, 1997). Overapplication of N fertilizer may actually decrease grain yield by increasing susceptibility to lodging (Pham et al., 2004) and damage from pests and diseases (Cu et al., 1996).

China is currently the world's largest consumer of N fertilizer. In 2006, annual N fertilizer consumption in China was 31 million metric tons or 31.7% of the global N consumption (Heffer, 2008). About 18% of this N was used for rice production in China (Heffer, 2008). China accounts for about 19% of the world's rice planting area and produced 29% of global rice production in 2006 (FAOSTAT, 2008). Rice crops in China use about 36% of the total N fertilizer used for rice production in the world (Heffer, 2008).

There are several sources of information on the average N rate of rice production in China. The International Fertilizer Industry Association (IFA, 2002) reported that China's national average N rate for rice was 145 kg ha^{-1} in 1997. Based on data from 1995 to 1997, the average rate of N application for rice production in China was 180 kg ha^{-1} (FAO, unpublished data, 2001). If we use the data for the rice planting area from FAOSTAT and N consumption for rice from Heffer (2008), China's national average N rate for rice was 193 kg ha^{-1} in 2006, about 90% higher than the world average (FAOSTAT, 2008; Heffer, 2008). Nitrogen rates of 150 to 250 kg ha^{-1} are common (Wang et al., 2001; Peng et al., 2006). In Jiangsu Province, China, the average N rate reached 300 kg ha^{-1} in some counties (Q. Zhu, pers. commun., 2001). Average rice grain yield in China was 6.27 t ha^{-1} in 2006, about 53% higher than the world average (FAOSTAT, 2008). Rice crop in China received twice as much N fertilizer as in Japan but the grain yield was similar in the two countries.

Fertilizer N use efficiency can be measured in different ways using N-omission plots (Novoa and Loomis, 1981). The yield increase that results from N application in comparison with no N application is defined as the agronomic N use efficiency (kg grain yield increase per kg N applied). The apparent recovery efficiency of fertilizer N is used to express the percentage of fertilizer N recovered in aboveground plant biomass at the end of the cropping season. Yield response of rice crop to N fertilizer application is defined as N response, which is calculated as the difference between yields with and without N fertilizer (IRRI, 2006). The partial factor productivity from applied N is the ratio of grain yield to N applied and it provides an integrative index that quantifies total economic output relative to utilization of all N resources in the system, including indigenous soil N and fertilizer N (Cassman et al., 1996b). This parameter for quantifying fertilizer N use efficiency does not need N-omission plots.

The high rates of N fertilizer input and improper timing of N application in China have led to low recovery efficiency and agronomic N use efficiency. Zhu (1985) reported that recovery efficiency in China was less than 30% for ammonium bicarbonate and 30–40% for urea. Li (1997) estimated that recovery efficiency for rice in China was around 30–35%. However, Li (2000) observed that the average recovery efficiency of rice in Jiangsu Province was only 20%. This was further confirmed by Wang et al. (2001), who reported that recovery efficiency of farmers' N fertilizer practice was 18% in an on-farm experiment conducted in Zhejiang. Peng et al. (2006) found that recovery efficiency of farmers' N fertilizer practice was 20 to 30% in four provinces in China.

Yoshida (1981) estimated agronomic N use efficiency to be 15–25 kg rough rice per kg applied N in the tropics. Cassman et al. (1996b) reported that agronomic N use efficiency was 15 to 18 kg kg^{-1} N in the dry season in the farmers' fields in the Philippines. In China, agronomic N use efficiency was 15–20 kg kg^{-1} N from 1958 to 1963 and declined to only 9.1 kg kg^{-1} N between 1981 and 1983 (Lin, 1991). Since then, agronomic N use efficiency has further decreased in China because of the increase in N rate (Peng et al., 2002). Wang et al. (2001) reported that agronomic N use efficiency of farmers' N fertilizer practice was 6.4 kg kg^{-1} in Zhejiang. Peng et al. (2006) reported that rice yield increases by only 5 to 10 kg for every kg of N fertilizer input by using farmers' N fertilizer practice in China.

2 Principles and Procedures of Site-Specific N Management (SSNM)

Site-specific N management (SSNM) was developed to increase fertilizer N use efficiency of irrigated rice (Dobermann et al., 2002). In SSNM, N application is based on the crop demand for N. Climatic factors (solar radiation and temperature) and indigenous N supply largely affect crop N demand. Indigenous N supply includes N from soil mineralization, irrigation water and crop residues. Soil chemical analysis is not reliable to quantify indigenous N supply for paddy soil (Cassman et al., 1996a). Therefore, the measurement of grain yield in N-omission plots is used to obtain field-specific estimates of the indigenous N supply (Cassman et al., 1996b). During the growing season, leaf N status measured with a chlorophyll meter (SPAD) or leaf color chart is a good indicator of crop N demand (Peng et al., 1996). Both the SPAD and leaf color chart provide a good estimation of leaf N content on a leaf-area basis (Peng et al., 1993; Yang et al., 2003). In-season upward or downward adjustments of predetermined N topdressings at critical growth stages are made based on SPAD or leaf color chart readings at these stages. In this approach, the timing and number of N applications are fixed while the rate of N topdressing varies across seasons and locations.

In current SSNM practice, four steps are needed to estimate total N rate based on indigenous N supply capacity and target yield: (1) set an attainable yield target based on 85% of yield potential, (2) estimate indigenous N supply – yield without N fertilizer, (3) estimate N response – the difference between target yield and yield without N fertilizer, and (4) estimate N rate based on N response and agronomic N use efficiency. A realistic target agronomic N use efficiency is chosen by considering the yield and agronomic N use efficiency of the location in previous seasons (IRRI, 2006). The total N is distributed at one day before transplanting, midtillering, panicle initiation and heading with approximate proportions of 35, 20, 30 and 15%, respectively (Peng et al., 2006). The pre-determined N rate is 30 kg ha^{-1} at midtillering and 40 kg ha^{-1} at panicle initiation. The actual rates of N topdressing at midtillering and panicle initiation are adjusted by ±10 kg N ha^{-1} according to leaf N status measured with a SPAD or leaf color chart. When leaf N is below the low level of the SPAD or leaf color chart thresholds, N rate will be increased by 10 kg ha^{-1}; when leaf N is greater than the upper level of the SPAD or leaf color chart thresholds, it will be decreased by 10 kg ha^{-1}. No adjustment in N rate is needed when leaf N is within the thresholds. At heading, N is applied only if leaf N is below the SPAD or leaf color chart thresholds. We used the same SPAD and leaf color chart thresholds across different growth stages for a given variety (Peng et al., 1996). The SPAD and leaf color chart thresholds vary with varieties, which needs to be determined experimentally. Japonica generally requires higher thresholds than indica. For example, SPAD thresholds of 35–37 were used for indica varieties and they were 2 units higher for japonica varieties (Huang et al., 2008). The SPAD value of 35 corresponded to a leaf color chart score of 3.2, 4.8 and 5.2 for the leaf color charts developed by the International Rice Research Institute (IRRI), Zhejiang Agricultural University, China, and University of California-Davis, USA, respectively (Yang et al., 2003).

The procedures of SSNM can be simplified in two ways. Firstly, if there is no N-omission plot and its grain yield is unknown, one can estimate the N response based on the information of climatic yield potential and soil fertility (i.e. skipping the first two steps in estimating total N rate). Secondly, if farmers do not want to measure leaf N status using the SPAD or leaf color chart, upward or downward adjustments of N topdressings can be made based on visual leaf N status (IRRI, 2006).

Since 1997, SSNM has been evaluated in farmers' fields in eight major irrigated rice domains in Asia (Dobermann et al., 2002), including rice farms in Zhejiang Province, China (Wang et al., 2001, 2004). Across all sites in Asia, average grain yield increased by 11% and average recovery efficiency increased from 31% to 40%, with 20% of all farmers achieving more than 50% recovery efficiency (Dobermann et al., 2002).

3 IRRI-China Collaboration on SSNM Research

IRRI scientists in collaboration with partners in China have started research work on improving rice N fertilization through SSNM in China since 1997 (Wang et al., 2001). Field experiments were conducted initially in Zhejiang province in 1997 and gradually expanded to Guangdong, Hunan and Jiangsu provinces in 2001, to Hubei province in 2003, and to Heilongjiang province in 2005 (Peng et al., 2006). These six provinces occupy about 45% of the rice planting area in China. Collaborating organizations in China are Zhejiang University, Guangdong Academy of Agricultural Sciences, Hunan Agricultural University, Yangzhou University, Huazhong Agricultural University, Northeast Agricultural University, China Agricultural University and the Chinese Academy of Sciences.

From 1997 to 2000, on-farm demonstration of SSNM was conducted in Zhejiang province in farmers' fields by comparing SSNM with farmers' N fertilizer practice (Wang et al., 2001, 2004). From 2001 to 2003, SSNM was compared with other N treatments including real-time N management, farmers' N fertilizer practice, modified farmers' N fertilizer practice, and three fixed-N split treatments in the replicated on-farm trials in Zhejiang, Guangdong, Hunan and Jiangsu provinces (Peng et al., 2006). In real-time N management, N was applied only when the leaf N content was below a critical level. The farmers' N fertilizer practice was modified by reducing the total N rate by 30% and this reduction was restricted to within 10 days after transplanting. The three fixed-N split treatments had total N rates of 60, 120 and 180 kg ha^{-1} with 35% applied at basal, 20% at midtillering, 30% at panicle initiation, and 15% at heading. From 2005 to 2007, SSNM was compared with the real-time N management using four SPAD thresholds in the replicated on-farm trials in Guangdong, Hunan, Hubei and Jiangsu provinces. Overall, SSNM performed better than the real-time N management and the modified farmers' N fertilizer practice because the total N rate of SSNM was closer to the optimal level. In on-farm demonstration and farmer participatory research, we focused mainly on SSNM technology for improving fertilizer N use efficiency in irrigated rice in China.

On-farm demonstration of SSNM was done in the six provinces from 2003 to 2007. Only one location was chosen in each province. About 10 farmers from each location participated in the demonstration experiment. Each farmer's field had three treatments: farmers' N fertilizer practice, SSNM and N omission. The field was divided into two equal parts with a levee, and farmers' N fertilizer practice and SSNM treatments were randomly assigned to each half of the field. The N-omission plot (5 m × 5 m) was installed in the half of the field with SSNM treatment. In the farmers' N fertilizer practice, farmers apply N fertilizer (form, rate and timing) based on their own decision. Other crop management practices including P and K application were identical among the three treatments.

We used farmer participatory research for on-farm testing of N fertilization by standard and farmer-modified SSNM for irrigated rice in Guangdong, Hunan, Hubei and Jiangsu provinces from 2003 to 2005 (Hu et al., 2007). Twelve to 15 farmers were randomly selected in each study village in each year for a dialogue with the research team and for a rapid rural technology assessment. Based on the information obtained from the rapid rural technology assessment, modified SSNM schemes were developed through dialogue between a research team and farmers at a workshop in each village. Modification mainly involved decreasing the number of N fertilizer topdressings and increasing the rate of basal N application. Based on the farmers' willingness, 144 farmers were selected to conduct an experiment to compare SSNM or modified SSNM with farmers' N fertilizer practice.

Data from on-farm field trials, on-farm demonstration and farmer participatory research in the six provinces in China from 2001 to 2007 were pooled to compare SSNM with farmers' N fertilizer practice and other N treatments. The same dataset was used to determine the relationships among yield with N fertilizer, zero-N yield, N response and agronomic N use efficiency. There were 544 observations for these analyses. Data from the on-farm demonstration of SSNM in Zhejiang province from 1997 to 2000 were published elsewhere (Wang et al., 2001, 2004) and not included in the analyses of this paper.

4 Key Research Findings

There were wide ranges in both grain yield and total N rate across all experiments (Fig. 1). Grain yield varied from 4 to 10 t ha^{-1} with an average of 7.28 t ha^{-1},

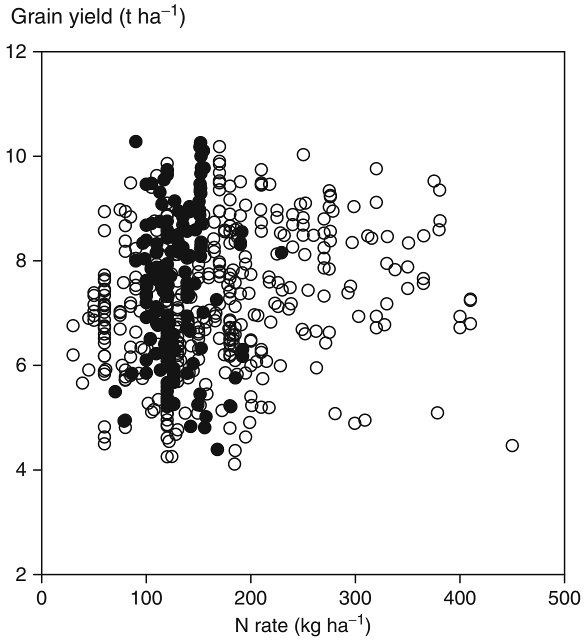

Fig. 1 Grain yield and N rate of site-specific N management (solid symbols) and other N treatments (open symbols). Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007

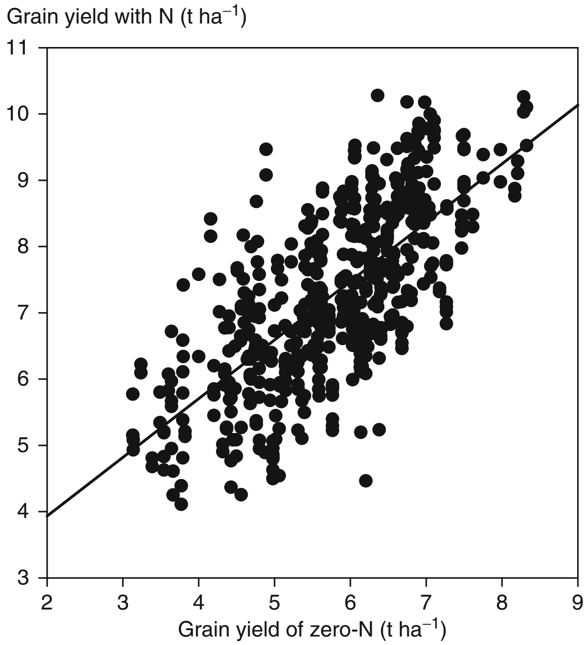

Fig. 2 Relationship between grain yield with and without N fertilizer. Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007. $y = 2.16 + 0.886x$ ($r = 0.70$; $n = 544$)

while total N rate ranged from 50 to 400 kg ha^{-1} with a mean of 157 kg ha^{-1}. SSNM was able to narrow the range of total N rate to 80–200 kg ha^{-1} with a mean of 120 kg ha^{-1}. There was no correlation between grain yield and total N rate. This poor relationship was caused by many factors including location, season, variety, pest damage, other crop management practices, etc., which affected grain yield aside from total N rate.

We observed a relatively high indigenous N supply capacity in irrigated rice fields in China compared with other major rice-growing countries. Crop N uptake at maturity when N fertilizer was not applied was in the range of 90–100 kg ha^{-1}, compared with 60–70 kg ha^{-1} under tropical conditions (Dobermann et al., 2003). Grain yield of zero-N control ranged from 3 to 8 t ha^{-1} and averaged at 5.77 t ha^{-1} (Fig. 2). There was a positive relationship between grain yields with and without N fertilizer.

Averaged across 107 farmers who conducted on-farm demonstration, SSNM produced 5% higher grain yield than farmers' N fertilizer practice (Table 1). The yield increase was associated with the reduction in insect and disease damage and improved lodging resistance of rice crop under the optimal N inputs (Zhong et al., 2006a, b). This small yield increase was achieved when total N rate was reduced from 195 to 133 kg ha^{-1}, a 32% saving of N fertilizer over farmers' N fertilizer practice. The N response of SSNM was 28% higher than that of farmers' N fertilizer practice. Consequently, SSNM almost doubled farmers' N fertilizer practice in agronomic N use efficiency, and had 55% higher partial factor productivity of applied N than farmers' N fertilizer practice. Similar results were obtained when SSNM was compared with other N treatments such as real-time N management, farmers' N fertilizer practice, modified farmers' N fertilizer practice, and fixed-N split treatments in 25 replicated on-farm trials in five provinces between 2001 and 2007 (Table 2). Yield increase was insignificant but SSNM saved 25% N fertilizer over other N treatments. SSNM increased N response by 15%, agronomic N use efficiency by 55%, and partial factor productivity of applied N by 38% compared with other N treatments. In farmer participatory research, the rate and distribution of N fertilizer during the growing season of

Table 1 Grain yield, total N rate, yield response to N application, agronomic N use efficiency, and partial factor productivity of applied N (PFP) of farmers' fertilizer practice and site-specific N management (SSNM). Data were from on-farm demonstrations conducted by 107 farmers from six provinces in China between 2003 and 2007. Average grain yield of zero-N control was 5.69 t ha^{-1} across the 107 farmers

Parameters	Farmers' practice	SSNM	Difference (%)
Grain yield (t ha^{-1})	7.08 b	7.47 a	5
N rate (kg ha^{-1})	195 a	133 b	38
N response (t ha^{-1})	1.39 b	1.78 a	25
Agronomic N use efficiency (kg kg^{-1})	7.1 b	13.4 a	61
PFP (kg kg^{-1})	36.3 b	56.2 a	43

Within a row, means followed by different letters are significantly different at the 0.05 probability level according to the least significant difference (LSD) test.

Table 2 Grain yield, total N rate, yield response to N application, agronomic N use efficiency, and partial factor productivity of applied N (PFP) of fixed N-rate treatments and site-specific N management (SSNM). Data were from 25 on-farm field experiments conducted in five provinces in China between 2001 and 2007. Average grain yield of zero-N control was 5.87 t ha^{-1} across the 25 experiments

Parameters	Other N treatments	SSNM	Difference (%)
Grain yield (t ha^{-1})	7.45 a	7.69 a	3
N rate (kg ha^{-1})	161 a	120 b	29
N response (t ha^{-1})	1.58 a	1.82 a	14
Agronomic N use efficiency (kg kg^{-1})	9.8 b	15.2 a	43
PFP (kg kg^{-1})	46.3 b	64.1 a	32

Within a row, means followed by different letters are significantly different at the 0.05 probability level according to the least significant difference (LSD) test.

modified SSNM were in between those of SSNM and farmers' N fertilizer practice (Hu et al., 2007). Both SSNM and modified SSNM, compared with farmers' N fertilizer practice, maintained rice yields with significantly less N fertilizer and no significant increase in total labor input. The reduction in N fertilizer input averaged 48 kg ha^{-1} for SSNM and 23 kg ha^{-1} for modified SSNM.

Large variation in N response was observed across 544 observations (Fig. 3). The 1.5 t ha^{-1} average N response across all experiments is very low compared with the average N response of 3.0 t ha^{-1} observed under tropical conditions (Dobermann et al., 2003). Negative N response occurred when the application of N fertilizer actually reduced grain yield. Yield reduction was often observed under excessive N input due to greater pest damage and lodging. There was no correlation between N response and total N rate. High total N rates did not result in high N response. The N response was correlated positively with grain yield when N was applied (Fig. 4). There was a negative but weak correlation between N response and the grain yield of zero-N control.

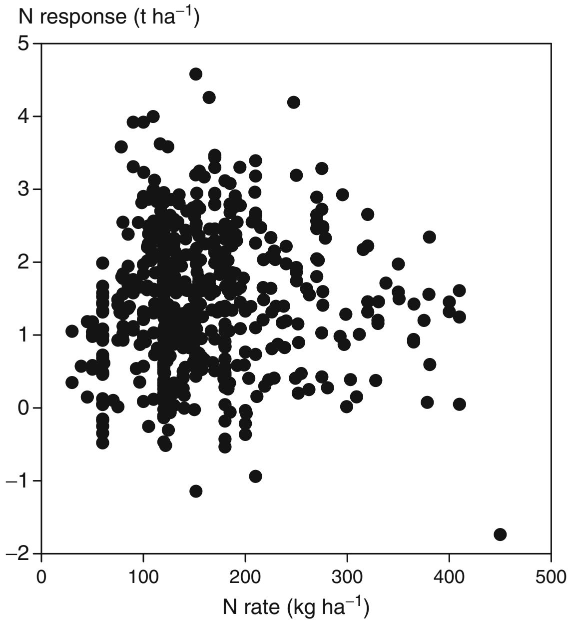

Fig. 3 Relationship between yield response to N application (N response) and N rate. Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007

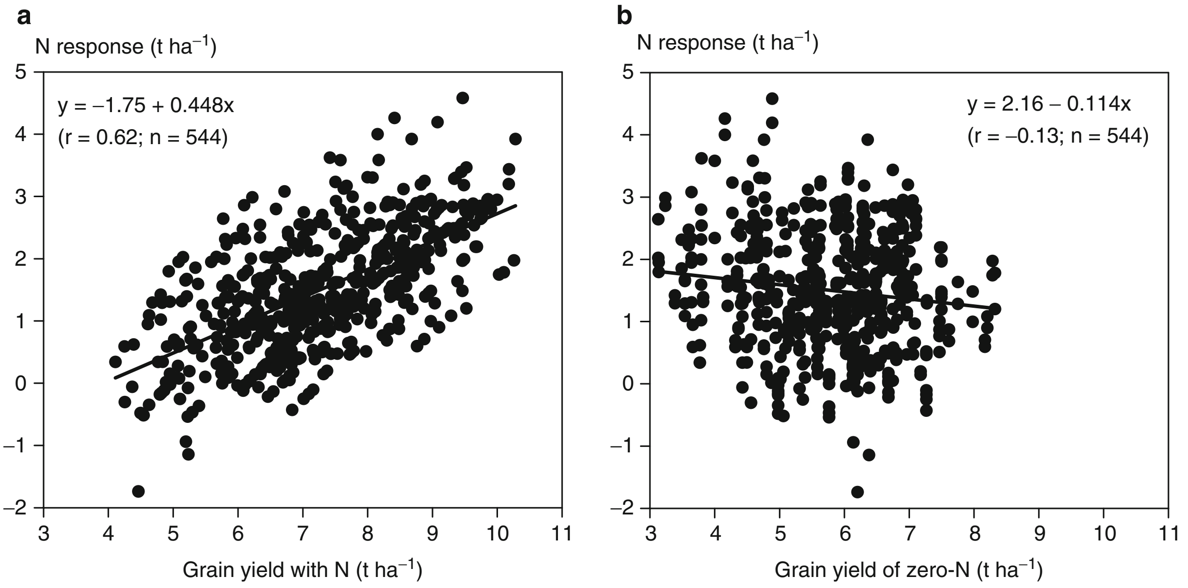

Fig. 4 Relationship between yield response to N application (N response) and grain yield with N fertilizer (**a**) and grain yield without N fertilizer (**b**). Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007

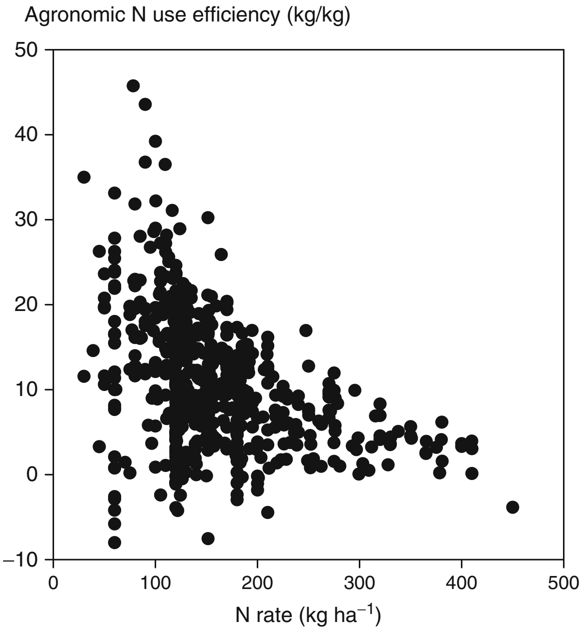

Fig. 5 Relationship between agronomic N use efficiency and N rate. Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007

There was a negative relationship between agronomic N use efficiency and total N rate (Fig. 5). Large variation in agronomic N use efficiency was observed when total N rates were between 80 and 120 kg ha^{-1}. When total N rates were greater than 300 kg ha^{-1}, agronomic N use efficiency was below 10 kg kg^{-1}. Negative agronomic N use efficiency was observed when grain yield was reduced by the application of N fertilizer. Like N response, agronomic N use efficiency was correlated positively with the grain yield of N-applied treatments but negatively with the grain yield of zero-N control (Fig. 6).

Grain yield and total N rate were generally higher in the North than in the South. Grain yield of zero-N control suggests that indigenous N supply was the lowest in Guangdong compared with the other five provinces. The N response and agronomic N use efficiency were the highest in Heilongjiang compared with the other five provinces. Therefore, there is a great potential to improve N management through SSNM in the provinces where total N rate is high and agronomic N use efficiency is low.

The main reason for poor fertilizer N use efficiency of rice crop in China is that most rice farmers apply excess N fertilizer, especially at the early vegetative stage (Peng et al., 2006). High indigenous N supply capacity and low N response were not considered by rice researchers and extension technicians when N fertilizer rate was determined for recommendation to rice farmers in China. This explains why SSNM usually have

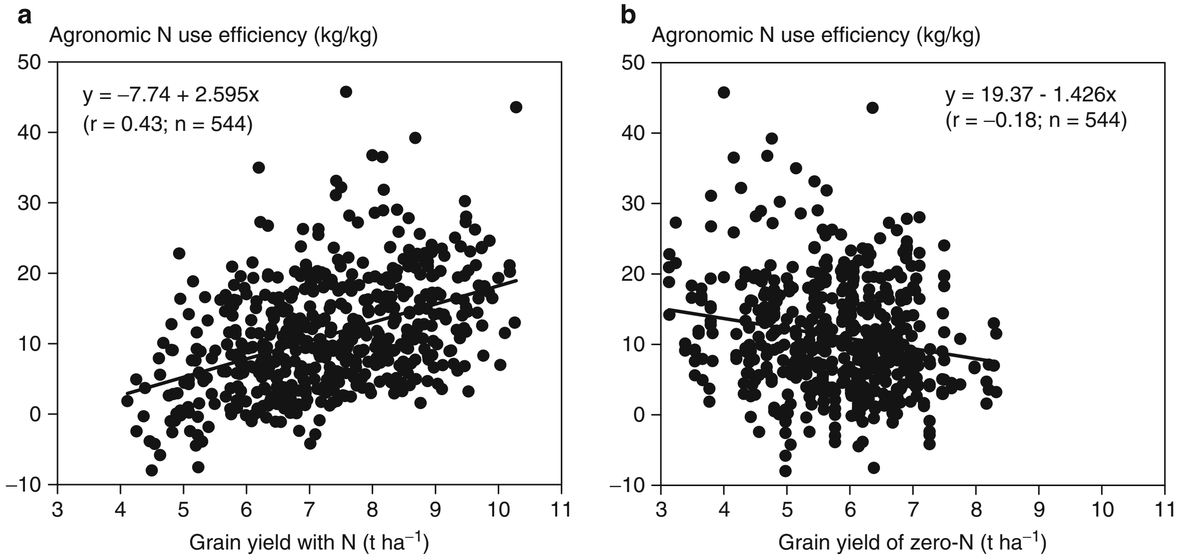

Fig. 6 Relationship between agronomic N use efficiency and grain yield with N fertilizer (**a**) and grain yield without N fertilizer (**b**). Data are from on-farm field trials, on-farm demonstration and farmer participatory research conducted in six provinces in China from 2001 to 2007

a lower total N rate than farmers' N fertilizer practice in China. A twelve-season long-term field experiment demonstrated that a decrease in total N rate may reduce indigenous N supply capacity, but will not cause yield reduction in subsequent rice crops as long as optimal N management is practiced (data not shown).

5 Remarks on Site-Specific N Management

Improved N management such as SSNM increases both grain yield and fertilizer N use efficiency compared with farmers' N fertilizer practice in China. This is achieved by reducing total N rate and by reducing N rate during the early vegetative stage. The total N rate is reduced because of small N response and high target agronomic N use efficiency. The reduction in N rate during the early vegetative stage is considered because irrigated rice soils in China have a high indigenous N supply capacity, which provides sufficient N for the early vegetative growth. In implementing the SSNM procedure, we have two steps to determine the right N rate for rice crop. The first step is done before crop establishment. The total N rate is estimated based on N response and target agronomic N use efficiency. The percentage of the first N application is decided based on previous experimental results and indigenous N supply capacity. The second step is the upward or downward adjustments of topdressing N rate by 10 kg N ha^{-1} at critical growth stages based on leaf N status.

Several limitations are associated with SSNM. First of all, grain yield of zero-N control varies with cropping history, variety, climatic conditions and crop management practices. As a consequence, N response is not very stable for a given location across seasons. Secondly, agronomic N use efficiency is affected by many factors such as N response and total N rate. Therefore, total N rate determined by N response and agronomic N use efficiency provides only an approximate range. Thirdly, varietal differences in tillering capacity and early growth vigor are not considered when the percentage of the first N application is decided. Finally, the in-season adjustment of N rate by ± 10 kg ha^{-1} may be inadequate because more adjustment could be needed when yield level and the magnitude of N response are high. Because of these limitations, only 3–5% increase in grain yield can be achieved by SSNM in China. Furthermore, SSNM improved agronomic N use efficiency only up to an average of 15 kg kg^{-1} in China. Future research should focus on accurate determination of N response and agronomic N use efficiency using weather data such as solar radiation and air temperature, and information on variety and soil characteristics. Experiments are needed to test if in-season adjustment of N rate should be linked with the difference between actual SPAD readings and SPAD threshold values.

Several problems exist in the adoption of SSNM technology. First of all, information on the yield of N-omission plots and target yield are not available to many farmers. Secondly, N response is variable and largely affected by variety, crop management practices, season and location. Thirdly, some farmers are reluctant to invest time in monitoring leaf N status using the leaf color chart. Many farmers have difficulty in determining leaf color chart readings accurately. Fourthly, frequent changes in varieties require adjustments in leaf color chart threshold values. Finally, the procedures for implementing SSNM could be complicated for many farmers. These problems may be overcome by developing remote sensing technology such as a canopy reflectance sensor or satellite remote sensing for determining the timing and rate of fertilizer N topdressing during the rice-growing period (Xue and Yang 2008).

Despite the above limitations, a decade of collaborative research work on SSNM between IRRI and Chinese scientists has changed the perception of researchers and farmers about N fertilizer management in irrigated rice in China. The following concepts are accepted now by many rice researchers in China: (1) target yield and indigenous N supply capacity should be considered when total N rate is determined; (2) determination of N application rate at basal and during the early vegetative stage should also be based on indigenous N supply; (3) the rate of N topdressing depends on leaf N status and crop N demand, and (4) regional blanket recommendation for N management will not work well. Nowadays, many researchers have developed local N management practices based on SSNM principles and by integration with other management practices. Many farmers who have been exposed to SSNM understand that it is not true that “the higher the N fertilizer input, the higher the grain yield” and that “the greener the leaves, the better the rice crop”. They know that it is necessary to reduce N input at basal and the early vegetative stage and increase N input at late stages. The dynamic change in leaf N status has been embedded in some farmers’ minds. They can judge visually when and how much N should be applied without using the leaf color chart.

6 Conclusions

After one decade of research on SSNM in China and other Asian rice-growing countries, we believe SSNM is a matured technology for improving both fertilizer N use efficiency and grain yield of irrigated rice crop. Our study suggests that there is potential for large-scale dissemination of SSNM technology in China. Accurate estimation of N response and selection of the right agronomic N use efficiency are needed for a better performance of SSNM. In-season adjustment in N rate should also consider the differences in location, season and variety. SSNM principles are useful for researchers to develop N management practices for their local conditions. Our challenges are to further simplify the procedure of SSNM and to convince policy-makers of the effectiveness of this technology in order to facilitate a wider adoption of SSNM among rice farmers in China. Future research is needed to develop SSNM based on remote sensing technology so that SSNM can be practiced on a large scale.

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Solid–Liquid Separation of Animal Slurry in Theory and Practice

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Contents

Abstract Animal slurry contains plant nutrients that are essential for crop production. However, intensive livestock production may lead to a surplus of plant nutrients on farms and, as a consequence, discharge or emission to the environment. In order to ensure that the slurry applied to fields matches the nutrient requirements of the crops, techniques have been developed to reduce the nutrient content of slurry by means of separation. This review discusses the separation technologies used for animal slurry treatment and the physical and chemical processes involved in separation. These processes need to be understood before efficient, reliable and cheap separation technologies that take into account the actual properties of slurry and the likely end-use of the separation products can be developed. A simple separation efficiency expression can be used to assess the efficiency of slurry separation. It is indeed important to measure the amount and composition of the slurry before treatment, the dry-matter-rich fraction and the liquid fraction. The separation efficiency of mechanical separators for the removal of dry matter and phosphorus (P) is ranked as follows: centrifugation > sedimentation > non-pressurized filtration > pressurized filtration. In general, the separation of total N and NH_4^+ follows the same pattern, but the separation efficiency is lower than for dry matter and P. Treatment with a flocculant before separation improves separation efficiency significantly. Of the polyacrylamide polymers tested, high-molecular-weight, linear cationic polymers with a medium charge density (20–40 mol%) were found to be the most efficient flocculants. The best mechanical separation techniques for flocculated slurry are screens or filter belts. The separation efficiency of polyacrylamide-treated slurry can be improved by

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*, DOI 10.1007/978-94-007-0394-0_43,

from Hjorth et al., Agron. Sustain. Dev. 30 (2010) 153–180. DOI: 10.1051/agro:2009010

adding a multivalent ion to coagulate particles and for precipitation of phosphorus. Aluminium sulfate ($Al_2(SO_4)_3$) or ferric chloride ($FeCl_3$) seem to be very efficient for improving the mechanical separators. Alternatively, the mineral struvite ($MgNH_4PO_4$) may be formed by changing the slurry characteristics, such as by the addition of magnesium (Mg) or by increasing the pH to 9. The struvite crystals are removed during solid–liquid separation. The products of the solid–liquid separation may be further treated by evaporation, membrane filtration or ammonia stripping in order to obtain the desired end-products; however, low-maintenance and/or cost-efficient operation of these post-treatments has not yet been demonstrated. The separation should be developed as a whole-system approach, paying attention to parameters such as the value of end-products, environmental consequences and economy.

Keywords Manure • Phosphorus • Nitrogen • Sedimentation • Centrifugation • Filtration • Coagulation • Flocculation • Struvite

1 Introduction

Livestock production increased towards the end of the 20th century and, due to increasing demand in developing countries, production is expected to increase further in Asia and South America (FAOSTAT, 2006). Globally, the intensification of agricultural systems increases the environmental impact of food production, as high concentrations of livestock increase the risk that organic waste nutrients are used for crop production in excess of crop requirements.

This section briefly discusses current problems related to discharge and the unsustainable use of slurry, with a focus on how to optimize separation as a sustainable and cost-effective treatment option for animal slurry.

Livestock slurry contains phosphorus (P) and potassium (K), which have fertilizer values equivalent to those of mineral fertilizers, while its nitrogen (N) content has a lower and more variable fertilizer value than that of commercial fertilizers. If applied at a rate higher than plant uptake, there is a great risk of nutrient leaching and runoff that will pollute surface- and groundwater (Burton and Turner, 2003). This leads to an increased need for water purification to provide safe drinking water supplies. Slurry is also harmful to the environment when discharged directly into receiving waters (Rattanarajcharkul et al., 2001; Steinfeld et al., 2006; Petersen et al., 2007; Vu et al., 2007). Negative effects from heavy applications of slurry may include salinization in semi-arid regions, toxic concentrations of heavy metals, and decreased soil aeration (Bernal et al., 1992, 1993). Larger production units may result in higher local emissions of odor and ammonia gas from housing and stores. Intensive livestock production may also lead to higher energy use for the transport of livestock wastes to be recycled in crop production elsewhere (Sørensen et al., 2003; Sørensen and Møller, 2006), and the risk of spreading disease among livestock will increase.

Until recently, European farmers have tried to minimize the environmental problems caused by slurry management through recycling on farm. It has been recognized that livestock slurry represents a valuable resource that, if used appropriately with minimal loss, can replace significant amounts of mineral fertilizers (Bouwman and Booij, 1998; Le, 1998). However, an increasing number of large, intensive livestock production units with insufficient area for the sustainable recycling of slurry nutrients have emerged in recent years. This specialization in livestock production tends to weaken the link between livestock and plant production and increases the farm's impact on the environment. To facilitate the recycling of slurry produced on these farms, there is a need to transport the slurry to farms specializing in crop cultivation. The cost of transporting slurry may be reduced, and its fertilizer value increased, by separating the slurry into (1) a liquid fraction intended for on-farm use, and (2) a dry-matter- and nutrient-rich fraction that can be exported to farms with few or no animals (Møller et al., 2000; Sørensen et al., 2003). Separation may also contribute to a reduction in odor emission (Zhang and Westerman, 1997) and assist in producing energy-rich biomass that can be used for incineration or biogas production (Møller et al., 2007a; Hjorth et al., 2009).

In Asia, slurry from pig farms is often separated in-house on small- to medium-sized farms by manual scraping, while on large farms the solid fraction is separated from the liquid fraction by filter separators or decanter centrifuges (Ong, 2002; Vu et al., 2007). The liquid fraction is transferred to fish ponds, where it fertilizes aquatic plants that are eaten by herbivorous fish

(Vu et al., 2007). The solid slurry produced by pigs and cattle that is scraped off the floor is composted, and an organic fertilizer is produced that is sold to vegetable and fruit producers as a highly valued product for soil amelioration.

In Europe and North America, the solid fraction is often not considered to have much value and slurry processing is therefore considered as an additional cost of livestock production (Petersen et al., 2007). Thus, the challenge is to reduce the cost and improve the efficiency of the processes while adding value to the separation products – especially the solid fraction, which has a high energy and P content. In this context, it is important to understand that slurry management consists of several interrelated operations carried out from the time the slurry is removed from the animal house until it is used for bioenergy production and to fertilize crops. Therefore, a whole-system approach should be considered when developing the technology for optimizing the recycling of plant nutrients in livestock production and optimizing green energy production using livestock slurry (Petersen et al., 2007).

It should be borne in mind that the separation of animal slurry may create new problems; for example, a change in the ratio of plant nutrients to heavy metals in the biomass. A recent study showed that solid–liquid separation with flocculation as a pre-treatment transferred zinc (Zn), copper (Cu) and cadmium (Cd) to the solid fraction (Møller et al., 2007b). The additives used when optimizing the separation of slurry (e.g. polymers and Al) may also pose an environmental problem (Nahm, 2005; Schechter et al., 1995).

Overall, slurry separation and recycling of organic matter and plant nutrients can mitigate the environmental hazards described above, since it will contribute to the optimization of crop fertilization, thereby reducing the need for external supplies of N, P, K and micronutrients.

An effective separation system can only be designed if the following three points are taken into account:

1. the chemical and physical properties of the slurry;
2. the desired end-products;
3. the potential separation techniques, including pre- and post-treatments and combinations of different techniques.

Knowledge of these variables will provide the information needed to produce separation products that can be used efficiently in crop production. Desirable separation products would, for instance, comprise a liquid fraction containing N, P and K at a ratio equivalent to the plant demand, and a solid fraction with high P, a relatively high N content (including organic N), and a high content of DM, which could be used as a carbon source for energy production (Hjorth et al., 2009).

A thorough understanding of the physical and chemical processes involved in the separation of slurry is required in order to develop reliable, efficient and cheap separation technologies, taking into account the actual slurry properties and the end-use of the separation products. This review aims to present a characterization of animal slurry relevant for slurry separation, and to describe concepts of separation optimized to treat slurry with the specified characteristics. The review includes the presentation of separation techniques, including the use of additives as pre-treatments.

2 Slurry Production

The management of slurry will affect its physical and chemical characteristics. Animal housing may be designed so that both solid and liquid slurry are produced. Alternatively, some animal houses are designed that have slatted floors with cellars or channels beneath. Below the slats, a slurry consisting of a mixture of feces and urine, strewing material, and spilt feed and water is collected.

The range of animal housing design and methods of slurry collection, storage and handling reflect the large differences in climate and production objectives throughout the world. Slurry management systems have been developed in order to reduce the manual labor required. The animal slurry in these systems is mainly stored and transported from animal houses via deep pit, pull plug, pit recharge and flushing systems (Menzi, 2002; Arogo et al., 2003). There is little or no seasonal variation in untreated slurry composition (Petersen and Kjellerup, 1990; Conn et al., 2007), which reflects the fact that the diets given to the animals and the slurry management normally do not vary significantly throughout the year. With respect to the separation of animal slurry, the slurry removal system and method of storage is important because the physical and chemical characteristics of the slurry will change due to microbial transformation processes during storage (Hindrichsen et al., 2006). Slurry characteristics may also be changed by introducing additives or changes in feed, as these changes will be reflected in slurry composition (Otto et al., 2003). The composition of the slurry also varies significantly with depth in the slurry store due to sedimentation and surface crust formation (Burton and Turner, 2003).

Separation of urine and feces may be carried out in the animal house. In cattle houses with tethered cattle, the manure may be separated into solid and liquid fractions by collecting the solids from the floor behind the animals while the liquid is drained through gutters. In these houses, the collected solid manure mainly contains feces and straw and the liquid slurry contains a mixture of water, urine and soluble fecal components (Sommer and Hutchings, 2001; Sommer et al., 2007). In modern pig houses, the animals move freely on slatted floors, and therefore in-house separation of the slurry needs to be carried out below the slatted floor (Kroodsma, 1986; Lachance et al., 2004). However, farmers are still reluctant to install this technology because of problems related to equipment maintenance; instead, the separation of mixed urine and feces, i.e. slurry, will be the focus of this review.

3 Nitrogen and Phosphorus Transformation during Storage

The distribution of nitrogen (N) and phosphorus (P) between the solid and liquid slurry fractions is affected by the microbial transformation of organic matter that takes place during storage before slurry separation. As shown in Sect. 4, the N and P are distributed between a solid phase (particles) and a liquid phase, and this distribution is affected by excretion and storage.

3.1 Nitrogen

In livestock farming, usually not more than 5–45% of the nitrogen (N) in plant protein is transformed into animal protein, depending on the type of animal and livestock management (Oenema et al., 2001). The remaining 55–95% is excreted via urine and dung as organically bound N. Following deposition on the floor of the animal house or in pastures, a major fraction of the organic N is rapidly hydrolyzed into ammonium (NH_4^+) by the enzyme urinase, which is present in the feces (Sommer et al., 2006).

Immobilization of inorganic N into organically bound N is a microbial process, which depends on the C:N ratio in the degradable organic compounds. Slurry mixtures have C:N ratios in a range between 4 for pig slurries and 10 for cattle slurries (Chadwick et al., 2000). In general, there is no immobilization of N in slurry mixtures, because the C:N ratio of the easily degradable compounds is low (<15) (Kirchmann and Witter, 1989; Thomsen, 2000). During in-house storage, most of the digestible compounds containing N are transformed, and about 10% of the organic N is mineralized (Zhang and Day, 1996; Sørensen, 1998). During outdoor storage of slurry, little N is mineralized and it is assumed that about 5% of the organic N is transformed into inorganic N during the course of 6–9 months' storage (Poulsen et al., 2001).

Approximately 70% of the N in slurry is dissolved and most of the dissolved N is present as NH_4^+ (Christensen et al., 2009). The NH_4^+ in slurry, as well as in the liquid and solid separation fractions, may be lost due to volatilization as NH_3 ($pKa = 9.25$) or after microbial transformation as nitrous oxide or N_2 (Hansen et al., 2006). Thus, the total loss of gaseous N from animal housing and slurry storage ranges from about 10% of the excreted N in dung and urine from dairy cattle to $>30\%$ for pigs and poultry in intensive livestock operations (Oenema et al., 2001).

3.2 Phosphorus

Phosphorus is fed to the animals via plants, but also as additives in the form of inorganic phosphate. Cattle are able to utilize P very efficiently and only the plant P that is not mobilized and adsorbed in the digestive system is excreted. Most of this P is excreted in feces and very little in urine (Meyer et al., 2007). Higher amounts of dietary P may lead to higher P excretion in feces (Dou et al., 2003; Chapuis-Lardy et al., 2004).

The digestive system of pigs is not as efficient as that of cattle in absorbing P in feed, and pigs excrete about 50–60% of their P intake in feces and urine. Surplus P intake is excreted in urine. The enzyme phytase is added to pig feed to improve P uptake. Phytase degrades the organic P compound phytate and makes the P more easily available; consequently, the inorganic P supplement can be reduced and P excretion reduced significantly, i.e. by $>20\%$ (Pfeiffer, 1995; Han et al., 2001; Sommer et al., 2008). This reduction mostly affects the amount of P in urine (Poulsen, 2000) and is affected by P digestibility, the amount of feed in relation to the requirements of the pig, and the age and category of animal. Production systems will also affect P excretion; for instance, changing the group size of fattening pigs may increase welfare and reduce P excretion. In contrast, group housing of sows and the addition of straw may increase P excretion (Krieter, 2002).

In slurry $>80\%$ of the dissolved P is orthophosphate. The orthophosphate fraction may vary during storage, i.e. increase immediately after excretion and reach a maximum, and thereafter it will decrease slightly (Christensen et al., 2009).

4 Physical and Chemical Characterization of Slurry

Animal slurry from housing is a mixture of feces and urine, bedding material (e.g. straw, wood shavings, sawdust, sphagnum), spilt feed and drinking water, and water used for washing floors. Factors of importance for optimizing the efficiency of slurry separation include particle size, concentration of organic and inorganic components, pH and buffer systems of the slurry. The physical and electrochemical properties of the particles are also important for separation.

Water use will vary due to differences in animal housing systems and management; therefore, due to the amount of dilution, the composition of the animal slurry will vary. In pig slurry, in particular, the concentration of the components varies due to variations in use of water, for instance, because more water is used in sow than in pig houses (Conn et al., 2007), and because a large amount of water is used in Asian pig production to cool the pigs and to clean solid floors (Taiganides, 1992; Sommer et al., 2005). Thus, in slurry from finishing pig houses, the total N, P and K concentrations may be 2.3, 2.5 and 1.7 times greater, respectively, than in sow slurry (Conn et al., 2007). Also, the average DM content in slurry from finishing pigs is six times greater in European slurry than in Asian slurry (Sommer et al., 2005).

4.1 Particle Size

Particle size distribution in the slurry is important for separation; for example, filtration will only retain particles above a certain size, and during sedimentation particles in the range between 1 nm and 1 μm (i.e. colloids) are subject to Brownian motion and move by diffusion in the liquid, and therefore settle very slowly or not at all.

The amount of DM in the particle size fraction below 0.025 mm is greater in pig slurry than in cattle slurry; 66–70% and 50–55% in pig and cattle slurry, respectively (Møller et al., 2002; Sommer et al., 2008). Feed composition may affect particle size distribution; for instance, the proportion of DM in the particle size fraction >1 mm was lower in slurry from cows fed only roughage compared with slurry from cows whose diet was supplemented with concentrates (Nørgaard, 2006; Sommer et al., 2008). The effects of diet and category of animal are also reflected in the variation in total solids found in the <0.125 mm category of fresh feces from cattle, being 50%, 41% and 44% (± 1.4) for lactating cows, heifers and calves, respectively (Meyer et al., 2007).

Microbial transformation of the organic pool (see Sect. 3) will change the particle size distribution. The amount of organic matter in slurry decreases due to microbial transformation into CH_4 and CO_2 during anaerobic storage. The DM content may decrease by 25% during 5 months of storage at 20 °C (Møller et al., 2002). At low storage temperatures, anaerobic transformation of organic material – and therefore DM reduction – will be significantly lower (Christensen et al., 2009), i.e. about 10 times lower at 10 °C than the reduction at 20 °C (Sommer et al., 2006).

Anaerobic digestion reduces the DM concentration of animal slurry, and changes the particle size distribution. It has been observed that particles <10 μm account for 64% of DM in raw slurry, while it increases to 84% of DM in anaerobically digested slurry (Massé et al., 2005). In contrast, the concentration of particles <1.6 μm is reduced most during anaerobic storage in slurry stores (Møller et al., 2002). In anaerobic digesters, stirring and high temperatures facilitate microbial degradation of large particles, leaving relatively small particles with slowly degradable material. In a cold unstirred slurry, the slow transformation of particles primarily degrades small particles, transforming only a minor fraction of the large particulate material.

In cattle slurry >80% of N and P is in the particle fraction below 0.125 mm (Meyer et al., 2007), and a very detailed analysis of particle size fractions showed that in slurry more than about 70% of the undissolved N and P was in the particle size fraction 0.45–250 μm (Massé et al., 2005). Because approximately 30% and 80% of N and P, respectively, is contained in particles (see Sect. 3), the small particles contain a large percentage of the total P in slurry. The addition of phytase to the diet of pigs or supplementing the diet of dairy cows with concentrates does not affect the distribution of total P in the different particle size fractions (Sommer et al., 2007).

4.2 Organic Components

The composition of the organic components may affect separation, as charge and pH will influence flocculation and also the cation absorption on and charge neutralization of the particles being separated from the liquid. Animal slurry contains short-chain volatile fatty acids (VFA) with 1–5 atoms of carbon, organic lipids, proteins, carbohydrates and lignin, which also contains non-carbohydrate organic components such as phenols (Møller et al., 2004; Massé et al., 2005; Christensen et al., 2009). Carbohydrates comprise the largest fraction of the organic material, followed by proteins, lipids, lignin and VFA. The organic components in slurry include compounds with the functional groups carboxylates, hydroxyls, sulfur hydryls and phenols (Massé et al., 2005) which, at the pH interval in slurry, will contribute to a negative charge of both dissolved and particulate organic matter.

Most of the P in animal slurry is in the particle fraction of slurry, and >30% is dissolved in the liquid phase (Fig. 1). One study showed that organic P in solution only constitutes about 5% of the P in slurry (Fordham and Schwertmann, 1977a). The remaining P is mainly associated with the particle fraction, where it may be bound inorganically in crystalline form or is adsorbed onto particles.

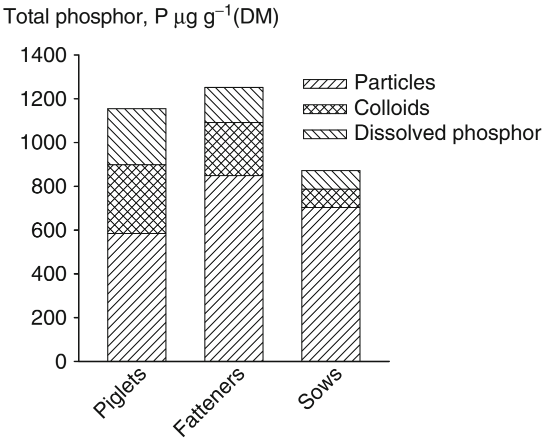

Fig. 1 Distribution of phosphorus between the particles, colloids and dissolved fraction of pig slurry (adapted from Christensen et al., 2009)

4.3 Inorganic Components

The separation efficiency of different technologies is greatly affected by the slurry composition; for instance, large minerals will end up in the solid fraction. In addition, electrical conductivity will affect flocculation, and the relative concentration of cations will affect crystallization of compounds such as struvite.

The composition of ions in animal slurry varies significantly among slurry from different animals and is affected by the diets fed to the animals and slurry management (Table 1). Electrical conductivity depends on the concentration and species of ions in solution and varies from 0.008 to 0.026 $\mathrm{S\,cm^{-1}}$ (Sommer and Husted, 1995a; Christensen et al., 2009). Cattle slurry contains more sodium, potassium and calcium than pig slurry (i.e. 2–3 times more) due to an intake of roughage having a high content of these cations (Sommer and Husted, 1995a; Massé et al., 2007a). In cattle slurry, the content of these ions varies twofold due to variation in diets (Chapuis-Lardy et al., 2004).

The distribution of P between the solid and liquid phases is affected by pH, with more P being dissolved at a decreased pH. The distribution is almost constant down to pH = 6.5, whereas the concentration of dissolved P increases by a factor of 5 when pH is lowered from 6.5 to 5.5; mainly due to dissolution of struvite (Christensen et al., 2009). In slurry, P may be crystallized as struvite ($MgNH_4PO_4 \times 6H_2O$) and different forms of calcium phosphates (Fordham

Table 1 The composition of animal slurries is most variable both between samples of slurries from one site and between slurries from different sites

		Component content (g/kg slurry)									
Slurry origin		*DM*	Total-P	Total-N	TAN	K	Ca	Mg	Cu	Zn	Reference
Sows	Mean	23	0.8	3.2	2.0	2.0	0.7	0.2	0.0		Møller et al., 2007a; Conn et al., 2007
	Standard deviation	(15)	(0.2)	(0.9)	(0.7)	(0.3)	(0.4)	(0.1)			
Finishing pigs	Mean	67	2.1	7.5	4.5	3.3	2.1	0.9	0.0	0.1	Conn et al., 2007; Ye et al., 2005; Massé et al., 2005; Møller et al., 2004, 2007a; Saeys et al., 2005; Sommer and Husted, 1995a; Sommer et al., 2008
	Standard deviation	(26)	(0.8)	(2.5)	(2.1)	(1.1)	(0.9)	0.4	0.0	(0.1)	
Dairy cows	Mean	82	1.0	3.7	5.0	4.3	1.6	0.6	0.0		Amon et al., 2006; Clemens et al., 2006;Møller et al., 2007a; Sommer and Husted, 1995a; Sommer et al., 2008
	Standard deviation	(24)	(0.2)	(1.7)	(9.3)	(1.8)					

and Schwertmann, 1977b, c), i.e. calcium dihydrogen phosphate $Ca(H_2PO_4)_2$, calcium hydrogen phosphate $CaHPO_4$ and tricalcium phosphate $Ca_3\ (PO_4)_2$. The forms of phosphate will be affected by the pH of the slurry (Eq. (1)).

$$Ca_3(PO_4)_2 + 2H_2SO_4 + 4H_2O \leftrightarrow 2CaSO_4 \cdot 2H_2O + Ca(H_2PO_4)_2 \cdot 2H_2O \qquad (1)$$

Studies suggest that, in slurry, phosphate can crystallize as struvite (Fordham and Schwertmann, 1977b, c; Bril and Salomons, 1990; Sommer and Husted, 1995a; Gungor and Karthikeyan, 2005). Ammonium is present in large excess for struvite formation, thus crystallization is mainly controlled by magnesium concentration and pH.

An increase in the amount of iron (Fe^{3+}) dissolved in the slurry will reduce pH; for example, if the Fe^{3+} precipitates as crystalline iron phosphate.

$$Fe^{3+} + H_3PO_4^{n-3} \leftrightarrow FePO_4(s) + nH^+ \qquad (2)$$

Precipitation of calcite ($CaCO_3$) is mainly controlled by the concentration of calcium (Ca^{2+}), because carbonate (CO_3^{2-}) is present in large excess in slurry. Slurry pH is reduced significantly by adding Ca^{2+}, thereby precipitating CO_3^{2-} and producing the crystal calcite (Witter and Kirchmann, 1989; Husted et al., 1991).

$$Ca^{2+} + HCO_3^- + H^+ \rightleftarrows CaCO_3(s) + 2H^+ \qquad (3)$$

Part of the inorganic P may precipitate as calcium phosphate, or it may absorb to $CaCO_3$ (Fordham and Schwertmann, 1977a, b).

Most K^+ and NH_4^+ salts are very soluble, and struvite (see Sect. 6.2) is the only crystal that in practice contributes to settling or separation of ammonium (Bril and Salomons, 1990). Thus, most K^+ and NH_4^+ is dissolved in the liquid phase (Massé et al., 2005).

4.4 pH Buffer Systems

When considering which process or technology is optimal for the separation of slurry, the pH needs to be known, since struvite precipitation is greatly affected by pH, as is the precipitation of P and ammonia stripping. It has been shown that the main buffer components in animal slurry controlling $[H^+]$ are total inorganic carbon ($TIC = CO_2 + HCO_3^- + H_2CO_3$), total ammoniacal nitrogen ($TAN = NH_3 + NH_4^+$) and volatile fatty acids ($VFA = C_2–C_5$ acids) (Angelidaki et al., 1993; Sommer and Husted, 1995a, b; Vavilin et al., 1998). In stored slurry, the concentration of TIC may be larger than those of TAN and VFA; so this component is therefore of major importance in controlling slurry pH (Japenga and Harmsen, 1990;

Sommer and Husted, 1995b). In addition to inorganic buffers, negatively charged particles in the slurry will contribute to acid groups with pKa values from 5 to 9 (Christensen et al., 2009).

A number of processes will affect the pH of slurry; the emission of CO_2 and NH_3 is particularly important because emission of CO_2 will increase pH, and emission of NH_3 will reduce pH. Oxic degradation of organic material reduces the content of acids in solution and thereby increases pH. In contrast, anoxic processes will contribute to the formation of organic acids (e.g. VFA $= C_1–C_5$) and thereby reduce pH. In an anoxic environment, the content of organic acids may be reduced by methanogenic microorganisms by transformation into CH_4 and CO_2 which, in contrast, will increase pH.

Diet and feeding practices may affect the concentration of ionic species in the slurry and the pH, because the electric charge of the solution has to be neutral. At present, soya beans in the diet supply most of the crude proteins needed by pigs. As soya contains high concentrations of K^+, this will, when excreted, increase the pH of urine and slurry. Reducing the soya concentration in the diet and supplementing with amino acids will reduce the K^+ concentration and, because of the charge balance, the concentration of H^+ will increase (Sommer and Husted, 1995b). Thus, for pig urine and slurry, and for cattle urine, it has been shown that pH declines when cationic species in the feed are reduced; for pig slurry, a reduction of more than 1 pH unit has been observed within the range of traditional diets with and without the addition of amino acids and the reduction of soya (Canh et al., 1998; Portejoie et al., 2004).

The addition of multivalent ions (e.g. $FeCl_3$ or $Al_2(SO_4)_3$) or cations precipitating carbonate ions ($Ca(OH)_2$) will also affect slurry pH, as indicated in Section 4.3 (Husted et al., 1991; Henze et al., 1997; Estevez Rodríguez et al., 2005).

4.5 *Electrochemical Properties*

Particle charge and ionic strength, which affect the electrical potential around the particles, are of vital importance for the efficient use of flocculants.

Organic particles often have a negative surface charge and the particles will therefore repel each other (Gregory, 1989). It has been shown that, in pig slurry, the particle charge density is -0.18 meq g^{-1} organic solids (Christensen et al., 2009). Furthermore, due to their negative charge, organic particles contribute to the alkalinity of the slurry. The concentration of ions expressed as ionic strength has been shown to be high in most animal slurry studies, i.e. conductivity >10 mS cm^{-1} (Sommer and Husted, 1995b; Christensen et al., 2009). An increasing ionic strength of the liquid will affect processes such as flocculation due to a reduced electrostatic repulsion. Furthermore, at high ionic strength, a linear polymer will change to a more sphere-shaped form, which will lower the efficiency of the polymer (Gregory, 1973, 1989).

The negative charge of organic particles in animal slurry affects the distribution of the ions in solution by adsorbing cations close to the particles (Stern layer), with the remaining counter-ions being distributed in the diffusive layer, which also contains anions. These two layers of ions are referred to as the ‘double layer’. It is assumed that the interaction between charged particles is visualized as the overlap of diffusive layers of particles. Therefore, the electrical potential of interest, when considering interactions between two particles, is the potential at the boundary between the Stern and the diffusive layers, which is assumed to be equal to the electrokinetic or zeta potential (ζ, mV; Lyklema, 1977). The effect of the particle charge density will be affected by the extent of the diffusive layer, which decreases with increasing ionic strength. The influence of the particle charge density is therefore expected to be low in slurry with a high ionic strength.

4.6 *Physical Properties*

The density and viscosity of slurry has been correlated to the dry matter (DM) content. Landry et al. (2004) give a thorough review in connection with their own work on concentrated slurry. Their conclusion is that, for a DM between 0 and 50%, the density can be described by the following equations:

Dairy cattle:

$$\rho = 1000 + 14.6 \cdot DM - 2.38 \cdot DM^2 + 0.0367 \cdot DM^3 \quad (4)$$

Pig:

$$\rho = 1000 - 11.2 \cdot DM + 1.19 \cdot DM^2 - 0.0235 \cdot DM^3 \quad (5)$$

where ρ is the density (kg m^{-3}) and DM is dry matter in % (w/w). As seen from the equations, dairy cattle slurry has a lower density and pig slurry a higher density at the same DM content. The flow properties for slurry have, in general, been found to be non-Newtonian at values of DM above 5%, while at lower DM values the slurry behaves as a Newtonian liquid (Landry et al., 2004). In general, animal slurry with a higher DM content shows pseudoplastic behavior, but for simple flow considerations an apparent viscosity will often suffice. Landry et al. (2004) correlated their viscosity data, measured for a DM between 9% and 14% at 20 °C, as follows:

$$\text{Dairy cattle:} \quad \mu_{slurry} = 4.10^{-5} \cdot DM^{4.4671} \quad (6)$$

$$\text{Pig:} \quad \mu_{slurry} = 4.10^{-6} \cdot DM^{4.6432} \quad (7)$$

where μ_{slurry} is the apparent slurry viscosity (Pa s). Although these data should be used with caution, as Landry et al.'s (2004) comparison with other literature data shows, the conclusion is that pig slurry is less viscous than cattle slurry at a comparable DM content.

5 Solid–Liquid Separation

Different techniques for separating slurry in a DM-rich and a liquid fraction have been developed and are used on farms. Solid–liquid separation may be carried out in settling tanks, where the solids are removed from the bottom of the tank, or the settling may be forced using centrifuges. Solids may also be removed mechanically by forced filtration using screw presses or drainage through fabric belts or screens. In-house separation can be viewed as an alternative to these solid–liquid separation techniques; however, this technology is not fully developed and is not used in practice. It will therefore not be described further in this review (see Sect. 2). Numerous designs of solid–liquid separators exist for slurry mixed in the animal house, and the majority may be categorized as one of the four standard types of separators mentioned above.

When comparing results from different studies, it is an advantage to use one separation parameter expressing the efficiency of separation. The removal efficiency (R) expresses the efficiency of removal of a specific compound (x) from a slurry to the solid fraction. It is defined as follows:

$$R(x) = 1 - \frac{c(x)_{liquid}}{c(x)_{slurry}} \quad (8)$$

where $c(x)_{slurry}$ and $c(x)_{liquid}$ are the concentrations (g L^{-1}) of the species in consideration (dry matter, P, N) in, respectively, the slurry being treated in the separator and the liquid fraction that is produced. The greater the removal efficiency, the lower the amount of compound x remaining in the liquid fraction. The equation characterizes the efficiency of the separator with respect to the liquid fraction; however, it does not give any indication of the production of the solid fraction.

A separation index (E_t), on the other hand, expresses the distribution of the specific compound between the solid and liquid fractions:

$$E_t(x) = \frac{m_{x,solid}}{m_{x,slurry}} \quad (9)$$

where $m_{x,slurry}$ and $m_{x,solid}$ are the mass (g) of the compound in consideration in, respectively, the slurry being treated by the separator and of the solid fraction being produced. Hence, the values of both the liquid and the solid fraction are indicated. The larger the separation index, the greater the amount of compound x in the solid fraction.

The separation index, however, does not include information on the total amount of solid fraction relative to the total amount of liquid fraction. Thus, the simple separation index will theoretically be 50% if a machine separates the slurry into two equally sized fractions with similar contents of compound x. An improved expression for the separation may be obtained using the reduced separation index (E_t'):

$$E_t'(x) = \frac{E_t(x) - \frac{m_{solid}}{m_{slurry}}}{1 - \frac{m_{soild}}{m_{slurry}}} \quad (10)$$

where m_{slurry} and m_{solid} are the total mass (g) of slurry being treated and the total mass of solids being produced.

As only approximately 25% of the studies presented in the literature on solid–liquid separators contain sufficient data to assess E_t and of these only two-thirds contain sufficient data to assess $E_{t'}$, the separation index (E_t) has been used to compare efficiencies of separations in this review.

5.1 Sedimentation

5.1.1 Technical Description

Sedimentation in a thickener is an attractive option for separation, due to the low cost and simple technology. Most thickeners consist of a container that is cylindrical at the top and conical at the bottom. In batch operation, slurry is added to the top of the thickener (Fig. 2) and the solids settle at the bottom of the conical part from whence the solids can be removed (Suzuki et al., 2002; Loughrin et al., 2006). To encourage settling and increase the transfer of solids settled on the upper part of the conical section, small thickeners can be vibrated while, for larger thickeners, this can be achieved by using a rake. Thickeners can also be operated in continuous mode, where slurry is added continuously while solid and liquid phases are removed at the same rate as slurry is added. In this case, the slurry has to be added in the separating zone (Fig. 2). Alternatively, lagoons may be used as settling basins.

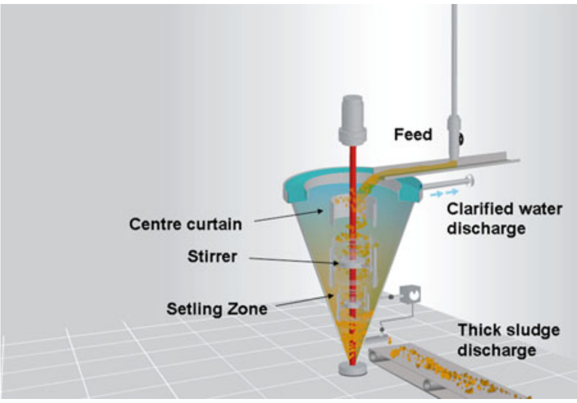

Fig. 2 A typical thickener used for sedimentation

5.1.2 Operation and Separation Efficiency

The time it takes for the solid to separate from the liquid phase can be estimated for dilute slurries from the terminal velocity of a single solid particle. The smallest particles will have the slowest terminal velocity and will therefore determine the final settling time. For small particles, the flow is laminar and the equation for the terminal velocity (v_{tg}) simplifies to (Foust et al., 1980):

$$v_{tg}(d_p) = \frac{(\rho_{solid} - \rho_{liquid}) \cdot g \cdot d_p^2}{18 \cdot \mu_{slurry}} \tag{11}$$

where μ_{slurry} is the viscosity of the slurry, ρ is the density of solid and liquid (see Sect. 4.5), g is the acceleration of gravity, and d_p is the diameter of the solid particle (all in SI units).

As seen, the settling velocity increases with the solid density compared with liquid density and particle size, but decreases with increasing slurry viscosity. As with solid density, because the viscosity and particle size vary from slurry to slurry, the terminal velocity can seldom be calculated in advance. At the same time, at least in the thickening zone of the thickener, the slurry cannot be assumed to be dilute and hindered settling will take place. Therefore, the terminal velocity as calculated in equation (11) alone cannot be used to determine the thickener size. Instead, the calculations or assessments have to be carried out based on laboratory settling experiments using the Coe–Clevenger method or the Talmadge–Fitch method, as described by Foust et al. (1980). However, the following general findings on slurry can be used as a yardstick.

Increasing the settling time increases the separation efficiency (Ndegwa et al., 2001; Converse and Karthikeyan, 2004). In a laboratory study, the settling of solids in pig slurry was studied for 4 hours. For slurry with an initial DM content of between 2% and 4%, the settling was observed to be completed within 1 hour. For pig slurry with a DM of 6% the settling time was over 4 hours, as was also observed for diluted slurries with 0.5% and 1% DM (Ndegwa et al., 2001). Interestingly, it was observed that the removal of DM was highest for 1% and 2% DM slurries (60% removal), while the DM removal was roughly 30% for the 0.5% and 4% slurries, and only 5% for the 6% *DM* slurry. The indications are that hindered settling and changes in slurry liquid properties slow down the settling velocity for pig slurries above 2% *DM*, while for very dilute slurries (i.e. 0.5% *DM*) the settling also slows down, presumably because at this low concentration fewer of the fine particles are co-precipitated with the larger faster-settling particles. Plant nutrients are not evenly distributed between particles of different density and size (see Sect. 4); in consequence, the settling of plant nutrients may not be linearly related to the settling of dry matter.

The mechanisms behind the slower settling velocities at higher *DM* seen in some studies are, for actual livestock slurries, due to a combination of hindered settling and increased density and viscosity of the slurry, as a large content of small particles will cause an increasing viscosity of the slurry liquid and thereby reduce the settling velocity (Henriksen et al., 1998a, b). It must be noted, though, that the effect of *DM* concentration on settling has not been observed in all studies (Gao et al., 1993). Also, the increased weight of the top sediment particles will squeeze water out of the thickening zone, causing turbulence that stirs up the particles; a mechanism that may be more pronounced in batch settling systems compared with technologies where the sediment is removed continuously (Foust et al., 1980). Fermentation and increased buoyancy of the particles due to the trapping of gas bubbles may reduce settling if the process is taking place over a long period of time, e.g. in lagoons; therefore it is recommended that slurry temperature should be kept below 16 °C (Meyer et al., 2007).

Cattle slurry has a higher viscosity than pig slurry at similar *DM* concentrations (see Sect. 4.5; Landry et al., 2004), most probably due to a larger amount of particles, which may contribute to a slower and less efficient settling of *DM* in cattle slurry. As for pig slurry, the *DM* settling of cattle slurry increases when *DM* concentration in the slurry increases; for example, from 0.1% to 1% (Moore et al., 1975). The settling rate of *DM* in cattle slurry decreases exponentially with time, and *DM* settling is almost at maximum after 1.5 hours (Moore et al., 1975). In contrast, because of the large P content in the small, slowly settling particles (Sect. 4.1), P settling may increase significantly over time and increase from about 50% settling after 4 hours to 75% settling after 48 days (Converse and Karthikeyan, 2004). On the other hand, the settling of total N may not increase with increased settling time (Converse and Karthikeyan, 2004). Most K and NH_4^+ is dissolved in the liquid phase (see Sect. 4.3; Massé et al., 2007a); therefore most K and NH_4^+ is recovered in the liquid phase after sedimentation of solids (Table 2; Massé et al., 2005).

Table 2 Separation indexes at sedimentation

Technical specifications	Slurry		Separation index (%)[2]					
Sedimentation time (h)	Origin[1]	Dry matter (%)	Volume	Dry matter	N-total	NH_4-N	P-total	Reference
Mean (standard deviation)			22 (4)	56 (10)	33 (2)	28 (2)	52 (21)	
0.2	P	–	–	51	–	–	17	Powers and Flatow, 2002
NA	P	2.0	18	63	–	–	65	Reimann, 1989
0.3	C	1.0	16	72	30	–	69	Sherman et al., 2000
4	C	1.3	25	52	35	27	42	Converse and Karthikeyan, 2004
24	C	2.5	25	42	33	28	46	Converse and Karthikeyan, 2004
1200	C	3.2	25	55	35	30	70	Converse and Karthikeyan, 2004

[1] P = pig, C = cattle.

[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

5.2 Centrifugation

5.2.1 Technical Description

Increasing the gravitational force can reduce the settling time needed to achieve a given separation efficiency. In practice, this is accomplished in decanter centrifuges, where a centrifugal force is generated to cause the separation.

There are vertical and horizontal types of decanter centrifuges. The horizontal decanter centrifuge (Fig. 3) uses a closed cylinder with a continuous turning motion. The centrifugal force separates solids and liquids at the wall into an inner layer with a high *DM* concentration and an outer layer consisting of a liquid containing a suspension of colloids, organic components and salts. The solid and liquid phases are transported to either end of the centrifuge by rotating the entire centrifuge at high speed and by simultaneously rotating the conveyor at a speed that differs slightly from the speed of the bowl (outer conical shell). The solid particles are conveyed towards the conical end and let out through the solid-discharge openings, whereas the supernatant flows towards the larger end of the cylinder formed by the bowl and the flights of the conveyor. During the transport of the slurry, the particles are separated from the liquid and the liquid phase is discharged through liquid-discharge openings at the wide end of the decanter centrifuge.

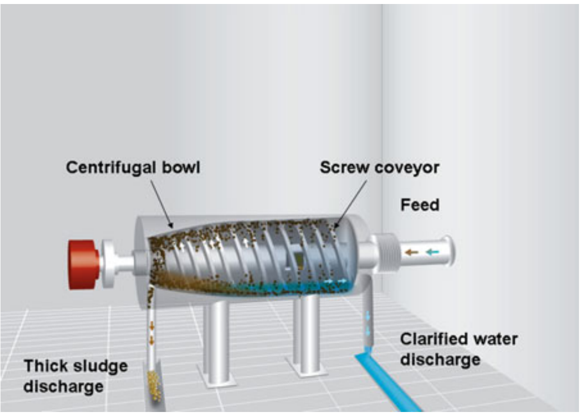

Fig. 3 A typical decanter centrifuge

5.2.2 Operation and Separation Efficiency

For small particles in laminar flow, the terminal velocity (v_{tc}) can be calculated as (Foust et al., 1980):

$$v_{tc}(d_p) = \frac{\left(\rho_{solid} - \rho_{liquid}\right) \cdot \omega^2 \cdot r \cdot d_p^2}{18 \cdot \mu_{slurry}} \qquad (12)$$

where r is the distance of the particles from the centrifuge's axis of rotation and ω is the angular velocity, all in SI units.

The only difference between the terminal velocity in a sedimentation tank and a centrifuge is the applied force; gravity versus centrifugal force. For simple laboratory centrifuges, the centrifuge efficiency can thus be related to the improvement in gravitational force, the G force (unit: g):

$$G = \frac{\omega^2 \cdot r}{g} \qquad (13)$$

The efficiency of full-scale decanter centrifuges, however, cannot be described as simply as that. This is partly because the distance that the particles travel in a radial direction is large; thus the distance the particles have to travel from the center of the centrifuge, and therefore the settling velocity, vary during the sedimentation process; and partly because the geometry of decanter centrifuges is quite complicated.

The performance of a decanter centrifuge is often described by its feed handling capacity (Q), which can be calculated as (Foust et al., 1980):

$$Q = \frac{\left(\rho_{solid} - \rho_{liquid}\right) \cdot g \cdot d_{pc}^2}{18 \cdot \mu_{slurry}} \cdot 2 \cdot \Sigma = v_{t,g} \cdot 2 \cdot \Sigma \qquad (14)$$

where Q is the volumetric feed rate, d_{pc} is the diameter of the smallest particle separated from the slurry in the centrifuge, and Σ is the sigma factor (m^2), all in SI units.

The sigma factor, Σ, is a property of the specific centrifuge geometry and G force and can only be calculated in advance for simple laboratory centrifuges. For full-scale centrifuges, Σ has to be obtained from experiments. As v_{tg} is a function of the slurry alone, and Σ a function of the centrifuge alone, the sigma factor can be used to compare the efficiency of different decanter centrifuges.

The dewatering volume of a decanter is considered to be the total volume (V) of the liquid zone in the cylindrical part of the drum. This volume may be changed by level regulators and the retention time (Rt) in seconds can be calculated as:

$$Rt = \frac{V}{Q} \quad (15)$$

where V is the dewatering volume of the decanter bowl (m^3).

From equations (14, 15), it is obvious that the volumetric feed rate, and therefore the retention time, depends on the chosen value of d_{pc} (the smallest particle to be separated). Reducing the feed rate and thereby increasing the retention time automatically leads to better separation but less economical performance of the centrifuge.

Increasing the retention time by reducing the volumetric feed rate has been observed to increase the efficiency of the separation of slurry (Table 3; Sneath et al., 1988; Møller et al., 2007a). In consequence, the separation of DM and P was found to be high in the laboratory study of Vadas (2006) (see Table 3) using the relatively long retention time of 600 s.

Increasing the dewatering volume within the centrifuge increases the retention time (Eq. (15)) but reduces the thickening zone (conical water-free part). Therefore, increasing the dewatering zone will increase the removal of DM from the liquid fraction. However, it will also reduce the drainage of water from the solid fraction (Reimann, 1989); hence the DM concentration in the DM-rich fraction will decrease.

Increasing the angular velocity of the decanter centrifuge will increase the DM concentration of the solid fraction (Eq. (15)). Thus, using a velocity as high as 2560 g, the decanter centrifuge produced a solid fraction with a DM of 40% (Møller et al., 2007a). However, increasing the angular velocity had no effect on the separation of the P, K and N (Møller et al., 2007a).

The separation efficiency of DM increases at increasing DM content of the slurry (Table 3). The opposite result may have been expected, as seen in the sedimentation studies, because a higher viscosity of the slurry may reduce the settling velocity of the small particles (Reimann, 1989). It was hypothesized by Sneath et al. (1988) that vigorous stirring of the slurry may enhance the attachment of small particles to larger particles, and thereby improve settling of the small particles. This effect declines with dilution of the slurry; it has been observed that in the liquid from separation of a low-DM slurry, 70% of the particles in the liquid were <4 μm, while 50% of the particles were <4 μm in liquid from separation of a high-DM slurry.

Table 3 Separation indexes at centrifugation

Technical specifications		Slurry		Separation index (%)[2]					
Centrifugation velocity (g)	Centrifugation time (s)	Origin[1]	Dry matter (%)	Volume	Dry matter	N-total	NH_4-N	P-total	Reference
Mean				14	61	28	16	71	
(standard deviation)				(7)	(16)	(10)	(8)	(14)	
1500	600	P	6.3	–	95	–	–	90	Vadas, 2006
2050	30	P	8.9	22	69	34	22	87	Møller et al., 2007a
2050	8	P	7.0	15	57	–	–	–	Sneath et al., 1988
2050	8	P	4.2	8	52	–	–	–	Sneath et al., 1988
2050	8	P	2.0	3	47	–	–	–	Sneath et al., 1988
2200	30	P	5.1	9	51	17	10	71	Møller et al., 2007a
2200	30	P	4.0	7	52	17	7	70	Møller et al., 2007a
2200	30	P	6.8	26	70	36	26	82	Møller et al., 2007a
4100	600	P	5.3	13	60	29	–	62	Møller et al., 2002
4100	600	P	2.6	5	33	13	–	66	Møller et al., 2002
–	–	P	7.0	16	70	32	–	52	Reimann, 1989
1500	600	C	8.0		86	–	–	83	Vadas, 2006
2200	30	C	7.0	23	63	29	16	55	Møller et al., 2007a
4100	600	C	6.4	21	65	49	–	82	Møller et al., 2002
4100	600	C	4.5	12	55	27	–	79	Møller et al., 2002
–	–	C	6.0	13	44	23	–	48	Reimann, 1989

[1] P = pig, C = cattle.

[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

As a consequence of a higher *DM* content of cattle slurry compared with pig slurry, the *DM* separation index is higher for cattle slurry separation than for pig slurry separation (Table 3). For assessment of the retention of slurry, it is reasonable to assume that decanter centrifuges can retain particles >20–25 μm in the *DM*-rich fraction (Sneath et al., 1988; Reimann, 1989; Møller et al., 2002).

Organic nitrogen and dissolved or adsorbed ammonium is related to the *DM* content of the solid fraction (see Sect. 3.1); therefore, total N separation is related to the *DM* content of the slurry being treated (Møller et al., 2002, 2007a; Sørensen and Møller, 2006). One should bear in mind that the relation between separation efficiencies and *DM* of treated slurry differs between centrifuges (Sneath et al., 1988; Møller et al., 2007a, b), and therefore an algorithm developed in one study cannot easily be applied to another. As decanter centrifuges do not retain the smallest particles, it is not surprising that no relation between separation of P and *DM* of treated slurry was observed (Møller et al., 2007a).

5.3 Drainage

5.3.1 Technical Description

Many different kinds of slurry separation equipment that filter out solids from liquids are commercially available (Fig. 4). These use screens and filter belts to retain the solid fractions. With simple screens and belt separators, the liquid is drained by gravity from solids in the separator. With a belt separator, the filter cake is continuously removed as the belt rotates, so that the raw-slurry loading area and solid-fraction unloading area change over and are cleaned continuously. Most of the tested filter separators are screens, which may have a variety of designs but often consist of a rotating perforated cylinder with a loading area at the top and a scraper to remove the solids. The liquid flows through the screen and is drained off.

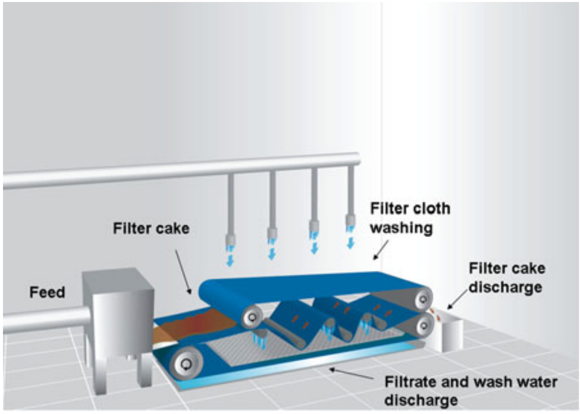

Fig. 4 A typical belt separator with pressure rolls

5.3.2 Operation and Separation Efficiency

The liquid flux through the filter is determined by the hydraulic resistance of the filter medium and the hydraulic resistance of the material deposited on the medium (i.e. the filter cake):

$$J = \frac{p}{\mu_{liquid}\left(R_m + SRF\,\frac{S\cdot V}{A}\right)} \tag{16}$$

where J is the flux (kg/m^2/s), μ_{liquid} is the viscosity of the permeate (Pa·s), R_m is the resistance to flow through the membrane (m^{-1}), S is the particle concentration in feed (kg/m^3), A is the filter area (m^2) and V is the filtrate volume (m^3). *SRF* is the specific filter cake resistance (m/kg), p is the pressure (Pa) and is related to the mass of the slurry (i.e. $p = \rho g h$), and h is the height of suspension above the filter cake (m), which decreases during the process.

Particles can adhere to or clog the filter media and thereby increase the resistance of the filter (Massé et al., 2005), and substances such as hair in pig slurry may cause an immediate build-up of a filter cake with a low *SRF* (specific filter cake resistance). Thus, when the filter cake is formed, small particles often clog the filter pores (Severin and Grethlein, 1996). A mixture of particles with a particle size distribution between 1 and 100 μm will produce a cake with a high *SRF*. This will reduce the liquid draining velocity from the filter cake and the effect will be a solid fraction with a low *DM* concentration (Karr and Keinath, 1978). Due to a higher fraction of larger particles (see Sect. 4.1), filter technology is more efficient in separating cattle slurry than pig slurry (Table 4).

As small particles are caught within the filter cake or adhere to the media, screens and filter will retain

Table 4 Separation indexes at drainage

Technical specifications		Slurry		Separation index (%)[2]					
Filter pore size (mm)	Filtration time (min)	Origin[1]	Dry matter (%)	Volume	Dry matter	N-total	NH_4-N	P-total	Reference
Mean (standard deviation)				23 (16)	44 (27)	27 (17)	23 (19)	34 (21)	
0.1	(x)	P	1.5–5.4	25	50	33	28	34	Holmberg et al., 1983
0.1	(4^*x)	P	1.5–5.4	45	67	51	47	59	Holmberg et al., 1983
0.11	120	P	6.3	–	87	–	–	53	Vadas, 2006
0.5	–	P	1.8	–	17	5	–	3	Pieters et al., 1999
0.8	–	P	1.5	–	18	–	–	–	Hegg et al., 1981
2.5	(x)	P	1.5–5.4	1	11	3	3	2	Holmberg et al., 1983
2.5	(4^*x)	P	1.5–5.4	13	23	16	15	23	Holmberg et al., 1983
3.0	–	P	5.7	30	62	34	–	39	Møller et al., 2000
0.11	120	C	8.0	–	84	–	–	62	Vadas, 2006
0.8	–	C	1.1	–	13	–	–	–	Hegg et al., 1981
0.85	–	C	7.7	4	14	–	–	–	Gilbertson and Nienaber, 1978
3.0	–	C	7.1	44	56	49	–	49	Møller et al., 2000
–	–	C	6	19	54	22	–	20	Pain et al., 1978
–	–	C	12	30	58	29	–	29	Pain et al., 1978

[1] P = pig, C = cattle.

[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

x: unspecified constant.

not only particles larger than the size of the mesh or the screen openings but also smaller particles. Therefore, one cannot assess filtering efficiency simply from particle size distribution and plant nutrients in different particle fractions and the size of the screen openings.

Increasing the retention time of the filter cake on the screen or the filter fabric will increase the drainage, and hence the *DM* concentration of the solid fraction produced. Retention time is often longer in laboratory studies than in pilot and full-scale tests; therefore, laboratory studies often give the most efficient separation of *DM* and P (Table 4) (Vadas, 2006). The best strategy is to determine the *SRF* in the laboratory and use it for the design of pilot or full-scale equipment.

Most P is contained in the small particles or is dissolved in the liquid (Fig. 1), while little N is found on particles larger than 0.125 mm (see Sect. 4.2). Therefore, relatively little P and N is retained on screens or filter fabrics, which only retain large particles (Meyer et al., 2007). Nevertheless, some N, P and K are retained in the filter cake because the cake has a high water content (50–80%-volume is not uncommon) with dissolved N, P and K (Pain et al., 1978; Holmberg et al., 1983; Møller et al., 2000).

5.4 Pressurized Filtration

5.4.1 Technical Description

The typical equipment used for filtration with applied pressure is a screw press or a press auger. In a press auger separator or a screw press separator, the effluent is transported into a cylindrical screen with a screw (Fig. 5). The liquid will pass through the screen and be collected in a container surrounding the screen. At the end of the axle the *DM*-rich fraction will be pressed against the plate and more liquid pressed out of the solid fraction. The solid phase will drop from the opening between the plate and the opening of the cylindrical mesh.

5.4.2 Operation and Separation Efficiency

The liquid flux through the filter medium can be determined from equation (18) by setting p equal to the applied pressure. According to filtration theory, *SRF* (specific filter cake resistance) is constant during constant pressure filtration; however, for a

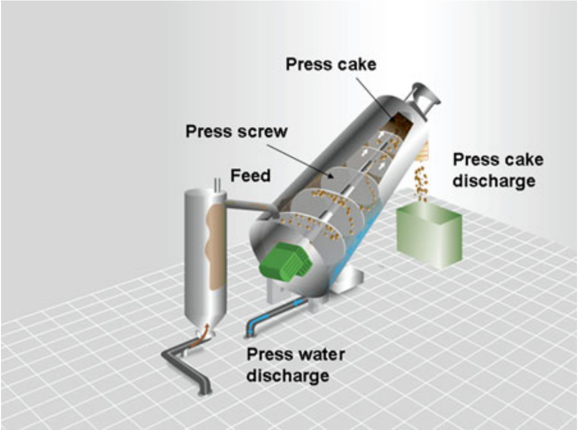

Fig. 5 A typical screw press

complex organic suspension such as slurry, *SRF* often increases during the process. The increasing *SRF* has been ascribed to sedimentation (Christensen and Dick, 1985), small particles blocking the pores in the cake (Sørensen et al., 1995), and a time-dependent compression of the cake (Christensen and Keiding, 2007). The slurry filter cake is compressed during pressure filtration; hence the *SRF* is several orders of magnitude higher for pressure filtration than for gravity drainage (Hjorth et al., 2008).

The cake compression ensures that the screw press can produce a solid fraction with a high *DM* content; often being twice as high as for gravity drainage (Møller et al., 2000). Increasing the applied pressure will increase the *DM* content of the solid fraction. Although aggregation of particles on the filter may, to some degree, contribute to the retention of small particles in the screw press, this has no significant effect, as the applied pressure forces small particles through the filter pores. A large proportion of small particles is therefore found in the liquid fraction after separation (Møller et al., 2002). Thus, the filter cake contains little N, P or K, because these are primarily found in the liquid phase and in the small particles which are drained off the filter cake with the permeate. In consequence, the plant nutrient separation efficiency of the screw press is low (Table 5).

Table 5 Separation indexes at pressurized filtration

Technical specifications		Slurry		Separation index (%)[2]					
Filter pore size (mm)	Filtration time (min)	Origin[1]	Dry matter (%)	Volume	Dry matter	N-total	NH_4-N	P-total	Reference
Mean				11	37	15	–	17	
(standard deviation)				(15)	(18)	(17)	(–)	(14)	
0.7	–	P	5.7	7	28	7	–	15	Møller et al., 2000
0.75	2	P	6.3		64	–	–	46	Vadas, 2006
0.9	–	P	5.7	5	28	6	–	12	Møller et al., 2000
1.0	–	P	5.3	4	27	7	–	7	Møller et al., 2002
–	–	P	1.8		51	31	–	42	Pieters et al., 1999
–	–	P	1.8		26	11	–	7	Pieters et al., 1999
–	–	P	6.3	7	21	4	–	13	Pos et al., 1984
0.75	2	C	8.0		64	–	–	28	Vadas, 2006
0.9	–	C	7.1	3	14	4	–	7	Møller et al., 2000
1.0	–	C	4.5	2	13	4	–	8	Møller et al., 2002
1	–	C	5.2	10	46	5	–	3	Wu, 2007
1.0	–	C	6.4	5	30	8	–	15	Møller et al., 2002
1.6	–	C	3.8	6	20	–	–	–	Pos et al., 1984
3.0	–	C	7.1	13	40	13	–	21	Møller et al., 2000
3.2	–	C	5.0	13	37	–	–	–	Pos et al., 1984
–	–	C	5.2	13	35	36	–	–	Pos et al., 1984
–	–	C	17.7	60	77	60	–	–	Menear and Smith, 1973

[1] P = pig, C = cattle.

[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

5.5 Concluding Remarks about Slurry Separators

In general, centrifugation is the most efficient technique for the separation of DM and P (see Tables 2–5) and filtration is less efficient for separating DM, N and P. To some extent, the separation of NH_4^+ follows the same pattern. The poorest N and P separation is achieved using pressurized filtration. The advantage of pressurized filtration, though, is the production of a solid fraction with a high DM concentration of the solid fraction. Therefore, drainage is sometimes followed by pressurized filtration to obtain both high separation efficiency and high DM concentration.

The choice of separators will depend on the objective of the separation. The goal may be to reduce DM content in the slurry to ease the transport of slurry in pipes or channels, reducing the risk of sedimentation or blockage of the pipes and channels, in which case simple screens or filters may be useful. This simple separation will also produce a liquid fraction that will contribute to a homogeneous spreading of plant nutrients on the field and reduced ammonia and odor emissions (Rubæk et al., 1996; Hansen et al., 2006). If the cost of separation has to be low and retention time is no problem, then sedimentation is a cheap technique that will reduce the plant nutrient composition of the slurry efficiently.

A screw press seems to be a good choice if the objective is to produce biomass with a high DM concentration suitable for incineration (Table 5). The filtration technologies may retain up to about one-quarter of the N and P in the slurry. In some cases this may be sufficient to achieve a harmonious balance between the amount of plant nutrient applied to the field and the plant demand on the livestock farm.

Of the above-mentioned solid–liquid separation techniques, the decanter centrifuge is the most efficient in retaining P and at the same time producing a DM-rich fraction low in water. In addition, this technique may produce a liquid fraction with a N:P:K ratio similar to the N:P:K requirements of the crop.

The produced solid and liquid fractions may be treated further to obtain valuable slurry products (see Sect. 7). The solid–liquid separation may be improved further by an initial addition of chemicals (see Sect. 6).

The major disadvantages of the above-mentioned solid–liquid separation technologies are that they require investment in expensive equipment, have a limited life span, and require maintenance, process control systems and an external power supply in order to run. These extra costs need to be balanced against the reduction in investment in and cost of slurry transportation between animal houses, slurry storage facilities and fields.

6 Pre-treatment: Chemical Additions

Solid–liquid separation technologies often have a limited capacity to retain plant nutrients in the solid fraction and thereby produce a liquid fraction with a composition that fulfils the needs of the livestock and plant producer.

Additives such as brown coal, benthonite, zeolite, crystals, chitosan and efficient microorganisms are used by livestock farmers and have been examined in numerous studies (Kayser, 1997; Henriksen et al., 1998a; Sommer et al., 2005; Garcia et al., 2009); however, in this review, these additives will not be considered as a pre-treatment for solid–liquid separation, as reliable information about their efficiency is lacking.

The focus here is on examples of the most commonly used methods that have been developed and used as pre-treatments to sedimentation, centrifugation or filtration. This section concentrates on the theory and use of polymers and multivalent ions, as well as on struvite formation.

6.1 Precipitation, Coagulation and Flocculation

Flocculation, coagulation and precipitation are chemical pre-treatments that improve the mechanical solid–liquid separation of many suspensions (Gregory, 1973; Smith-Palmer et al., 1994; Ashmore and Hearn, 2000; Nowostawska et al., 2005). In most suspensions, colloidal particles will not aggregate because the particles are negatively charged and repel each other (Gregory, 1989). However, aggregation will be facilitated by adding (1) multivalent cations that cause coagulation and/or (2) polymers, whereby flocculation occurs. The addition of multivalent cations will also enhance the precipitation of P.

6.1.1 Theory

Large amounts of phosphate will precipitate following the addition of multivalent cations to the slurry (Hjorth et al., 2008) due to formation of, for instance, $FePO_4$, $Fe_5(PO_4)_2(OH)_9$ and $Ca_3(PO_4)_2$.

At the same time, the multivalent cations cause coagulation of the particles in the slurry (Barrow et al., 1997; Sherman et al., 2000; Hjorth et al., 2008). At coagulation, the multivalent cations neutralize (or partially neutralize) a particle's negative surface charge by adsorbing the oppositely charged ions to the particle surface, creating a double layer (see Sect. 4.5) and thereby removing the electrostatic barrier that prevents aggregation; a process termed 'charge neutralization' (Fig. 6a). An optimum dose exists, and overdosing occurs when the adsorbed ions reverse the surface charge, thus counteracting aggregation (Gregory, 1989).

The addition of polyelectrolyte polymers to slurry induces flocculation. Polymer bridging is the main reaction mechanism, whereas patch flocculation is of limited significance, and charge neutralization is not important (Vanotti and Hunt, 1999; Hjorth et al., 2008). The addition of polymers will cause flocculation of particles and of existing but smaller aggregates that have been produced due to coagulation; for example, induced by the addition of Fe^{3+} to the slurry.

Patch flocculation is the adsorption to particles of oppositely charged polyelectrolytes with a charge density much higher than the charge density of the particles. Thus, local positively and negatively charged areas are formed on the surface of the particles (Fig. 6b; Gregory, 1973). This results in a strong electrical attraction between the particles, especially when the electrical attraction extends far into the solution, i.e. at low conductivity (Gregory, 1973). A greater tendency to patch flocculation is expected for branched polymers compared with linear polymers, and for high-molecular-weight polymers compared with lower-molecular-weight polymers (Bouyer et al., 2001; Bratskaya et al., 2005). However, since the conductivity of slurry is relatively high (see Sect. 4.5) patch flocculation is of minor relevance.

Polymer bridging occurs when long-chain polymers adsorb to the surface of more than one particle, causing the formation of strong aggregates of large flocs (Fig. 6c; Gregory, 1989). At high conductivity of the slurry or at high doses of added polymer (see Sect. 4.5), the polymer coils up and forms loops and tails. Due to steric hindrance between the particles, the loop and tail formation leads to deflocculation (Gregory, 1973). The maximum adsorbed mass of polymers increases with increasing ionic strength (Eriksson et al., 1993). Polymer characteristics of importance to polymer bridging are molecular weight and the molecular structure, as large molecules with long tails are capable of catching by-passing particles relatively easily. Charge density is also important, as this affects the number of loops and tails that are formed and the interaction between polymer and particles (Larsson et al., 1999; Ashmore and Hearn, 2000; Bouyer et al., 2001).

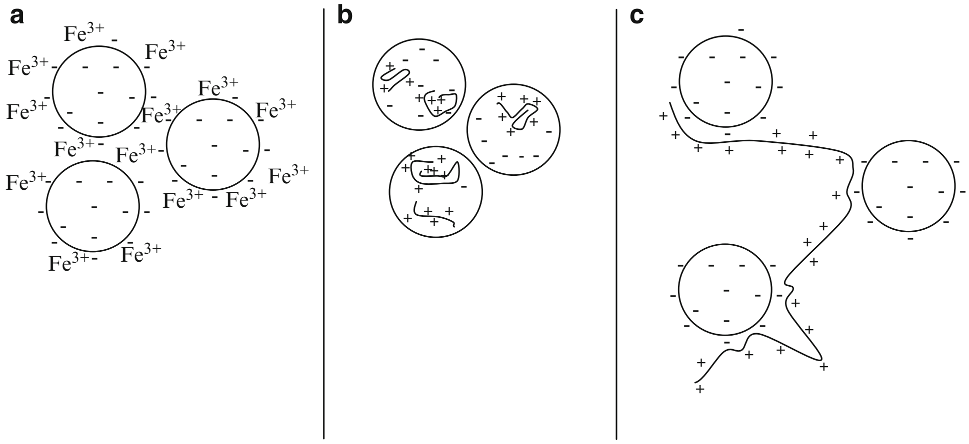

Fig. 6 Diagrammatic representations of **(a)** coagulation, **(b)** patch flocculation and **(c)** polymer bridging (adapted from Hjorth et al., 2008)

6.1.2 Technical Description

Multivalent ions and polymers need to be added carefully to the slurry in order to achieve satisfactory particle aggregation. If both additives are used, then the multivalent ion is added first to the slurry, which is then stirred to ensure homogeneous distribution of ions and dry matter (Fig. 7). Several minutes of slow stirring are necessary for the charge neutralization and coagulation to occur. Next, the polymer is slowly added in small doses during vigorous stirring, followed by slow stirring, which is necessary for polymer bridging and patch flocculation to occur. The shear applied (for example, by the impeller, i.e. time and stirring velocity), has a large impact on the formation of the aggregates; too low a shear causes the aggregates to be non-uniform and unstable with low particle catchment, while too large a shear causes the aggregates to be destroyed (Mikkelsen, 1999). After the addition and aggregation, the slurry may be transferred to ordinary solid–liquid separators (see Sect. 5; Fig. 7).

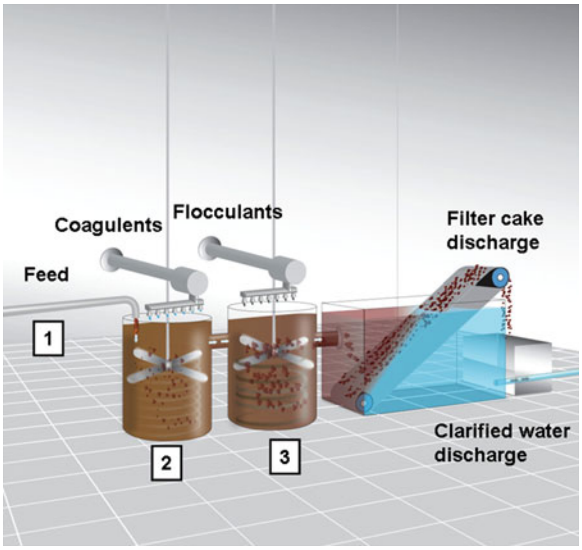

Fig. 7 Example of the use of additives and separating the solids from the liquids using a filter bed separator with containers and stirrers for treatment with (1) slurry pumped into the separator, (2) coagulants added to the slurry pumped into the first container, and (3) polymers added to slurry transferred from the first to the second container. The additives may be added into the tubes transferring the slurry to the separator and between containers, or the additives may be added to the slurry in the containers that are equipped with a stirrer: vigorous stirring may break up the flocs

6.1.3 Operation and Separation Efficiency

A broad range of multivalent cations intended to precipitate P and coagulate the slurry have been tested. These tests provide an opportunity to compare the efficiency of additives and to assess which multivalent ions provide the best slurry separation (Table 6). If the multivalent cation added to the slurry is calcium, the efficiency is ranked as $CaO > Ca(OH)_2$; if the multivalent ion is iron, the efficiency is ranked as $FeCl_3 > Fe_2(SO_4)_3 > FeSO_4$; while if the multivalent ion is aluminium, the efficiency is ranked as $Al_2(SO_4)_3 > AlCl_3$.

The use of the separation products should also be considered when selecting the multivalent ion. For example, a decrease in pH is observed upon addition of $FeCl_3$ and $Al_2(SO_4)_3$ (Hjorth et al., 2009), which could be an advantage with respect to NH_3 emissions; while CaO causes pH to increase, which may be preferable when a nitrification/denitrification step is to follow the separation (Szögi et al., 2006). The environmental consequences of applying multivalent cations should also be considered (Nahm, 2005).

The literature reports on a wide range of polyacrylamide (PAM) polymers that have been examined, with characteristics that may vary with respect to charge, charge density, molecular size and structure (Table 7). These studies therefore provide an opportunity to compare polymers and to give an indication of the polymer characteristics likely to provide the best slurry separation.

Most studies indicate that a cationic polymer is superior to anionic and neutral polymers (Table 7), which correlates well with the fact that the particles in animal slurry are mainly negatively charged (see Sect. 4.5). Polymers of medium charge density (20–40 mol%) have been shown to be most efficient in most studies, confirming the hypothesis that polymer bridging is the main mechanism behind flocculation in slurry (Vanotti and Hunt, 1999; Hjorth et al., 2008). The reason is that a polymer of medium charge density has a large number of charged sites and hence would have the ability to catch particles efficiently; however, a polymer of medium charge density also has many non-charged sites and will therefore not neutralize the particles completely, thereby leaving charges available on the particle surface for another polymer. This is supported by the finding that optimal separation has been observed at approximately 30% neutralization of the

Table 6 Comparison of precipitants/coagulants with respect to separation-relevant criteria

Slurry origin[1]	ID	Comparison Precipitant/coagulant		Criteria[2]	Reference
P	1	$FeCl_3$	$1 > 2 > 3 = 4$	pH reduction	Hjorth et al., 2009
	2	$Fe_2(SO_4)_3$			
	3	$AlCl_3$			
	4	$Al_2(SO_4)_3$			
P	1	$Fe_2(SO_4)_3$	$1 > 2$	Volume separation	Møller et al., 2007a
	2	$Al_2(SO_4)_3$	$2 > 1$	*DM*, N, P and NH4 separation	
P	1	$FeCl_3$	$1 > 2$	Liquid turbidity, volume separation	Estevez Rodríguez et al., 2005
	2	$Fe_2(SO_4)_3$	$1 = 2$	*DM*, VS, COD and P separation	
C	1	$Al_2(SO_4)_3$	$1 > 2, 3$	pH reduction	Karthikeyan et al., 2005
	2	$FeCl_3$	$1 = 2 > 3$	*DM*, P, N, NH_4 and TOC separation	
	3	CaO			
C	1	$AlCl_3$	$1 > 2$	pH reduction	Krumpelman et al., 2005
	2	$FeCl_3$	$2 > 1$	P separation	
			$1 = 2$	*DM* separation	
P	1	$Al_2(SO_4)_3$	$1 = 2 = 3 > 4 = 5$	*DM* separation	Powers and Flatow, 2002
	2	$FeCl_3$	$1 = 2 > 3 > 4 = 5$	Volume separation	
	3	CaO	$1 = 2 > 3 > 4 > 5$	P separation	
	4	$FeSO_4$			
	5	$CaCO_3$			
C	1	$Al_2(SO_4)_3$	$1 = 2$	*DM* separation	Dao and Daniel, 2002
	2	$FeCl_3$	$2 > 1$	pH reduction	
P	1	$Al_2(SO_4)_3$	$1 = 2$	*DM* separation	Zhang and Lei, 1998
	2	$FeCl_3$			
P	1	$FeCl_3$	$1 > 2$	*DM* separation	Ndegwa et al., 2001
	2	$Al_2(SO_4)_3$	$2 > 1$	P separation	
C	1	$FeCl_3$	$1 > 2$	Volume, P and N separation	Sherman et al., 2000
	2	$Al_2(SO_4)_3$	$2 > 1$	*DM* separation	
C	1	$FeCl_3$	$1 = 2 = 3$	*DM*, P separation	DeBusk et al., 2008
	2	$Al_2(SO_4)_3$			
	3	$AlCl_3$			
P	1	$FeCl_3$	$1 > 2 = 3$	*DM* separation	Gao et al., 1993
	2	$Ca(OH)_2$			
	3	$Al_2(SO_4)_3$			
C	1	CaO	$1 > 2 > 3$	*DM* separation	Barrow et al., 1997
	2	$Ca(OH)_2$	$1 = 2 > 3$	pH increase	
	3	$CaSO_4$			
C	1	$Fe_2(SO_4)_3$	$1 > 2$	*DM*, N and P separation	Barrow et al., 1997
	2	$FeSO_4$			
C	1	$FeCl_3$	$1 > 2$	*DM*, N and P separation	Barrow et al., 1997
	2	$Fe_2(SO_4)_3$			

[1] P = pig, C = cattle.

[2] *DM* = dry matter, COD = chemical oxygen demand, TOC = total organic carbon, VS: volatile solids.

particle surface charge (Hjorth et al., 2008). Further indications are that linear polymers are preferable to branched polymers, and polymers of large molecular weight are preferable to those of smaller molecular weight (Table 7). The reason seems to be the very efficient capture of smaller particles by the large, loose

Table 7 Comparison of flocculants (all polyacrylamide – PAM) with respect to separation-relevant criteria

Slurry		Flocculant (polymer)							
origin[1]	Id	Trade name	Charge	Charge density (mol%)	Structure	Molecular weight	Comparison	Criteria[2]	Reference
P	1	Superfloc c-2260	+	40	Linear	Large		P separation, Liquid turbidity	Hjorth et al., 2008
							1 > 2	Dewatering at drainage	
	2	Zetag 7878FS40	+	80	Branched	Medium	1 = 2	DM separation	
							1 < 2	Dewatering at pressurized filtration	
P	1	Superfloc c-2220	+	10	Linear	Large	5 > 1, 2, 3, 4	Liquid turbidity	Hjorth et al., 2009
	2	Superfloc c-2230	+	15	Linear	Large			
	3	Superfloc c-2240	+	20	Linear	Large			
	4	Superfloc c-2250	+	28	Linear	Large			
	5	Superfloc c-2260	+	40	Linear	Large			
C	1	MCP1	+	Medium	–	–	1 > 2 > 4 > 3	DM, VS and COD separation	Rico et al., 2007
	2	MCP2	+	Medium	–	–			
	3	MAP	–	Medium	–	–			
	4	SCP	+	Large	–	–			
P	1	Magnafloc 90L	–	10	–	–	1 = 2 > 3 = 4	DM separation	Szögi et al., 2006
	2	Magnafloc 120L	–	34	–	–		Dewatering at filtration/pressure	
	3	Zetag 7837	+	13	–	–			
	4	Zetag 7867	+	20	–	–			
P	1	NA	+	4	–	–	4 = 5 > 1, 2, 3, 6	DM separation	Vanotti et al., 2005
	2	NA	+	7	–	–	5 > 1, 2, 3, 4, 6	Dewatering at filtration/pressure	
	3	NA	+	13	–	–			
	4	NA	+	20	–	–			
	5	Magnifloc C-1596	+	40	–	Large			
	6	NA	+	70	–	–			
P	1	Nordfloc CE 169	+	60	Linear	Large	1, 2, 3 > 4	Dewatering at filtration/pressure	Estevez Rodríguez et al., 2005
	2	Nordfloc CE 169 WD	+	60	Linear	Large	1 = 2 = 3	DM, VS, COD and P separation	
	3	Nordfloc CE 129	+	40	Linear	Large			
	4	Nordfloc CE 166 TR	+	60	Branched	Large			

(continued)

Table 7 (continued)

Slurry origin[1]	Id	Flocculant (polymer) Trade name	Charge	Charge density (mol%)	Structure	Molecular weight	Comparison	Criteria[2]	Reference
C	1	Superfloc A 1885 RS	–	Large	–	–	5,6,7,8 > 1,2,3,4	DM and P separation	Timby et al., 2004
	2	Superfloc A 1883	–	Medium	–	–	1 = 2 = 3 = 4 = 5 = 6 = 7 = 8	Soluble reactive P separation	
	3	Superfloc A 1849 RS	–	Small	–	–			
	4	Superfloc N 1986	0	0	–	–			
	5	Superfloc C 1594	+	Medium	–	–			
	6	Magnifloc 240 G	+	Medium-large	–	–			
	7	Superfloc C 1598	+	Large	–	–			
	8	Magnifloc 255 G	+	Large					
C	1	C-1596	+	40	–	Large	1 = 2 = 3	DM seperation	Dao and Daniel, 2002
	2	C-581	+	100	–	Large			
	3	SD-2085	+	55	–	Medium			
P	1	Magnifloc 494 C	+	20	–	–		Volume, DM, COD, organic-N and organic-P separation	Vanotti and Hunt, 1999
	2	Magnifloc 985 N	0	0	–	– 1 > 2 = 3		NH4 and ortho-P seperation	
	3	Magnifloc 844 N	–	33	–	– 1=2=3			
P	1	Magnifloc 494 C	+	20	–	–	1 > 2 > 3 > 4	DM seperation	Garcia et al., 2007
	2	Excel Ultra 5000	+	75	–	–	1 > 2 = 3 > 4	COD seperation	
	3	Magnifloc 120L	–	34	–	–	1 > 3 > 2 = 4	P seperation	
	4	Magnifloc 985N	0	0	–	–			
P	1	Excel 5020	+	20	–	–	1 > 2 > 3 = 4	Volume and DM seperation	Vanotti and Hunt, 1999
	2	Excel 5020	+	40	–	–			
	3	Excel 5055	+	55	–	–			
	4	Excel 5000	+	75	–	–			
P and C	1	Magnifloc 240 G	+	Large	–	Large	4 > 1,2,3,5	DM seperation	Zhang and Lei, 1998
	2	Magnifloc 240 GL	+	Large	–	Large			
	3	Magnifloc 240 GSD	+	Large	–	Large			
	4	Magnifloc 255 G	+	Large	–	Large			
	5	Magnifloc 1596 C	+	Large	–	Large			
C	1	P 234 GD	+	–	–	–	1 = 2	DM, N and P seperation	Sherman et al., 2000
	2	PC 494	+	–	–	–			

[1] P = pig, C = cattle.

[2] *DM* = dry matter, COD = chemical oxygen demand.

flocs and/or that the shape of large, loose flocs is an advantage in the solid–liquid separators used to treat the slurry, especially when using separators where liquid is drained off the solids.

The separation products are often destined to be deposited in landfills or applied to cultivated fields; thus the environmental and health consequences of the applied polymer must be considered. The monomers of PAM used in most slurry separation studies (Table 7) can be toxic; however, a study on separated slurry products showed the risk to be minimal (Schechter et al., 1995). In the USA, these additives are considered to be 'generally recognized as safe' (GRAS) when added to the slurry below a specific application rate related to the end-use (Vanotti et al., 2002). However, there is a need for further studies on the efficiency of alternative polymer types for slurry separation, with the objective of replacing PAM with potentially less toxic polymers. Furthermore, when considering new polymers, one must take into consideration the toxicity of the organic components produced during degradation of the polymers.

The chemical and physical properties of slurry may vary (see Sect. 2) because slurry varies between livestock farms. During a separation run, slurry properties may vary even if the slurry is stirred when treated. Consequently, the optimal application rate of multivalent cations and polymers to slurry varies between farms and during a separation run. Various analytical methods have proved useful for evaluating the efficiency of different additives (Table 8). Of these, measurement of the *DM* content of the solids, dewatering ability, surface erosion from flocs, turbidity of the liquid fraction, capillary suction time, viscosity of the liquid fraction and floc size have proved to be useful as laboratory tests of slurry separation (Hjorth and Christensen, 2008).

The separation technique affects the separation efficiency of slurry treated with multivalent ions and flocculants. As for the combined solid–liquid separation technique, drainage appears to be superior when considering P and *DM* separation (Table 9), as pressure may disintegrate the flocs.

The addition of a coagulant/precipitant (a multivalent cation) increases the P separation (Table 9). There is a maximum multivalent ion amount above which no additional P will be retained in the solid fraction. The maximum separation index shows that 99% of the P in treated slurry is retained in the solid fraction (Sherman et al., 2000; Estevez Rodríguez et al., 2005). Due to coagulation, the addition of the multivalent cation may increase the *DM* and N separation (Barrow et al., 1997; Sherman et al., 2000). In theory, the multivalent ion may be overdosed when all dissolved P has

Table 8 Slurry characteristics potentially useful for assessing optimal coagulant and polymer dosage to slurry prior to separation

	Observed sample		
Slurry characteristics	Flocculated sample	Liquid fraction	Solid fraction
DM content		X	X
VS content		X	X
P content		X	X
N content		X	X
NH_4 content		X	X
Metal content		X	X
Dewaterability	X		
Settling	X		
Turbidity		X	
Viscosity		X	
Surface erosion from flocs	X		
Capillary suction time (CST)	X		
Floc size	X		
Particle dispersion ánalysis (PDA)	X		
Streaming current	X		

VS: volatile solids.

Table 9 Separation indexes following coagulation and flocculation

Technical description			Slurry		Separation index (%)[2]					
Separation technique	Coagulant/ precipitant	Flocculant	Origin[1]	Dry matter (%)	Volume	Dry matter	N-total	NH_4-N	P-total	Reference
Mean					22	70	43	20	79	
(standard deviation)					16)	(13)	(24)	(14)	(21)	
Sedimentation	$Al_2(SO_4)_3$	(none)	P	–	–	78	–	—	62	Powers and Flatow, 2002
Sedimentation	$FeCl_3$	(none)	P	–	–	82	–	–	64	Powers and Flatow, 2002
Sedimentation	$Fe_2(SO_4)_3$	(none)	P	–	–	58	–	–	38	Powers and Flatow, 2002
Sedimentation	$CaCO_3$	(none)	P	–	–	61	–	–	18	Powers and Flatow, 2002
Sedimentation	CaO	(none)	P	–	–	75	–	–	50	Powers and Flatow, 2002
Centrifugation	$CaCl_2$	(none)	P	3.1	10	34	1	10	59	Poels et al., 1988
Centrifugation	$CaCl_2$	(none)	P	3.3	10	64	28	21	86	Poels et al., 1988
Centrifugation	(none)	Optifloc c-6260	P	1.2	5	55	20	8	72	Møller et al., 2007a
Centrifugation	(none)	Optifloc c-6260	P	2.8	13	84	54	18	85	Møller et al., 2007a
Centrifugation	$Fe_2(SO_4)_3$	Optifloc c-6260	P	1.2	6	56	24	9	75	Møller et al., 2007a
Centrifugation	$Fe_2(SO_4)_3$	Optifloc c-6260	P	2.1	5	54	16	7	73	Møller et al., 2007a
Centrifugation	$Al_2(SO_4)_3$	Optifloc c-6260	P	2.1	10	73	35	15	91	Møller et al., 2007a
Centrifugation	$Al_2(SO_4)_3$	Optifloc c-6260	P	4.2	18	87	45	28	90	Møller et al., 2007a
Drainage	(none)	Superfloc c-2260	P	7.0	35	79	45	36	85	Hjorth et al., 2009
Drainage	$FeCl_3$	Superfloc c-2260	P	7.0	43	88	62	50	97	Hjorth et al., 2009
Drainage	$FeCl_3$	Nordfloc CE 169	P	2.5	–	70	–	–	99	Estevez Rodríguez et al., 2005
Drainage	$FeCl_3$	Nordfloc CE 169WD	P	2.5	–	65	–	–	99	Estevez Rodríguez et al., 2005
Drainage	$FeCl_3$	Nordfloc CE 129	P	2.5	–	70	–	–	99	Estevez Rodríguez et al., 2005
Drainage	$Fe_2(SO_4)_3$	Nordfloc CE 169	P	2.5	–	70	–	–	99	Estevez Rodríguez et al., 2005
Drainage	$Fe_2(SO_4)_3$	Nordfloc CE 169WD	P	2.5	–	67	–	–	99	Estevez Rodríguez et al., 2005
Drainage	$Fe_2(SO_4)_3$	Nordfloc CE 129	P	2.5	–	65	–	–	99	Estevez Rodríguez et al., 2005
Sedimentation	$Al_2(SO_4)_3$	(none)	C	1.1	54	92	83	–	99	Sherman et al., 2000
Sedimentation	$FeCl_3$	(none)	C	1.1	47	78	75	–	94	Sherman et al., 2000
Sedimentation	(none)	P-234GD	C	1.1	29	66	35	–	67	Sherman et al., 2000
Sedimentation	(none)	P C-494	C	1.1	29	67	36	–	67	Sherman et al., 2000
Drainage	$AlCl_3$	(none)	C	2.0	–	60	–	–	–	Timby et al., 2004
Drainage	$AlCl_3$	(undefine)	C	2.6	–	97	80	–	–	Worley et al., 2008
Pressurized filtration	CaO	MCP1	C	5.0	24	69	48	–	78	Rico et al., 2007

[1] P = pig, C = cattle.

[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

been precipitated and all particles have been charge neutralized. Overdosing will cause the separation indexes to decrease, because the particles then become positively charged and repel each other.

Polymer (flocculant) addition improves DM, N and P separation (Table 9), because floc formation increases the amount of DM retained in the solid fraction and also the N- and P-containing organic and inorganic particles. Depending on the polymer, the structure of the flocs varies. The addition of linear polymers of large molecular weight produces large, loose flocs; while the addition of branched polymers of small molecular weight produces small, dense flocs (Hjorth et al., 2008). As sedimentation and centrifugation efficiency depend on particle density, it may be advantageous to produce small, dense flocs using branched small-molecular-weight polymers. Alternatively, when using filtration without applied pressure, draining of the liquid should be eased by producing large, loose flocs, resulting in a filter cake with high porosity. Filtration with applied pressure is favored by non-compressible flocs; and thus small, dense flocs may be the floc structure giving the best separation.

Ammonium and K are dissolved in the liquid (see Sect. 4.3); hence the polymer will not improve the NH_4^+ or K separation. Due to the positive charges of NH_4^+ and K, a multivalent cation will not improve the separation through precipitation or charge neutralization. Adding polymers may increase NH_4^+ and K content in the solid fraction (Hjorth et al., 2009), because liquid with dissolved NH_4^+ and K is retained in the flocs, as floc liquid is difficult to remove by mechanical solid–liquid separation (Vesilind, 1994).

The charge of DM particles have to be neutralized. Therefore at increasing charge of the DM and increasing concentration of the particles demands increasing addition of multivalent cation and polymer to a successful separation (Zhang and Lei, 1998). In contrast, decreasing amounts of polymer are needed when coagulation is improved by pre-treatment with an increased amount of multivalent cations (Krumpelman et al., 2005; Hjorth et al., 2009).

The separation indexes of untreated slurry (Tables 2–5) are much lower than the indexes of slurry treated with flocculants and coagulants (Table 9), with flocculation having a larger impact on filtration than on centrifugation (Hjorth et al., 2008). Thus, separation is improved by flocculation and further improved when multivalent ions are also added to the slurry.

6.2 Struvite Crystallization

Slurry contains struvite or the inorganic components contributing to the formation of struvite crystals. Thus, physical and chemical changes in slurry may initiate struvite formation. This is a problem in biogas plants because struvite will build up on pipe surfaces and reduce the capacity of slurry transport, but struvite formation may also be used to remove P and NH_4^+ from the slurry.

6.2.1 Theory

Crystallization of phosphate is used to facilitate the sedimentation of phosphorus as particles. Animal slurry contains P in the form of struvite ($MgNH_4PO_4 \cdot 6H_2O$) and apatite $Ca_5OH(PO_4)_3$ (hydroxyapatite) crystals (see Sect. 4; Bril and Salomons, 1990; Greaves et al., 1999; Suzuki et al., 2002). Struvite has been shown to be the most significant of the solid forms of P in slurry, and struvite crystallization has been widely used as a method for separating P from wastewater and slurry (Ohlinger et al., 2000; Burton and Turner, 2003). Hydroxyapatite is not as attractive as a means of removing P from slurry and wastewater, because much calcium will have crystallized as calcite $CaCO_3$ in slurry.

$$\mathrm{Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O} \\ \leftrightarrow \mathrm{MgNH_4PO_4 \cdot 6H_2O(s)} \qquad (17)$$

The formation constant (K_0) of struvite is 1.41×10^{13} and the equilibrium ion-activity product (IAPeq) is 7.08×10^{-14}. In slurry, the product of $[Mg^{2+}]$, $[NH_4^+]$ and $[PO_4^{3-}]$ is generally lower than the conditional formation constant, due to low concentrations of dissolved magnesium (Mg^{2+}) (Sommer and Husted, 1995a; Nelson et al., 2003), so little or no struvite is formed. The dissolved Mg may be lower than that determined with traditional extraction and measuring techniques, because a major fraction of the Mg^{2+} ions may form complexes with dissolved organic matter (Bril and Salomons, 1990; Christensen et al., 2009). The concentration of PO_4^{3-} will also have a major impact on struvite crystallization. HPO_4^{2-} is a weak acid and at pH levels below pH 7 the formation of HPO_4^{2-} and a low concentration of PO_4^{3-} will reduce struvite crystallization (Nelson et al., 2003). In most slurries with a typical pH of 7.5–8.3, the NH_4^+ concentration is higher than the 1:1:1 ratio ($[Mg^{2+}]$:$[NH_4^+]$:$[PO_4^{3-}]$) for the formation of struvite and will not be the limiting factor in the reaction (Nelson et al., 2003). At high pH, the NH_4^+ concentration will decrease, and low NH_4^+ concentration may limit struvite formation (Buchanan et al., 1994). Optimal conditions for the sedimentation of struvite therefore occur at about pH 9 (Buchanan et al., 1994; Nelson et al., 2003).

6.2.2 Technical Description

In a slurry container, the slurry may be manipulated with the purpose of forming struvite that will precipitate. Afterwards the slurry can be transferred to a thickener or another type of solid–liquid separator (see Sect. 5). In the pilot plant described by Suzuki et al. (2002), the struvite was formed in a thickener, which was also used to remove the struvite. Alternatively, the struvite may be produced in a psychrophilic anaerobic sequencing batch reactor biogas plant, where the P settles as struvite and is removed with the sludge (Massé et al., 2007a), or struvite may settle in anaerobic slurry lagoons and be removed with the sludge (Nelson et al., 2003).

6.2.3 Operation and Separation Efficiency

P removal can be increased by adding Mg to the slurry being treated (Table 10). The addition of Fe and a base to the slurry will enhance dissolution of Mg and thereby increase P removal (Suzuki et al., 2002; Laridi et al., 2005). On the other hand, P removal may be low even after the addition of Mg and NH_3 if pH is low (Table 10; Sheffield et al., 2005).

Aeration of slurry or anaerobic digestion of slurry will increase pH and also reduce the organic matter content in the slurry (Suzuki et al., 2002; Massé et al., 2005). Both processes will greatly enhance crystallization of struvite due to an increase in the concentration of dissolved Mg^{2+} and PO_4^{3-}. Thus, aeration may produce a slurry with a mole ratio optimal for struvite crystallization, and increase struvite crystallization by a factor of about 10. In a continuous-flow pilot-scale sedimentation plant, this leads to the removal of 65–99% of P and 15% of total N (Table 10; Suzuki et al., 2002).

Crystallization is initiated by nuclei, which may be grains of sand, or by contributing energy to the nucleation process by using rakes (Battistoni et al., 2002). In most slurries, the amount of sand is generally high and one would not anticipate any need to enhance the nucleation process.

Table 10 Separation index at struvite precipitation and sedimentation

	Slurry		Separation index (%)[2]		
Addition	Origin[1]	Dry matter (%)	N-total	P-total	Reference
Mean			16	63	
(standard deviation)			(1)	(37)	
Mg, OH^-	P			85	Nelson et al., 2003
Mg, NH_3	P			60	Bowers and Westerman, 2003
Mg, increase pH	P	18		18	Suzuki et al., 2007
Mg, increase pH	P	18		49	Suzuki et al., 2007
Mg, increase pH	P			96	Burns et al., 2003
Mg, increase pH	P			98	Burns et al., 2003
Fe, OH	P	17		98	Laridi et al., 2005
Fe, OH	P	15		99	Laridi et al., 2005
Mg, NH_3	C			8	Sheffield et al., 2005
Mg, NH_3	C			19	Sheffield et al., 2005

[1] P = pig, C = cattle.
[2] Separation index is the mass of a compound in the solid fraction compared to the mass of a compound in the original raw slurry.

7 Post-treatment: Separation Techniques

Post-treatment separation techniques need to be employed when eco-efficient technical solutions to the processing of animal slurry are sought. The objective is to produce clean water, heat and power, fuel, and high-value N, P and K fertilizers from animal slurry – products that may not be obtained when using solid–liquid separation on its own. Some technologies have been tested on the laboratory scale, while a few have reached the farm pilot-production scale. The techniques described in this section have been chosen based on the criterion that sufficient knowledge is currently available to discuss their possible usefulness.

7.1 Evaporation

7.1.1 Technical Description, Operation and Separation Efficiency

Water and volatiles can be removed from the slurry or the liquid fraction by evaporation. The liquid is heated to boiling point, which for slurry will be a little over 100 °C at atmospheric pressure. At this temperature, both water and volatile organic compounds, such as free fatty acids and NH_3, will evaporate. This vapor phase has to be condensed in order to retrieve the energy used to evaporate the water and volatiles. The energy consumed in the process is high, as the heat needed for evaporation represents roughly 670 kWh per metric ton of water evaporated. The energy (q) consumed in a single-step evaporator can thus be estimated as:

$$q = \overset{\bullet}{m}_{water} \cdot \Delta H^{vap} \tag{18}$$

where ΔH^{vap} is the heat of evaporation (J kg^{-1}), $\overset{\bullet}{m}_{water}$ is the amount of water removed (kg s^{-1}), and q is the energy consumption (W).

To reduce the energy costs, evaporators can be operated either as single evaporators using recompressed steam, or in series where the vapor generated in the first evaporator is used as heating steam for the next evaporator. In order to transfer energy from the steam to the liquid in each evaporator, the steam temperature has to be higher than the liquid temperature. Therefore, each consecutive evaporator is operated at a lower temperature than the preceding evaporator. Boiling can thus only be achieved in each evaporator by operating each at a slightly lower pressure than the preceding evaporator. As the boiling point for slurry increases with solid content, the liquid slurry should enter the last evaporator and run counter-current to the vapor. Even though multi-step evaporation increases investment costs, experience from industrial applications shows that between three and six evaporators in series is economically viable (Foust et al., 1980; McCabe et al., 2005). In this way, a 92%-volume reduction can be achieved at an energy consumption of 120–130 kWh per ton slurry treated (Pedersen, 2004). No literature on the effect on N separation has been found.

7.2 Membranes

For small particles, membrane filtration of the liquid fraction could be an attractive supplement to solid–liquid separation (see Sect. 5). Furthermore, membrane separation may be used to separate and concentrate dissolved K, P and N nutrients, producing a nutrient-rich liquid phase and, in principle, pure water for reuse or safe discharge to the environment. A review on membrane processes for slurry treatment has recently been published (Masse et al., 2007b), which gives details of efficiency and usability, and therefore only a short description is included here.

7.2.1 Microfiltration and Ultrafiltration

7.2.1.1 Technical Description

Microfiltration removes solid particles in the range of about 0.1–10 μm, while ultrafiltration retains solid particles in the range from about 5 to 200 nm (Baker, 2004). Thus, they are well suited to remove nutrients associated with small particles such as P (Masse et al., 2007b).

The design of membrane units is based on the membrane flux (J_{liquid}), which is the amount of liquid removed per area of membrane. In micro- and ultrafiltration, J_{liquid} can be described by the general equation:

$$J_{liquid} = \frac{\Delta P}{\mu_{liquid} \cdot (R_m + R_{rev} + R_{irrev})} \tag{19}$$

where J is the flux (kg m^{-2} s^{-1}), μ_{liquid} is the viscosity of the permeate, R_m is the resistance to flow from the membrane (m^{-1}), R_{rev} is the reversible fouling resistance often ascribed to filter cake formation and concentration polarization (m^{-1}), R_{irrev} is the irreversible fouling resistance normally ascribed to pore blocking and adsorption of materials to the membrane (m^{-1}), and ΔP is the transmembrane pressure over the membrane (Pa).

During micro- and ultrafiltration, the slurry is pressed through a porous membrane. If dead-end filtration is used, a very dense filter cake build-up will occur. Therefore, micro- and ultrafiltration have to be carried out as cross-flow filtration, where only a fraction of the liquid is removed as permeate through the membrane, while solids and part of the liquid are retained as retentate. The cross flow removes most of the solids deposited on the surface, but some flow-controlled reversible filter cake formation cannot be avoided. Worse still, irreversible adhesion of minor particles can occur in the membrane pores, partly blocking the path for the liquid. In addition, bacterial growth can occur on the membrane surface, further reducing the flow through the membrane. This kind of membrane fouling will be especially severe for the treatment of slurry. Reversible filter cake formation can be removed by flushing with water. In contrast, the irreversible fouling can, at best, only partly be removed by cleaning the membrane in the system (CIP) with dilute base followed by dilute acid and, if necessary, enzymatic treatment.

Because of fouling, micro- and ultrafiltration membranes can only be used to separate pre-treated slurry, such as effluent streams from biogas reactors or runoff streams from centrifuges.

7.2.1.2 Operation and Separation Efficiency

For slurry microfiltration, transmembrane pressure will typically be around 100–180 kPa. At this pressure, filter cake formation and fouling become rate-determining, and increasing the transmembrane pressure no longer increases the flux over the membrane to any significant extent. Increasing the feed flow can increase the flux by reducing the filter cake thickness, but at velocities above around 2 m s^{-1} this becomes uneconomic (Owen et al., 1995). Therefore, the maximum flux attainable is limited to around 160 L·m^{-2}·h^{-1}, although long-term fluxes down to 10–40 L·m^{-2}·h^{-1} should be expected, due to fouling. Depending on the size distribution, a retention efficiency of 75% of DM can be expected (Masse et al., 2007b). Membrane filtration retains small particles; therefore, the large amount of P contained in particles between 0.45 and 10 μm is retained with membrane filtration, and a high P removal can be expected (Masse et al., 2007b). Dissolved N, K and DM will not be retained.

For ultrafiltration, transmembrane pressures up to 800 kPa can be used, depending on membrane pore size. The larger the pore size, the lower the transmembrane pressure (Baker, 2004). For filtered pig slurry, fluxes between 10 and 40 L·m^{-2}·h^{-1} at a transmembrane pressure of 100 kPa have been reported with 100% DM removal and a P removal efficiency of up to 87%, depending on operational conditions (Masse et al., 2007b). For partially digested non-filtered pig slurry, flushing the membrane every 5 minutes with permeate leads to a fall in flux of 25% over a 70-day period compared with a flux decline of 75% if only a single cleaning procedure is carried out (du Preez et al., 2005). In this study, though, the maximum flux was only 16 L·m^{-2}·h^{-1}.

7.2.2 Nanofiltration and Reverse Osmosis

7.2.2.1 Technical Description

The liquid fraction from an ultrafiltration membrane can contain large amounts of dissolved K^+, NH_4^+ and NH_3. Reverse osmosis and, to some extent, nanofiltration membranes can retain dissolved nutrients, and deliver high concentrate retentate and purified water permeate. Depending on the membrane chosen, nanofiltration will retain uncharged molecules larger than 200–400 Da and, to a lesser extent, smaller charged molecules such as Ca^{2+}, Mg^{2+} and NH_4^+. Nanofiltration can thus be used to remove DM and, to some extent, charged molecules. If a pure water permeate is needed, reverse osmosis can retain NH_4^+ and K^+ and, to a lesser extent, NH_3 (Masse et al., 2007b).

The fouling problems are even more severe for nanofiltration and reverse osmosis than for micro- and ultrafiltration. In connection with slurry, nanofiltration or reverse osmosis can therefore only be used for separation of dissolved components from the permeate produced in an ultrafiltration unit. The direct filtration of raw slurry will foul the membrane within minutes.

The water flux through nanofiltration and reverse osmosis membranes can be described (Baker, 2004) as follows:

$$J_{liquid} = A \cdot (\Delta p - \Delta \Pi) \tag{20}$$

where A is the water permeability (kg m^{-2} $s^{-1}Pa^{-1}$) and $\Delta \Pi$ (Pa) is the osmotic pressure difference between retentate and permeate. The maximum possible retentate concentration will be determined by the osmotic pressure difference and the maximum possible transmembrane pressure.

As concentration polarization influences the osmotic pressure on the membrane surface during nanofiltration and reverse osmosis, experimental permeability constants depend on the liquid cross flow and, because of unavoidable fouling, on time in use. As for micro- and ultrafiltration membranes, cleaning will need to be performed regularly in order to avoid fouling.

7.2.2.2 Operation and Separation Efficiency

For nanofiltration transmembrane operation, pressure will be around 350–3000 kPa. Although not well suited for the removal of K or N, nanofiltration membranes have been shown to be capable of removing up to 52% of the NH_4^+ and 78% of the K (Masse et al., 2007b) and, if a suitable membrane is chosen, all the soluble DM with a molecular weight above 200 Da will be removed.

For more demanding applications, reverse osmosis has to be used. The transmembrane pressure for reverse osmosis operations is typically around 3.5–6.5 MPa, although up to 150 MPa can be achieved for specially designed membranes. The retention of K is usually high and independent of pH but, as NH_4^+ is retained better than NH_3, the separation is very pH-dependent and also depends on the ionic strength of the retentate (Masse et al., 2008). Therefore, N separation will depend on pH, slurry origin and the final volume reduction of the retentate. For pig slurry, the retention of NH_4^+ thus decreased from 90% to 70% and for K from 93% to 87% when the reduction in retentate volume was increased from 50% to 90% (Masse et al., 2007b).

As the flux for nanofiltration and reverse osmosis is very dependent not only on fouling but also on the osmotic pressure of the retentate, the flux will change dramatically during a concentration process, with maximum fluxes reaching up to 65 L m^{-2} h^{-1} at the initial conditions, but approaching zero at the final reduction volume, where the osmotic pressure approaches the transmembrane pressure.

8 Conclusions

Separation indexes, which express the distribution of the specific component between the solid and liquid fractions (see Eqs. (5, 6)), have proved useful for the comparison of the efficiency of the different separation techniques. From a literature review it was found that only about 25% of the papers on solid–liquid separation included sufficient data to assess the separation index of the technology tested. This emphasizes the need for a standard method for characterizing slurry separation efficiency, including a thorough characterization of the animal slurry treated and the fractions produced, and flow measurements of the slurry being treated.

Slurry can be treated with separation technologies that produce a solid fraction in which much of the P and dry matter (DM) and significant amounts of the N in the slurry are retained. These technologies are simple to use and reliable. More complex technologies may, although with a relatively high energy consumption, remove nitrogen, including NH_4^+, from the slurry. By combining solid–liquid separation technologies with pre- and post-treatments, end-products having an optimal composition for a specific end-use may be produced.

In order to achieve these objectives, there is a need for studies on the physical and chemical properties of slurry and their effects on separation efficiency, which include a functional physical-chemical characterization of slurry and identification of how the treatment affects the physical-chemical characteristics of the separation product. Only if this knowledge is available can existing separation technologies be optimized and combined in a systematic way in order to achieve the desired end-products.

At present, the most efficient solid–liquid mechanical separators for the removal of DM, P and, to some extent, total N and NH_4^+, can be ranked in the following order: centrifuge > sedimentation > filtration without pressure > filtration with pressure. Struvite crystallization prior to the solid–liquid separation improves the N and P separation. Flocculation before separation also improves the separation of DM, N and P significantly. The best flocculant available at present (polyacrylamide – PAM) is a cationic, medium-charge-density (20–40 mol%), linear, large-molecular-weight polymer, and the best coagulants are $Al_2(SO_4)_3$ and $FeCl_3$. Filtration without pressure is the best slurry separation technique for slurry with added flocculants.

After the separation of DM, P and N from the slurry, the liquid fraction produced may be treated with membrane separation, evaporation of water or NH_3 stripping. Regarding membrane separation, attention must be paid to fouling problems. Stripping and evaporation have a high energy consumption, and efficient heat recovery or cheap surplus heat is necessary for these technologies to be of interest at present.

In order to set up an animal slurry separation operation, a whole-system approach is important either with or without pre- and post-treatments. Just as in any industrial farm operation, it is of paramount importance to balance investment, running and labor costs against product added value, environmental impact and process complexity. This seems to be a promising area for future intensive research and development.

Acknowledgements This study was supported by a grant from the Danish Agency for Science, Technology and Innovation to the ‘CLEANWASTE’ project, under the Environmentally Friendly Technologies program.

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E. Lichtfouse et al. (eds.), *Sustainable Agriculture Volume 2*,
DOI 10.1007/978-94-007-0394-0,