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Chapter 1
Ecological Intensification: Local Innovation to Address Global Challenges

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Abstract The debate on future global food security is centered on increasing yields. This focus on availability of food is overshadowing access and utilization of food, and the stability of these over time. In addition, pleas for increasing yields across the board overlook the diversity of current positions and contexts in which local agriculture functions. And finally, the actual model of production is based on mainstream agricultural models in industrialized societies, in which ecological diversity and benefits from nature have been ignored or replaced by external inputs. The dependence upon external inputs should exacerbate the negative impacts on the environment and on social equity. Strategies to address future global food security thus require local innovation to increase agricultural production in a sustainable, affordable way in the poorest regions of the world, and to reduce the environmental

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impact of agriculture and its dependence on non-renewable resources. Ecological intensification, the smart use of biodiversity-mediated ecosystem functions to support agricultural production, is portrayed as the most promising avenue to achieve these goals.

Here we first review examples of ecological intensification from around the world. Functional diversity at plant, field and regional scales is shown to hold promise for reducing pesticide need in potato production in the Netherlands, increasing beef production on the pampas and campos in south-east South-America without additional inputs, and staple crop production in various regions in Africa. Strategies range from drawing on high-tech breeding programs to mobilizing and enriching local knowledge and customs of maintaining perennials in annual production systems. Such strategies have in common that larger spatial scales of management, such as landscapes, provide important entry points in addition to the field level.

We then argue that the necessary innovation system to support transitions towards ecological intensification and to anchor positive changes should be built from a hybridization of approaches that favour simultaneously bottom-up processes, e.g. developing niches in which experiments with ecological intensification develop, and top-down processes: changing socio-technical regimes which represent conventional production systems through targeted policies. We show that there are prospects for drawing on local experiences and innovation platforms that foster co-learning and support co-evolution of ecological intensification options in specific contexts, when connected with broader change in the realm of policy systems and value chains. This would require dedicated system innovation programmes that connect local and global levels to sustainably anchor change towards ecological intensification.

Keywords Food security • Agroecology • Soil rehabilitation • Livestock • Innovation systems • Transitions

1.1 Introduction

The discourse that dominates the debate on current and future global food security places emphasis on the need to intensify agricultural production in order to meet the demands of a growing world population (e.g. Huang et al 2002; Godfray et al. 2010). It is often assumed that agricultural production will have to increase by 70 % to be able to feed nine billion people by the year 2050, as a result of both population growth and expected changes in human diets associated with rising average incomes in developing countries (Tilman et al. 2011). Since the increase in food production that may be expected from agricultural land expansion is calculated to be in the order of 15 % (Lambin and Meyfroidt 2011), it is further assumed that agricultural production can only be increased through raising average crop and animal yields. This is a rather simplistic view on how to address the challenge of global food security. It is based on a large number of assumptions and only partially true. It justifies

further intensification of industrial agriculture in the global "North", with all the environmental problems that this entails (e.g., Geiger et al. 2010) in the name of helping the poorest of the poor. And it is shared among the principal international actors of the agricultural sector, i.e., research organisations and consultative panels, the agro-chemical and breeding industries, most national governments, and numerous members of the academia (cf. Tittonell 2014).

Meeting food security anywhere in the world requires addressing its four pillars: availability, access, utilization and the stability of all these over time (Pinstrup-Andersen 2009). At global scale, current food production (around 2700 Kcal person^{-1} day^{-1}) is enough to meet the demands of human kind (between 1800 and 2200 Kcal person^{-1} day^{-1}), as estimated by the World Health Organisation (2013). Yet 805 million people go hungry for more than 6 months every year (WFP 2012). It is also true that as humans we are climbing up in trophic levels due to increased consumption of animal protein (Bonhommeau et al. 2013). Recently, however, more detailed nutritional studies examining global diets and human requirements of various food items revealed that while the current production of vegetables, nuts, fruits, milk and edible seeds are insufficient to meet world demands, the production of whole grains and fish are about 50 % higher than human requirements, while the production of red meat is 568 % higher than required for a healthy diet (Murray 2014 – Institute for Health Metrics and Evaluation, www.healthdata.org). This suggests that the assumption that food production must increase is only true for certain food items (e.g., vegetables by 11 %, seeds and nuts by 58 %, fruits by 34 %, etc.). It is also clear that the problem of food security is not primarily one of availability, but primarily one of access to food.

But it is not just a problem of food distribution. To address food insecurity in rural areas of sub-Saharan Africa, for instance, it is not enough to produce large amounts of food in the American Midwest or in the Pampas of Argentina. The agricultural production from these regions is subject to multiple demands, from the food, livestock or chemical industries, or from the energy sector, all of which are often more attractive and logistically easier to meet than the needs of poor rural dwellers in developing countries. Addressing global food security requires local solutions. In other words, food must be produced where it is most needed. Paradoxically, most poor people around the world live in rural areas and own small pieces of land; most of the hungry of the world are farmers who can potentially produce their own food. Their ability to do so is hampered by different factors, including access to agricultural inputs, knowledge and technologies, socio-political instability, lack of governance or weak institutions, climate change, demographic pressure and natural resource degradation (UNCTAD 2013, 2014; WFP 2013).

The current model of agricultural intensification that fails at feeding the world today cannot be expected it to feed the world in 2050. This model, deployed in the developed world during the post-war period, had enormous consequences for the environment, and has been largely dependent on non-renewable resources and on subsidies from other sectors of national economies. Most poor countries in the developing world, where agriculture may generate up to 70 % of the national income, are not in a position to subsidise their agriculture at the levels observed in industrialised countries – where agriculture represents only 3 % of their economy

(Koning 2013). On the other hand, the model of intensification issued from the 'green revolution' in the 1960s and 1970s did not have the positive impacts that were expected in the poorest regions of the world, in spite of the subsidies and international aid that were deployed to that effect. Current per capita food production and average agricultural yields in most of these regions remain at the same levels as 50 years ago (FAO 2014). It did, however, have negative environmental and social impacts around the world (Freebairn 1995; Matson et al. 1997; Maredia and Pingali 2001; IAASTD 2009; UNCTAD 2014). New forms of agricultural intensification are needed, both to increase agricultural production in the poorer and currently less productive areas of the world, where people go hungry, and to reduce the environmental impacts and the dependence on non-renewable resources of industrialised agriculture.

We hypothesise that food production can increase where needed and at the same time be sustainable by making intensive and smart use of the natural functionalities that ecosystems offer. Approaches to agricultural intensification that rely largely on ecosystem functions have been grouped under the generic term of ecological intensification (Dore et al. 2011). Yet, ecological intensification, which takes different forms around the world, is not a universally applicable set of guidelines on how to farm sustainably (Tittonell 2014). It requires local innovation, local adaptation, and the creation of favourable socio-technical regimes that allow for such local diversity. In other words, it can only provide local solutions to global problems. The objective of this chapter is to examine examples of ecological intensification around the world, from small-scale family agriculture to high input western farming systems, and to reflect on the diversity of intensification pathways. Many of these examples, however, emerged within specific geographical, social and economic niches, and the question is how to scale them out and anchor them in mainstream systems. Hence, what kind of innovation environment would be necessary to foster ecological intensification? At the end of this paper we reflect upon the attributes and possible structure of an innovation system that can support the transition towards ecologically intensive ways of farming.

1.2 Intensify, Extensify, Detoxify

Current food production in the most productive areas of the developed world represents only a fraction of global food production, as illustrated for cereals in Fig. 1.1. For example, the total cereal production of all countries in which the average cereal yield is greater than 6 t ha^{-1} year^{-1} (most of western Europe and North America) represents barely 12.5 % of the world cereal production. Half of the total cereal production in the world comes from countries where the average yields are lower than 3 t ha^{-1} year^{-1}, whereas the poorest countries in the world produce average yields of around 1.3 t ha^{-1} year^{-1}. This analysis suggest that further increasing yields in developed countries to be able to feed the world is not justified, as even doubling

Contribution to world total production (%)
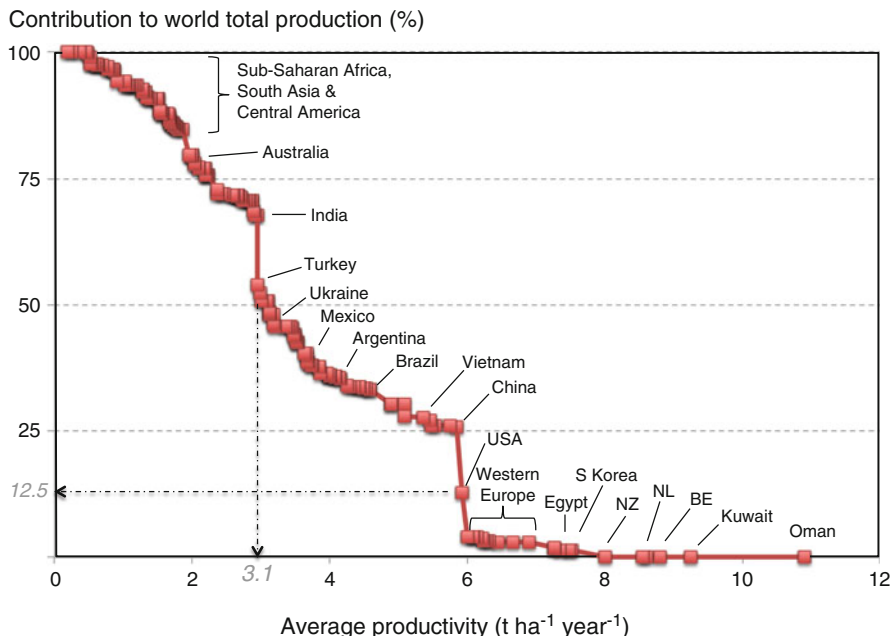
Fig. 1.1 Average cereal productivity per country and their cumulative contribution to total world production. *Dash-dotted lines* indicate (*vertical*) that 50 % of the total world production is realized in countries where average yields are lower than 3.1 t ha^{-1}, and (*horizontal*) that all the cereal production in the countries where average yields are higher than 6 t ha^{-1} (from USA to Oman) together represent 12.5 % of the world total

production in these countries will still contribute a relatively small fraction of the world demand. Besides, barely 20–30 % of the energy contained in the agricultural produce from these systems is delivered to the food chain, while the rest is lost in the process of transformation of grain into meat, bioenergy or other industrial products (Cassidy et al. 2013). Since yield gains in response to input intensification follow the law of diminishing returns, increasing average yields by e.g. 1 t ha^{-1} in countries and regions where yields are already high requires larger investments (and potentially greater environmental damage) than in regions where yields fluctuate around 1.3 t ha^{-1}. Industrial agriculture consumes most of the energy, water and nutrient inputs available at global level, pushing their international price to levels that make them prohibitive for smallholder farmers in the global South.

On the other hand, since agriculture represents an important economic activity in many developing countries, and the major form of livelihood for the rural poor, increasing agricultural productivity in the currently less productive countries and regions of the world is imperative. About 50 % of the food consumed worldwide is produced by low-input, smallholder family agriculture. These systems occupy approximately 20 % of the area available for agriculture in the world, and often not the most productive land within a country (FAO 2012). Some of such systems

rely on local genetic resources, institutions and traditional practices that in some cases may be millennia old. These systems are often termed 'organic by default' because they use very few or no external inputs. But for all the genuine attractiveness of traditional practices and natural resource management systems, it is obvious that they are unable to feed a currently increasing urban population in developing countries (Tittonell and Giller 2013). They were developed in a different historical context, in which most of the human population in the world still lived in rural areas. Their intensification is urgently needed. But, what form of intensification?

Over the last years, environmental concerns have increasingly influenced the terminology used to describe and communicate the need to increase agricultural productivity worldwide (e.g., 'sustainable intensification', 'eco-efficiency', 'more with less', etc.) but they did not influence the technological paradigm around intensification much beyond a recognised need for precision agriculture to improve physical and economic efficiencies (e.g., Cassman 1999). Such a view still assumes that the technologies of industrialised agriculture are effective at increasing yields anywhere in the world. Current efforts in this direction are placing emphasis in reducing yield gaps between actual and potential yields around the world (e.g. van Ittersum et al. 2013). Yet, closing yield gaps does not necessarily imply moving towards higher resource use efficiency (van Noordwijk and Brussaard 2014). In particular, the role that biodiversity can play in increasing efficiencies has been often overlooked (e.g., Kremen and Miles 2012), and there is increasing evidence on the benefits from diverse soil communities, beneficial arthropods or from agro-ecosystem diversification contributing to increased food production and reduced reliance on non-renewable resources (e.g., Bommarco et al. 2013; Fonte et al. 2012; Lin 2011).

We know that current levels of investment in terms of assets, labour and external inputs and current levels of attainable productivity differ widely worldwide (Fig. 1.2). Contextual demographic and socio-political pressures in the South condemn smallholder systems to very resilient poverty traps (Tittonell 2013), while economic pressures push farmers to unsustainable over-investment and indebtedness in the North (Van der Ploeg 2009). Serious investments in research are needed on ecological intensification in the South and on 'extensification' based on ecological principles in the North to allow moving from regime 1 (red line) to regime 2 (blue dotted line) in Fig. 1.2, and serious policies, institutions and territorial development are needed to shift to regime 3 (green dotted line). The set of actions in research, development and policy necessary to address the global food problem, which is not only one of food insufficiency but also of obesity, malnutrition, overconsumption, and waste, can be summarised as follows: intensify in the South, extensify in the North, and detoxify everywhere. In the following section, we describe examples of ecological intensification strategies from contrasting agricultural systems around the world, but all of them based on putting biodiversity to work for agriculture.
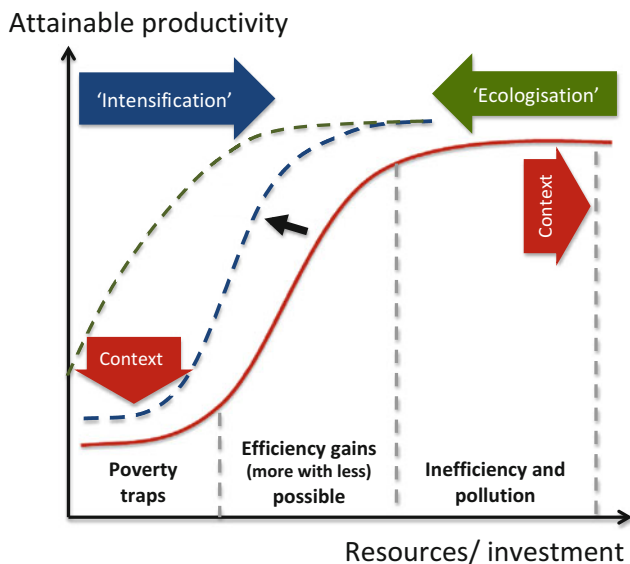
Fig. 1.2 Attainable productivity, contexts and pathways. The *red* curve (*solid line*) describes the current situation where institutional and political contexts create situations of poverty traps or of inefficiency and pollution. The zone of the curve where efficiencies are greater often corresponds to agricultural systems in emerging and developing economies (cf. Fig. 1.1). The ecological intensification *arrows* describe desirable directions of change: 'ecologisation' involving efforts to maintain productivity while reducing fossil fuel inputs, and 'intensification' to increase productivity per unit area in an affordable and sustainable manner (From: Tittonell 2013)

1.3   More with Less, the Same with Less, More with More or More with the Same?

In this section we will show successful practical examples of ecological intensification that lead to producing more value(s) with less resource investments, reducing the damage to nature and society. Non-exhaustively, we focus on strategies to reduce agrochemical inputs in high output agricultural systems, on the key roles that livestock may play in preserving nature and facilitating synergies, on the integration of annual and perennial species, and on the rehabilitation of degraded soils, particularly in Africa.

1.3.1   Designing Plant Disease-Suppressive Landscapes

Potato late blight caused by *Phytophthora infestans* has been estimated to result in a cost of M€ 4800 globally due to application of fungicides and residual yield loss (Haverkort et al. 2008). In the Netherlands, conventional potato production resulted in some 10 kg active fungicidal ingredient per ha being used in 2008 (CBS 2014) on

165,000 ha (Haverkort et al. 2008), making it the most pesticide-consuming crop in the country. Cultural means of control such as early cropping, strip cropping and reduced N application have been found to somewhat reduce disease pressure in organic production systems (Finckh et al. 2008). Eradication of sources of pathogen inoculum is an important means of control. In the Netherlands, the removal of potato volunteers and heaps of culled potatoes is compulsory by law to protect (seed) potato production. Breeding for resistance provides only temporary relief due to the aptitude of the pathogen to quickly overcome plant resistance by genetic mutation (Haverkort et al. 2008; Haas et al. 2009). It is thus evident that no silver bullet approach to disease control exists, and that smart combinations of multiple means are called for.

Skelsey et al. (2009) evaluated the combination of mixing cultivars with different resistance genes at field and regional scales with a set of disease management options. They explored virtual landscapes in which a susceptible and a partially resistant cultivar were grown in different spatial patterns. Disease appeared at a random location in the landscape and the resulting spores spread depending on atmospheric conditions (Skelsey et al. 2008). Spore viability was assumed to decrease with time and solar (UV) radiation levels. The epidemiological model, the spore viability model and the atmospheric dispersal model were all evaluated with field data. All scenarios were considered over 10 years of Dutch weather conditions, assuming 25 % of the area to be planted to potato. Random aggregation of resistant and susceptible potato fields was compared with block, strip or clustered arrangements of fields, considering also the shape and orientation of fields relative to the predominant wind direction. At the field scale, genetic monocultures were compared with different ratios of randomly mixed susceptible and resistant plants.

Results showed that donor landscapes as far away as 16 km could infect receptor landscapes, confirming the observation that the pathogen can travel large distances. Weather over the 10 simulation years caused considerable variation in final disease levels, indicating that stochastic effects play an important role in this ecosystem. Reducing the fraction of potato in the landscape, reducing the fraction of susceptible potato cultivars and orienting narrow and long fields perpendicular to the dominant wind direction all reduced percentage infected potato area at the end of the season. However, the strongest reduction in final disease level was consistently found when susceptible and partially resistant cultivars were mixed within each field. These results were confirmed by previous experiments at field level (; Bouws and Finckh 2008; Andrivon et al. 2003) and used to design new experiments to explore optimum spatial arrangements and cultivar mixtures (Fig. 1.3a, b).

In a complex strip cropping experiment in 2014 potatoes were grown in pure and mixed plots of potato cultivars. Due to the early onset of potato late blight (*Phytophthora infestans*), the yields were severely reduced by the disease. Pure plots of the partially resistant cultivar Raja had significantly lower yields than mixed plots of partially resistant cultivars of Raja and Connect mixed with resistant varieties of Carolus and Sarpo mira. The progress of the disease in the mixed plots was much lower than in the pure Raja plots (Fig. 1.4a). Analysis per cultivar showed that the contribution per cultivar was not uniform (Fig. 1.4b). The cultivar Connect was

Fig. 1.3 (**a**) A homogeneous, healthy potato crop at flowering in sandy soils near Wageningen, The Netherlands; (**b**) Detail of a potato cultivar mixture after a strong *Phytophthora* infestation, showing differences in susceptibility between cultivars – infested plants had been already removed from the field; (**c** and **d**): Cattle and sheep grazing together in bio-diverse, native grasslands of eastern Uruguay; (**e** and **f**): images of the same wheat crop growing in the open (*left*) or under the canopy of *Faidherbia albida* trees (*right*) in Ethiopia – both photos were taken the same day and at the same time (Photo credits: **a**–**c**: P. Tittonell; **d**: W. Rossing; **e** and **f**: F. Baudron)

responsible for 73 % of the total tuber yield of 31 t ha^{-1}, while the remainder was equally distributed over the other three cultivars. The yield of Raja in pure and mixed plots were the same but the mixed plots yielded larger potatoes, and roots were more evenly distributed over the soil layers.

From these (preliminary) results it is clear that (i) cultivar growth characteristics in mixed stands are crucial for reaping the full benefits of mixed cultivar cropping and (ii) the diversification of the genetic composition of potatoes at field scale thus appears as a promising option to reduce disease spread. It has also been argued that deployment of several genotypes in one field would expose all genotypes to inoculum pressure and might exacerbate selection of virulent spores. This debate is as yet unresolved and may well call for a re-assessment of the trade-off between efficiency and stability (e.g. Bousset and Chèvre 2013).
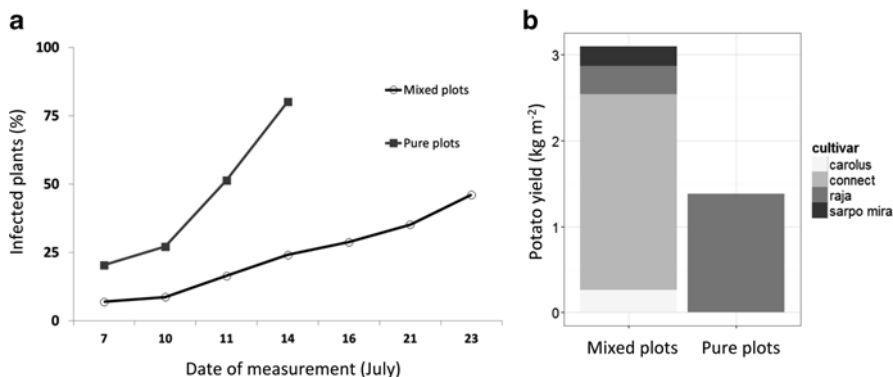
Fig. 1.4 (**a**) Disease progress in pure and mixed plots expressed as the number of plants infected over time; (**b**) Yield of the mixed and pure plots and the contribution to the total yield per cultivar. Preliminary data from an on-going experiment on the organic farm De Droevendaal, Wageningen, The Netherlands

1.3.2 Beef Production on Natural Grasslands

Extending over parts of Argentina, southern Brazil and Uruguay, the Pampas and Campos comprise 500,000 km^2 of natural grasslands that are mainly used for grazing cattle and sheep (Fig. 1.3c, d). The region is a hotspot for biodiversity of native C3 and C4 grasses and leguminous species. Some 450 grass species and 150 leguminous species are used as forages. In addition to biodiversity and livelihoods for 500,000 farmers, most of them family farmers, the Pampas and Campos provide a range of supporting, regulating and cultural ecosystem services of local and global importance (Sala and Paruelo 1997). Low productivity levels,, many cases around 60–80 kg meat equivalent ha^{-1} year^{-1} (Nabinger et al. 2000) make family farms on the more productive soils economically vulnerable to conversion to large-scale arable cropping of soybean and forestry monocultures. Ecologically, conversion to cropping will reduce biodiversity and make the region more vulnerable to droughts and soil erosion events, the frequency of which is predicted to increase due to climate change (Marengo et al. 2012). It will also cause rapid loss of carbon from soil stocks (DuPont et al. 2010), resulting in reduced water holding capacity (Alliaume et al. 2013) and plant growth limitation. Such threat is not hypothetical. Nabinger et al. (2009) report annual rates of decrease of natural grassland area in the region of about 1 % (440,000 ha year^{-1}) over the past four decades.

We postulate that a way out is to increase grass and livestock productivity on family farms to provide an economically viable alternative to a sell-out to soybean and pulpwood producers (Rossing et al. 2014). Overgrazing is considered as the main cause of low productivity, particularly on family farms where large numbers

of cattle culturally display wealth. Based on an analysis of long-term experiments in southern Brazil, Carvalho et al. (2011) described how a strategy of what they call 'moderate grazing' lifted productivity levels from 60 kg ha^{-1} year^{-1} to 170 ha^{-1} year^{-1}. This strategy involved allowing cattle to harvest vegetation with a forage allowance equivalent to 8–12 % of their live weight, leaving sufficient biomass for the sward to quickly re-grow and avoid loss of solar radiation interception as is the case when overgrazing. The diversity of C3 and C4 species enabled stabilization of production rates with C3 species being more productive under cooler conditions of winter and C4 species under warmer and drier conditions of summer. This required avoiding grazing during seeding times of both species types. A subsequent productivity increase to an average of 230 kg ha^{-1} year^{-1} was obtained from managing the dominance of grass tussocks, which appear as a result of differential grazing pressure on species of high and low palatability for livestock. Thus, increases from 60 to 230 kg ha^{-1} year^{-1} seem possible by changing management, without adding external inputs.[1]

These ideas were implemented in a co-innovation project with family farmers and local research and extension services in Uruguay, started in 2011 (Aguerre et al. 2015). Frequent interaction between researchers, extension teams and pilot farmers resulted in a comprehensive diagnosis of main productivity constraints and in sufficient trust on the part of the farmers to start to implement changes. Preliminary results after implementation show that by reducing the stocking rate (average −8 %) and the sheep/beef ratio (average −34 %, min. −17 %, max. −64 %), the standing biomass and consequently the forage allowance increased by 79 % and 88 %, respectively (Ruggia et al. 2015). These changes, together with adjustments in animal management, resulted in an increase in calving percentage from 62 % to 77 %, meat equivalent production per ha (including wool) from 100 to 124 kg ha^{-1} year^{-1} (representing 16–64 % on-farm increases), without increase in inputs or investments in infrastructure. As a result, net incomes increased on average from 58 to 97 US\$ ha^{-1}. No less important is the fact that higher grass biomass resulted also in less soil losses by erosion, greater systems' adaptability to erratic rainfall, net carbon sequestration and more favourable habitats for biodiversity. But the preliminary results of this project also indicate that improving grazing management requires redesign of strategies across fields and over time at farm level to purposefully incorporate diversity across multiple spatial and temporal scales.

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[1] Carvalho et al. (2011) also describe possible next intensification steps which all involve using external inputs, such as liming to increase pH, and N, P and K fertilizers and to replace native species by exotic, high production species in sown pastures. While this will substantially increase meat production levels, it will imply sacrificing the ecosystem services associated with the natural grasslands and making livestock production more vulnerable to climate change.

1.3.3 Creating Synergies through the Integration of Annual and Perennial Species

Simply by their presence, trees alter the local environment and affect other species, positively and/or negatively (Bruno et al. 2002). For example, retaining scattered trees in fields is very common in Ethiopia, and these trees affect the crop growing under or nearby the canopy in numerous ways. In the Central Rift Valley of Ethiopia, an area characterized by low and erratic rainfalls (comprised between 500 and 800 mm) and high evapo-transpiration rates, wheat is commonly grown on the heavier soils, where *Faidherbia albida* is the most common tree scattered in the landscape. Although *F. albida* is well known in literature for its reverse phenology, it generally sheds its leaves in winter and produces new shoots in summer (as other trees do) in the Central Rift Valley, probably because of heavy pruning at the beginning of summer (*F. albida* branches are extensively used for fencing). Nevertheless, *F. albida* was shown to have a facilitative effect on wheat, as the crop growing under its canopy is generally more productive (Hadgu et al. 2009). Recently, Shiferaw et al. (2014) analysed *F. albida*-wheat interactions in farmer fields (Fig. 1.3e, f), looking at the effect of the trees on microclimate, soil moisture, crop diseases, and the resulting effect on wheat development and productivity.

At anthesis, a critical stage of wheat development, air temperature under the canopy of *F. albida* was found to be significantly lower than outside the canopy during the day (Fig. 1.5a). Around midday, the temperature under the canopy was up to 5 °C lower than outside the canopy. The protection of the crop from excessive radiation at critical times by a tree canopy has been documented previously for other crops and trees (e.g. Ong et al. 2000). Soil moisture was also found to be higher under the canopy as compared to outside the canopy of *F. albida*, particularly during the early crop development (first 30 days) and grain filling stage (100–110 days after planting) (Fig. 1.5b). This may be the result of a reduction in soil evaporation (Ong et al. 2000) and/or a redistribution of soil water from the deep horizons to dry surface horizons – a phenomenon known as hydraulic lift (Burgess et al. 1998). Using the Normalized Difference Vegetation Index (NDVI) as a proxy, the crop growing under *F. albida* was found to be more vigorous than the sole crop throughout the season. The incidence and severity of *Fusarium* wilt (at anthesis) and head smut (at maturity) were also lower for wheat growing under *F. albida* canopy compared to wheat growing in the open.

These benefits were found to result in wheat producing 23 % more grain and 24 % more straw under the canopy of *F. albida* compared to sole wheat.

A different way of integrating annual and perennial plant species in agricultural landscapes is through biomass transfers. These may include transfer of tree leaf litter, of leaf biomass from trees and shrubs, or of woody biomass from these perennials. In a context in which crop residues are not available in sufficient amounts to sustain soil organic matter, or when most of this biomass is used to feed livestock, mulching with locally available woody biomass may represent a viable alternative to maintain or improve soil fertility. Experience from semi-arid zones of West
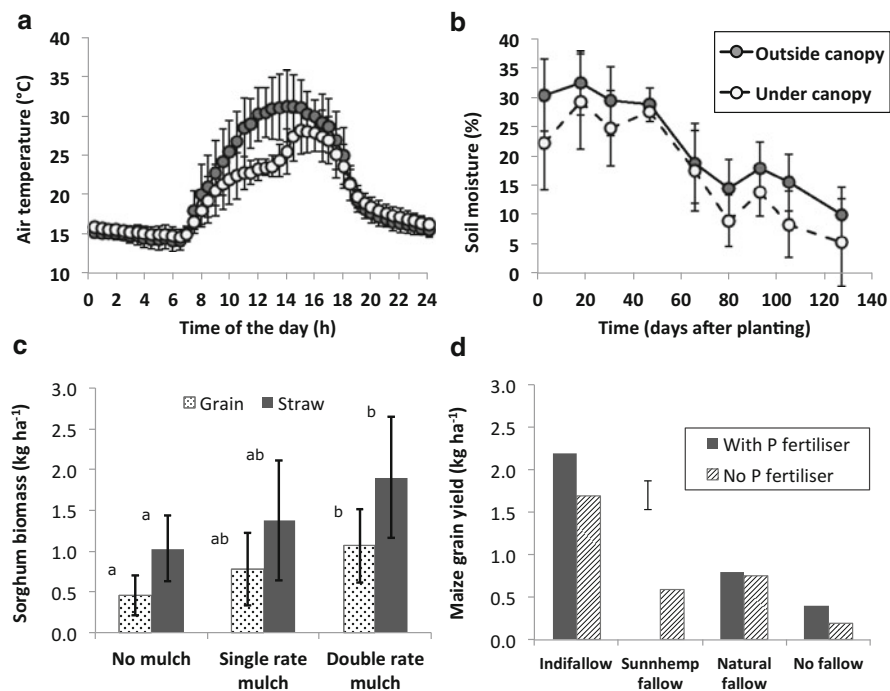
Fig. 1.5 (**a**) Air temperature during anthesis of wheat and (**b**) topsoil (up to a depth of 10 cm) moisture content throughout the season, under and outside the canopy of *F. albida* in Ethiopia; (**c**) Sorghum grain and straw yield on non-productive farmers' fields with application of ramial wood biomass in Burkina Faso: *Piliostigma reticulatum* biomass applied as mulch at rates of 1 t ha^{-1} (single) and 2 t ha^{-1} (double), versus control without mulch (Ouédraogo 2014). (**d**) Maize grain yields grown after 1-year indifallows (indigenous legume species), sunnhemp improved fallows, natural fallows and continuous maize with and without mineral fertilisers in degraded sandy soils of Zimbabwe. *SSP* simple super phosphate (From: Nezomba et al. 2010)

Africa, from Senegal to Niger, shows that farmers have developed innovative temporal and spatial management of native evergreen woody shrubs that grow spontaneously on farmer fields during the dry season (i.e. *Piliostigma reticulatum*) to provide in-situ organic mulching material (Yélémou et al. 2013a). Use of shrub fallows in farmers' fields has been documented since the 1970s but only recently have shrub-crop associations been proposed as an ecological intensification mechanism for agro-pastoral systems in semi-arid West Africa (Lahmar et al. 2012; Tittonell et al. 2012). The presence of these woody shrubs in the landscape reduces erosion and intercepts wind-driven organic particles, surface soil sediments and nutrients (Dossa et al. 2013). Shrubs are pruned prior to the onset of the rainy season and fresh matter is applied on soils as mulch to maintain/enhance soil organic matter, water retention, and infiltration before the main crop is sown – sorghum or millet, usually inter-cropped with cowpea (Kizito et al. 2012; Yélémou et al. 2013b). When crops are harvested at the end of the rainy season, shrubs re-gain biomass and

restore root reserves that carry them through the dry season. Farming families use woody branches with a diameter >2 cm as firewood. Hence, most of the woody organic matter applied on the fields consists of leaves and small-diameter branches, which decompose at a rate suitable for farmers to stop burning this biomass (Diack et al. 2000).

Shrub-crop associations were monitored in 2013 on farmer fields in Yilou, Burkina Faso (13°01′ N, 01°32′ W), and based on observed local management practices a series of on-farm trials of 300–900 m² plots were established in areas with homogeneous distribution of vegetation (average 500 shrubs ha⁻¹) and that farmers signalled as non-productive. Each plot was divided in three equivalent sections where standing woody shrub biomass was cleared and fresh matter was applied as three mulch treatments (Fig. 1.5c). In the first treatment (T1), the aboveground biomass of standing *Piliostigma* was applied as mulch at a rate of 1 t ha⁻¹ mulch; the second treatment (T2) received 2 t ha⁻¹ mulch, with biomass from standing Piliostigma in these and in the control (T0) plots. Sorghum-cowpea intercrops were established on plots using reduced tillage techniques. Sorghum grain and straw yields measured at harvest showed significant responses to the application of 2 t ha⁻¹ shrub biomass, although yields remained low for all treatments. When no woody mulch was applied, average sorghum grain yields were 460 kg ha⁻¹, versus 1063 kg ha⁻¹ when 2 t ha⁻¹ of fresh woody mulch was applied (Ouédraogo 2014). Although effects of these biomass amendments to soil are currently being assessed in the mid- to long term, both on farmer fields (e.g. Félix et al. 2015) and on experimental station (e.g. Barthès et al. 2014), these preliminary results show promise, as boosting crop biomass production (including roots) is the first step towards higher soil fertility regimes in cropping systems. This experience could be an incentive for collective shrub densification options to support crop productivity through woody biomass applications, especially in contexts of continued cultivation without fallows.

1.3.4   Restoration of Exhausted, Degraded Soils

After the Ethiopian drought and famine of the 1980s, various land rehabilitation techniques were implemented in the country (Hurni 1988). These included 'grazing exclosures' i.e. the exclusion of livestock from highly degraded common rangelands in order to rehabilitate them (Mengistu et al. 2005). Communities still had access to fuel and fodder from grazing exclosures through controlled cut-and-carry. The positive impact of exclosures on soil conservation, soil fertility build up, watershed hydrology and biodiversity is well documented (Asefa et al. 2003; Mengistu et al. 2005; Descheemaeker et al. 2006; Mekuria and Veldkamp 2012; Corral-Nuñez et al. 2014), and has been also applied to farmland, with the aim of conserving soil and water and improving crop productivity (Nedessa et al. 2005). Households involved in this collective action maintain their livestock in a year-round stall-feeding. Baudron et al. (2015) evaluated the impact of 8 years of farmland exclosure

in the Central Rift Valley of Ethiopia. 'Exclosed farms' (EF) and neighbouring 'open grazing farms' (OF) had significantly different feed and fuel use strategies. Compared to OF livestock, EF livestock depended less on cereal residues and more on biomass from on-farm trees and grass from the communal rangeland. Similarly, EF depended less on cereal residues and cattle dung for fuel and more on tree biomass (both from the farm and from the communal land). Because of these different patterns of feed and fuel use, more biomass – in the form of crop residue, manure and compost – was available as soil amendment. This translated into significantly more fertile soils (soil organic matter content in the topsoil of 2.7±0.9 % vs. 1.5±1.1 %) and significantly higher tef yields in EF as compared to OF (2200±715 vs. 1303±483 kg ha^{-1}). However, farmland exclosures may only be feasible in particular geographic locations. They will be difficult to implement in densely populated regions with a large proportion of the land allocated to crops, where the basic infrastructure such as physical barriers preventing outside livestock to access the area is not present, or where local institutions prevent any form of 'privatisation' of biomass resources (e.g. Andrieu et al. 2015). Other options to restore degraded soils are need in such places.

Southern Africa is largely a food deficit zone due to poor inherent soil fertility of granite-derived soils that predominate in many parts of the region (Mapfumo and Giller 2001; Nyikahadzoi et al. 2012). The soils typically contain about 10 % clay and over 80 % coarse sand, and are inherently deficient in N, P and S. Yet, these soils are home to >65 % of the Zimbabwean population who derive their livelihoods from maize-based smallholder farming systems. While the main source of livelihood is integrated crop and livestock farming, yields of staple crops average 0.8 t ha^{-1}, and complete crop failure primarily due to lack of external nutrient inputs is common in what has been described as a 'no fertilizer no crop' scenario (Mapfumo et al. 2001). Maize monocropping and associated agronomic packages typically derived from conventional (industrial) agriculture have resulted in abandonment of large tracts of degraded lands due to degradation and loss of economic returns to the limited external nutrient inputs that farmers can afford and to their family or hired labour. This has led to increased food deficits and agricultural expansion into marginal/fragile areas traditionally reserved for either livestock grazing or wildlife. The region therefore faces two main challenges to the growing calls for intensification: (i) restoring productivity of these abandoned field soils, and (ii) developing mechanisms to increase productivity on these lands and limit encroachment into natural resource areas that provide diverse ecosystems services underpinning socioecological resilience at community scale.

Current cropping practices have resulted not only in multiple plant nutrient deficiencies (Masvaya et al. 2013; Manzeke et al. 2012; 2014), but also in critically poor fertilizer responses. This has strong implications on major investments made by governments, NGOs and other development partners in fertilizer supply schemes including subsidy programs. In response to this problem, researchers from University of Zimbabwe and its partners under the Soil Fertility Consortium for Southern Africa (SOFECSA) introduced the concept of indigenous legume fallows to generate much needed high quality biomass to stimulate biological activity and

subsequently productivity of these abandoned soils (Mapfumo et al. 2005; Nezomba et al. 2010). Indigenous herbaceous legumes growing naturally under different agro-ecologies were identified through participatory approaches. Farmers in contrasting agroecological contexts were able to distinguish the legumes, which are often viewed simply as 'weeds' and are generally unpalatable to livestock. Criteria for field identification and seed collection were developed jointly with communities, opening opportunities for field testing the population dynamics and growth performance of the legumes when sown in mixtures in farmers' fields. This provided a new dimension of improved fallows: the Indifallow.

Successful stands were better established by mixing species of prostrate growth habit such as *Crotalaria pisicarpa*, *Indigofera demissa*, *I. praticola* and *Tephrosia radicans* and erect types such as *Crotalaria ochroleuca*, *C. laburnifolia* and *C. cylindrostachys*. Major costs for establishing these self- regenerating and nitrogen-fixing legumes were largely labour for initial seed acquisition and sowing. The studies identified the following as key criteria for selection of candidate species as Indifallows:

   (i) A long-lived seed bank
  (ii) Rapid establishment and growth
 (iii) Adaptation to poor soils with limited availability of phosphorus
 (iv) High N_2-fixing potential and shoot N concentrations under local conditions
  (v) Abundant seeding to allow ready propagation and seed collection to reinforce
       populations
 (vi) Easy to remove should weeding be required

The legumes that best fit these characteristics are largely annuals, biennials or short-lived perennials (Mapfumo et al. 2005). Persistence of these legumes under farming systems dominated by crop–livestock interactions in Zimbabwe suggests that they are either not palatable to livestock and therefore survive grazing or are adapted to grazing.

A unique characteristic of the indifallows has been their capacity to accumulate biomass yields exceeding 6 t ha^{-1} on soils with very low levels of phosphorus, and their response to mineral P fertilization, giving biomass yields exceeding 10 t ha^{-1} (Nezomba et al. 2010). The indifallows therefore accumulate high amounts of N-rich biomass on soils that otherwise fail to sustain productivity of common crops. Maize grown after the indifallow yielded significantly higher than that grown under either natural fallow or continuous, fertilized maize (Fig. 1.5d). When used in the context of integrated soil fertility management (ISFM), indifallows proved an appropriate entry point for kick-starting the productivity of soils abandoned by farmers for their loss of productivity. The indifallows increased soil biological activity and favoured growth of subsequent maize crops in rotation, particularly when aided with P fertilizer (Nezomba et al. 2015). Indifallows now hold potential as a local ecological approach upon which traditional ISFM options can build upon to restore productivity of degraded and so called non-responsive agricultural soils increasingly abandoned by farmers (e.g. Rusinamhodzi et al. 2013; Nezomba et al. 2015).

1.4 How to Foster Innovations, and How to Anchor Change

The various examples described in the previous section illustrate a diversity of ecological intensification pathways, from individual actions of farmers at field or farm level, to community efforts at landscape and territory scale. In the first example on ecologically intensive disease management in potato production, the actual implementation of the proposed genetic diversification by farmers will require addressing a range of challenges. From an agronomic perspective, the question is which cultivars can be combined synergistically e.g. in terms of nutrient uptake, or at least without major competition effects. But also both upstream and downstream value chain partners will need to accept changes from the usual practice of single cultivars. Upstream, seed companies will need to breed with mixtures of their own and other companies' cultivars in mind, and adjust their relations with growers to allow them to source the best mixtures. Downstream, retail will need to resolve the question of selling mixtures or separating cultivars after harvest. Adjustments in harvesting machinery will be needed to arrive at planting patterns that balance the need for diversity with the need for technical simplicity. To benefit from diversification at landscape scale, regional adjustments among farmers[2] and their seed companies need to be made. This indicates that the complexity inherent to ecologically intensive management, which is also knowledge intensive, calls for innovative approaches to support such transitions and anchor positive changes through strong links between the ecological and social sub-systems (Olsson et al. 2014; Foran et al. 2014).

1.4.1 Ecological Intensification Transitions through the Perspective of Niches, Regimes and Landscapes

To understand the challenges that a transition to ecological intensification faces, insights can be mobilized from innovation studies on how established worldviews, paradigms and sunk investments in physical and market infrastructure create path dependencies and keep food production systems in both developed and developing countries 'locked-in' (Elzen et al. 2012a; Horlings and Marsden 2011; Pant 2014; Vanloqueren and Baret 2009). Several related approaches to study such complex innovation processes co-exist and are complementary (such as socio-ecological systems thinking, innovation systems approach, political ecology – Foran et al. 2014; Olsson et al. 2014). Insights from these approaches have informed the strand of so-called system innovation or transition studies which conceptualize current food systems as a 'socio-technical regime' (Fig. 1.6a), a set of dominant technologies,

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[2] While this proposition raises eyebrows in the potato sector, regional coordination among Dutch farmers on mowing regimes of grasslands for bird protection has proven to be possible and fruitful (Schekkerman et al. 2008).
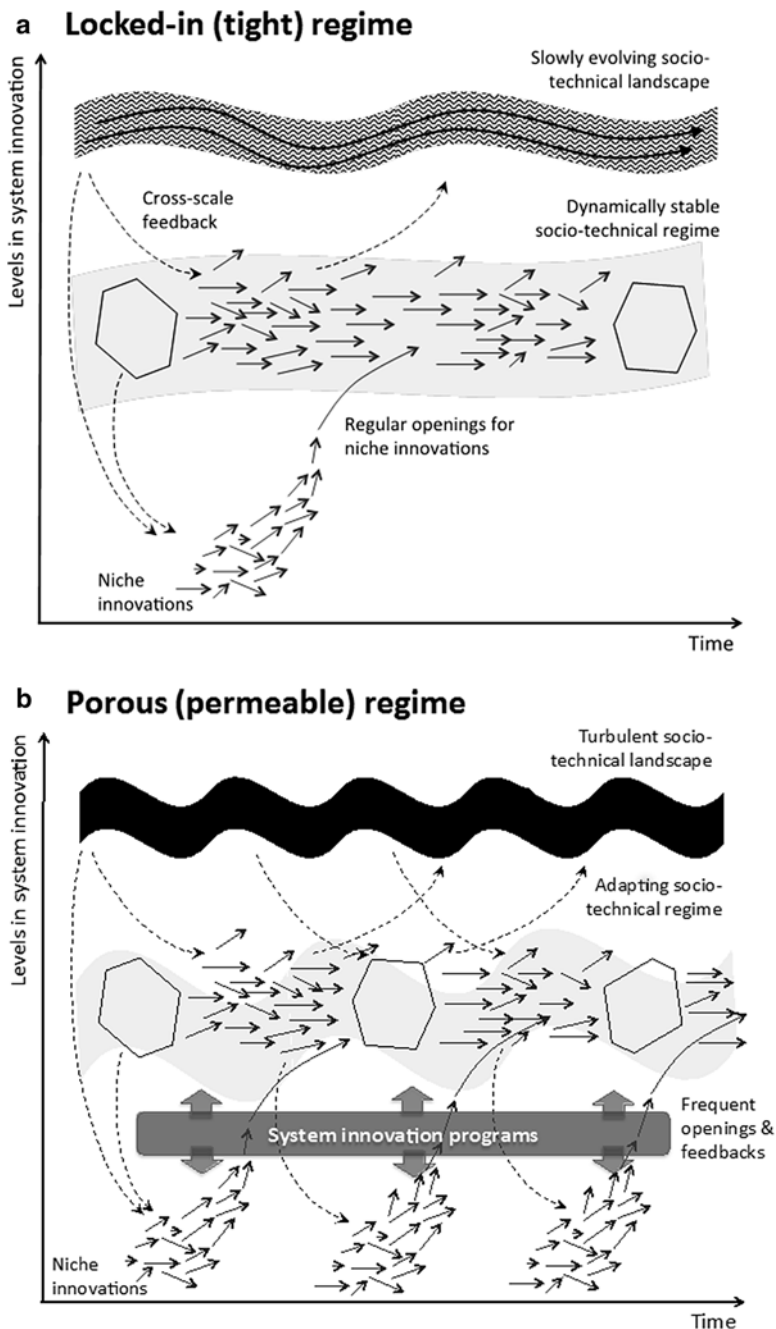
Fig. 1.6 Graphical representation of socio-technical landscapes, regimes and niche innovations, inspired on the diagram of Geels and Schot (2007). (**a**) Stable landscapes that lock-in niche innovations; (**b**) turbulent regimes that open up opportunities under landscape pressures, indicating the place of programs to support system innovation by facilitating niche experimentation and anchoring

practices and organizational and institutional arrangements (Fünfschilling and Truffer 2013; Holtz et al. 2008; Hounkonnou et al. 2012) within production systems, value chains and agricultural innovation systems (understood here as the 'support structures' for innovation – Klerkx et al. 2012).

In this model, deviant ways of practicing agriculture take place in so-called niches, where novelties are developed competing with the existing socio-technical regime while trying to grow in importance (Roep et al. 2003; Wiskerke and van der Ploeg 2004). While originally it was thought that such niches would 'overthrow' the socio-technical regime, later studies indicate that niche activity spurs changes in the regime, i.e. the regime starts to change from within (Geels and Schot 2007). For example, organic agriculture started as a niche, but has now become more prominent and has hence influenced certain parts of the socio-technical regime in its favour (Smith 2007), and was partly absorbed by this socio-technical regime as it has become conventionalized in some cases (Darnhofer et al. 2010). Development of niches and changes in socio-technical regimes are influenced by a broader socio-technical "landscape", which represents broader developments in natural and socio-economic systems that may provide triggers for change (e.g. climate change, economic crises, environmental pollution, etc.).

Often niches start as a response to dissatisfaction with current regime practices, and self-organize to start realizing alternatives (Elzen et al. 2012a; Fressoli et al. 2014; Roep et al. 2003; Smith and Seyfang 2013), but they can also be stimulated through dedicated support policies (Elzen et al. 2012a; Geels et al. 2008). Within the ecological intensification movements (i.e., agroecology, organic farming, permaculture, etc.), much grassroots activity or 'bottom-up' innovation by pioneers can be witnessed (Kirwan et al. 2013; Sage 2014), focused on 'anchoring' ecological intensification. As has become clear from some of the examples in the previous section, this goes beyond working on improved farming systems, but is also about creating favourable input supply systems, value chains and policy environments (Roep et al. 2003; Klerkx et al. 2010; Blesh and Wolf 2014). Such anchoring consist of 'cognitive anchoring' (changing mindsets and capabilities for ecologically intensive production), 'network anchoring' (building support networks and changing existing production and market configurations) and 'institutional anchoring' (changing rules, regulation and standards unfavourable for ecological intensification) (Elzen et al. 2012b).

A main question is how to accelerate and support such grassroots innovation activity with a view to anchoring ecological intensification niches, i.e. what are the roles of government policies and science in this transition (i.e. top-down innovation support to complement bottom-up actions by pioneers or champions) (Brussaard et al. 2010; Caron et al. 2014; Duru et al. 2014). As Westley et al. (2011) argue, a combination between bottom up activity and top down action is most effective, what Elzen et al. (2012a) call 'dual track governance' in which a co-innovation approach involving collaborative work and learning between different stakeholders is advocated (Dogliotti et al. 2014; Klerkx and Nettle 2013). However, government policies and science agendas are often part of the socio-technical regime (Sumberg et al. 2012; Thompson and Scoones 2009; Vanloqueren and Baret 2009; Foran et al.

2014) and do not accommodate paradigms on ecological intensification and support of niches. This requires that government policies acknowledge diversity in development directions for the agricultural sector (Scoones and Thompson 2009; Brooks and Loevinsohn 2011). Here lays an important role for grassroots movements in influencing the political agendas (Fressoli et al. 2014).

1.4.2   Linking Top-Down and Bottom-Up Approaches

As regards concrete instruments and interventions to support and govern transition towards enhancing the anchoring of ecological intensification, focusing both on bottom-up grassroots activities, top-down action, there are several promising examples:

1. Grassroots learning and experimentation at the level of farming systems can be a fruitful way of expanding principles of ecological intensification among farmers. Co-learning approaches enhance scope and capacity of farmers to understand, adapt and apply principles, and use of Learning Centres in Southern Africa is a notable example (Mapfumo et al. 2013). Farmer-driven research (Waters-Bayer et al. 2009) and farmer field schools (Friis-Hansen and Duveskog 2011) were also found to deliver contextually embedded farming systems. A risk however is that farmer field schools can be captured to serve other people's purposes and lose their farmer-driven and experimental character (Sherwood et al. 2012). Farmer-driven experimentation can also become isolated from larger regime and landscape developments if not properly connected (Elzen et al. 2012a), so that broader anchoring can be inadequate. Also, formal scientific knowledge may be under-utilized while it can help in re-designing and prototyping farming systems and help legitimizing the claims made about the benefits of ecological intensification (Bos et al. 2009; Caron et al. 2014; Lamine 2011).

2. To overcome some of the weaknesses of a purely bottom-up approach and enable broader anchoring, so-called 'hybrid forums' are needed, where niche and regime players negotiate change (Elzen et al. 2012b). This resonates with the increasingly popular concept of innovation platforms, where multiple stakeholders coordinate amongst themselves for co-innovation and enhance co-evolution between technical, social and institutional innovations to ensure effective anchoring at different levels in agricultural systems (e.g. farming system, value chain, policy environment, science system) by means of, for example, reformulating research agendas, and changing regulations and value chain standards (Duru et al. 2014; Kilelu et al. 2013; Pant 2014). While these platforms generally promote inclusiveness of stakeholders and co-innovation (Kilelu et al. 2013; Swaans et al. 2014), they are not without caveats as they are the scene of power imbalances and political struggle (Cullen et al 2014). This may lead to regime players stalling advancements of platforms to protect vested interests, and platforms being used to push externally imposed objectives and ignore local dynamics

(Cullen et al. 2014; Kilelu et al. 2013). This points to the need for adequate facilitation and monitoring, and for working with dedicated 'innovation champions' (Kilelu et al. 2013; Klerkx et al. 2010, 2013) or what have been called 'institutional entrepreneurs' (Van Paassen et al. 2014; Westley et al. 2013; Farla et al. 2012) who can make linkages between different levels and scales in systems (Klerkx et al. 2010; Olsson et al 2014).

3. While platforms generally are useful for enhancing co-evolution and may bring about conditions for broader scaling of practices towards ecological intensification (Millar and Connell 2009), they are also cost intensive. The high cost of innovation platforms implies that permanent innovation support systems such as agricultural research, extension and advisory services must support the learning needed for transitions at the farming system level via regular contacts with farmers. This requires a joint learning process between farmers, researchers and advisors, through an intensive relationship. As many countries nowadays have (semi) privatized research and extension systems with different type of providers, it is essential that these systems are orchestrated and supported to build capacities to support learning on ecological intensification (Chantre and Cardona 2013; Klerkx and Jansen 2010).

In summary, to stimulate transitions towards ecological intensification by stimulating niche activity, and make the link with regime activities, with an awareness of changing landscape factors, simultaneous work is needed at different levels combining bottom up and top-down action (Elzen et al. 2012; Westley et al. 2011; Olsson et al 2014), both oriented towards present ecological intensification efforts and desired future systems. For example, in the case of small-scale beef production in Uruguay, the position of farms in value chains should be considered along with the necessary support of farmer organisations to implement ecologically intensive management. While most of the current family farms in Uruguay produce for a bulk market, purposefully designed ecological intensification strategies may also help in (i) accessing market niches that fetch higher prices when consumers are aware of the multiple functions of natural grassland-based production systems, or (ii) accessing more competitive private credit when greater resilience of the ecologically intensive systems can be demonstrated. Earlier approaches with integrated 'system innovation programmes' fostering innovation networks and innovation platforms have shown these can be vehicles to connect grassroots activities with top down formal support, balancing farming system level work with reconfiguration of policy systems, science and advisory systems, and value chains (Elzen et al. 2012a; Fischer et al. 2012; Veldkamp et al. 2009; Wijnands and Vogelezang 2009) (see Fig. 1.6b). Such programmes can also foster learning amongst champions or institutional entrepreneurs in different projects in ecological intensification niches to support technological innovations and create joint capacity for lobbying for institutional change.

1.5 Outlook

We illustrated the potential of local ecological intensification strategies with detailed evidence from a number of examples that span contrasting agricultural systems and contexts around the world. An example from a high input agricultural system such as potato production in The Netherlands illustrated that even in the most industrialised systems in the world it is still possible to intensify agriculture ecologically. An example from beef production in the Pampas native grasslands of South America showed how livestock production is not necessarily always unsustainable or detrimental for the environment. Examples of integration between crops and shrub perennials in semi-arid Burkina Faso showed how native woody biomass could support the restoration of soil productive capacity and enhance yields within one year in farmers' fields. The analysis of agricultural production systems that reproduce the ecological structure of the native savannah in the Ethiopian highlands showed that biodiversity should not only be seen as a 'service' from farming landscapes but rather as the basis for their functioning. In Zimbabwe, on some of the world's most challenging sandy soils known for their low inherent P and N levels, naturally occurring herbaceous legumes grow to kick-start soil productivity in fields abandoned by smallholder farmers due to poor soil fertility, leading to staple maize yields beyond attainable average on smallholder farms.

These examples on ecological intensification of crop and livestock systems are not isolated or anecdotal, and they are certainly not the only ones in which biodiversity supports efficiency in agriculture. We chose these examples to embrace cultural, economic, and geographical diversity, and to illustrate that strategies for ecological intensification differ in complexity, contexts and scales. Due to inherent biases in current research and development paradigms towards industrial forms of agriculture as the convention, these localized "islands of success" are often circumscribed – widening the knowledge gap that separates local meanings of food systems from 'idealistic' forms of industrial agriculture as an approach to feeding the world. The increasing emphasis on research and development approaches hinged on co-learning, participatory, and innovation platforms has yielded much needed insights on the value of 'hybridizing' bottom-up and top-down approaches, connecting local experimentation with formal innovation systems. To enhance the transition to ecological intensification, this does, however, require that agricultural innovation systems of the different countries should recognize and foster *diversity*, and enable experimentation in the niches of ecological intensification. For this purpose, dedicated 'system innovation programmes' which build on the experience of pioneers and innovation champions and strengthen these with formal support (scientific support, facilitation of innovation platforms and farmer learning networks) could be an option. Since the niches of ecological intensification are not just confined to single countries and their agricultural innovation systems, transnational learning and action is key in this process (Coenen et al. 2012; Diaz Anadon et al. 2014).

1.5.1 More with Less?

In the most productive and industrialised areas of the world the concept of 'more with less' is certainly engaging but rather utopic, as these agricultural systems operate mostly beyond their physical and economic efficiencies already (cf. Fig. 1.2). It is hard to get 'more' form these systems and this should not be a priority from a global food security perspective, as such production does not contribute to alleviate hunger in the poorest regions of the world (cf. Fig. 1.1). The greatest contribution to humanity from the most productive and industrialised areas of the world would be to maintain current productivity using less inputs of non-renewable resources and reducing their huge environmental impact; in other words, producing "the same with less". In the most unfavourable regions of the world, where agricultural productivity is poor as the result of interacting biophysical, socio-economic and political factors, the concept of "more with less" is also inappropriate. Investments are needed in production resources, infrastructure, education and knowledge to foster agricultural productivity in a sustainable manner. This requires both technological and institutional innovation (Tittonell 2014), and supportive policies to make investments possible (e.g., consolidation of land rights). In these regions, we should probably speak of "more with more" or "more with the same". Agriculture alone cannot solve poverty in the least favoured regions of the world, but it can contribute to alleviate the cruel reality of thousands of rural families.

1.5.2 Livestock as Part of the Solution

Livestock is increasingly perceived as a global environmental threat, for example because of its implication to climate change (Steinfeld et al. 2006). At local-level, livestock grazing is also recognized as a driver of land degradation (Lal 1988). Heavy grazing may lead to soil compaction, soil erosion, riverbank erosion, and shifts in vegetation such as woody plant encroachment (Evans 1998; Sharp and Whittaker 2003). Heavy grazing is often the result of the increase in livestock number in parallel with a gradual conversion of rangelands into croplands, fuelled by demographic pressure. Excluding livestock from farmlands – and confining them into zero-grazing units – is often mentioned as a precondition to the implementation of sustainable land management options such as conservation agriculture or agroforestry (Franzel et al. 2004; Erenstein et al. 2008). In opposition to that view, we argue here that grazing is a fundamental ecological function that should be maintained in agroecosystems and integrated with crop production, particularly in low-input systems such as those of South America and sub-Saharan Africa.

Herbivores tend to by-pass the slow litter decomposition pathway, by returning to the soil labile organic materials rich in nutrients – such as urine and faeces – that stimulate soil microorganisms (McNaughton et al. 1997). In addition, certain plant species appear to respond positively to grazing, increasing their productivity through

compensatory growth (Agrawal 2000), and increasing the nutrient concentration of their roots and foliage through nutrient reallocation (Hiernaux and Turner 1996). The production of greater quantities of richer biomass generally has a positive effect on soil microorganisms and soil fertility. Moreover, grazing may increase root exudation by these plants, with a resulting stimulation of soil microorganisms (Hamilton and Frank 2001). By definition, forages are plant species that respond positively to grazing: it is likely that these mechanisms apply to most forage species (e.g. perennial ryegrass and clover, as demonstrated by Bardgett et al. 1998).

Integrating forages to existing cropping systems and grazing these fields during a pasture phase is thus likely to be beneficial for soil fertility. This is illustrated by the findings of Franzluebbers and Stuedemann (2009) showing that soil organic carbon and total soil nitrogen after grazing may be higher than after haying, and even higher than in a non-harvested control. Grazing fallow land between cropping sequences may also control pests and weeds (Hatfield et al. 2007a, b). Integrating pasture phases grazed by ruminants in farming systems dominated by crops may also increase profit and financial stability (Russelle et al. 2007). In addition, forages used in pastures are generally perennial plants that offer a permanent soil cover that controls erosion more efficiently, are characterized by a longer photosynthetic period resulting in a higher light use efficiency, and have a more developed and deeper root system that stores more carbon and captures more water and nutrients than annual crops (Glover et al. 2010). Pastures – as undisturbed land units with permanent vegetation cover – also play an important role in maintaining biodiversity within agricultural landscapes (Bretagnolle et al. 2011).

1.5.3 From Fields to Landscapes, from Individuals to Communities

The landscape is the most relevant scale at which the various components of the agricultural system need to be integrated. This resonates with the idea already mentioned that biodiversity is not simply a "service" from agriculture. Current agro-environmental payments in the European Union, for example, are based on the principle of rewarding farmers for the maintenance of biodiversity. But biodiversity – planned or unplanned – is not acknowledged as a service provider to farming (e.g. natural predators, microclimate effect, etc.). Perhaps the greatest difference between the concepts of sustainable and ecological intensification resides here: in seeing biodiversity as a service in itself, or as the necessary ecological structure to support agricultural production. This echoes the land sharing/sparing debate (Baudron and Giller 2014). It is generally established that for biodiversity, land sparing may be more desirable than land sharing in several cases (Balmford et al. 2012), except for open-habitat species that may depend on farmland (such as

European farmland birds, Wright et al. 2012) or in cases where farmland is structurally very similar to the native vegetation and supports high biodiversity (e.g. tropical agro-forests; Clough et al. 2011). However, agricultural production systems may be more stable and less vulnerable with land sharing than with land sparing, because of stronger interactions between cultivated and uncultivated patches (denser networks in a landscape mosaic), and due to more gradual gradients between the two land uses (Loeuille et al. 2013). And, since most of the ecological functions necessary to sustain agriculture operate at the landscape rather than individual field or farm levels, ecological intensification requires collective rather than individual actions.

1.5.4 A Dialogue of Wisdoms

Options for the ecological intensification of agriculture can be inspired by the type of interactions between structures and functions that can be observed in nature (e.g. Malézieux 2012), by the practical experience of local indigenous knowledge (e.g., Khumairoh et al. 2012), and by combining these with the latest scientific knowledge and technologies. Ecological intensification calls for a constant dialogue between the practical wisdom of farmers and our own scientific wisdom. Success in promoting integrated soil fertility management in Southern Africa that was described in Sect. 1.3.4 was achieved following the introduction of learning centres, which are interactive non-linear and field-based learning platforms bringing together farmer communities, researchers, extension and other development practitioners and service providers (e.g. Mapfumo et al. 2013). Their study proved that co-learning with communities could unlock innovations enabling them to harness resources within the bounds of their contexts to increase productivity and find pathways to achieving food and nutrition security. Ecological intensification not only has the potential to increase agricultural production, but also to support the development of capabilities and skills to manage biodiversity in complex systems, as the perceived extra labour provides jobs that are meaningful and empowering for local communities, and incentives to contribute, share, and evaluate observations and ideas for every participating farm member in all parts of the agroecosystem (Timmermann and Félix 2015). Thus, as the private sector will continue to invest in patentable technologies – understandably – to reinforce their position in the current socio-technical regime, the key role of the public sector should be to reinforce the diversity of approaches, prioritizing alternative rather than mainstream technologies, creating favorable 'openings' in established socio-technical regimes, and embracing the complexity and the associated transaction costs of system innovation programs or what could be called 'co-innovation systems'. In other words, investing in the creation and support of new niches rather than supporting technological 'solutions' that are already embedded in current regimes.

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Chapter 2
The Hidden and External Costs of Pesticide Use

Denis Bourguet and Thomas Guillemaud

Abstract A fair evaluation of the net benefits provided by pesticides is essential to feed the current debate on their benefits and adverse consequences. Pesticides provide many benefits by killing agricultural and human pests. However, they also entail several types of costs, including internal costs due to the purchase and application of pesticides, and various other costs due to the impact of treatments on human health and the environment. Here, we provide a comprehensive review of these costs and their evaluation. We define four categories of costs: regulatory costs, human health costs, environmental costs and defensive expenditures. Those costs are either internal to the market, but hidden to the users, or external to the market and most often paid by a third party. We analysed 61 papers published between 1980 and 2014, and 30 independent dataset. Regulatory costs reached very large values, e.g. US$4 billion yearly in the United States in the 2000s. However, if all regulations were respected, these costs would have jumped to US$22 billion in this country. Health costs studies generally did not take into account fatal cases due to chronic exposure such as fatal outcomes of cancers. Doing so would have increased estimates of health costs by up to tenfold, e.g. from US$1.5 billion to US$15 billion in the United States in 2005.

Most environmental impacts have never been quantified in the literature. Environmental costs were nevertheless estimated to up to US$8 billion in the United States in 1992. Although defensive expenditures have rarely been considered in the literature, they include at least the extra cost of the part of organic food consumption due to aversive behavior linked to pesticide use. This cost reached more than US$6.4 billion worldwide in 2012. Our review thus revealed that the economic costs of pesticide use have been seldom considered in the literature and have undoubtedly

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been strongly underestimated in the past. Despite this underestimation, we found that overall hidden and external costs ranged from US$5.4 million in Niger in 1996 to US$13.6 billion in the United States in 1992. We perform an updated and more complete retrospective evaluation of these costs in the United States and show that they probably reached the value of US$39.5 billion per year at the end of the 1980s-start of the 1990s. We also re-evaluate past benefit-cost ratio of pesticide use in various countries and reveal that the cost of pesticide use might have outreached its benefits, e.g. in the United States at the start of the 1990s. We finally advocate that the key impact to be evaluated is the cost of illnesses and deaths triggered and favored by chronic exposure to pesticides. The benefit-cost ratio of pesticide use may have easily fallen below 1 if this cost had been taken into account. The quantification of this key cost is therefore urgently required for a more accurate evaluation of pesticide use and for regulatory purposes.

2.1   Introduction

High levels of agricultural productivity will be required to sustain the world population, given current population growth rates. Between 1960 and 2000, the Green Revolution increased global food production by a factor of two to three (Evenson and Gollin 2003). However, the approaches used to increase production damaged many ecosystems, rendering them more vulnerable to pests. The control of these pests is essential if we are to maintain the high levels of productivity required to meet demand. The growth of the world population has also been accompanied by a desire to improve the length and quality of human life. With people living longer and in better health, food demands have increased, also necessitating the effective control of pests.

Organisms harmful to humans, their environment and production can be controlled in many different ways. Pesticides are one of the most widely used and effective tools for this purpose. Almost two billion people work in agriculture, and most use pesticides to protect their crops or livestock. Pesticides are also widely used in gardens and around the home, in the framework of public health programs. Pesticide sales increased by a factor of 20–30 between the 1960s and 1990s (Oerke 2006). Pesticide use has continued to increase over the last two decades in most developing countries, e.g. Thailand during the 1990s and 2000s (Praneetvatakul et al. 2013) and Pakistan during the 1990s (Khan et al. 2002). Moreover, contrary to what is commonly believed, pesticide use has remained stable in several developed countries, e.g. the United States (Osteen and Fernandez-Cornejo 2013), mostly due to an increase in herbicide use (Schreinemachers and Tipraqsa 2012). Overall pesticide

consumption is currently close to two to three million tons per year (United States Environment Protection Agency 2011), 45 % of all pesticides being used in Europe, 25 % in the United States, 4 % in India and 26 % in the rest of the world (De et al. 2014). Total expenditure on pesticides is about US$40 billion per year (Popp et al. 2013).

Despite the high cost of their purchase, the widespread application of pesticides has been favored by the benefits they provide. In particular, they have increased crop and livestock yields and, in some circumstances, have improved human health, e.g. by killing vectors of human pathogens, and quality of life, e.g. by killing troublesome organisms (Cooper and Dobson 2007).

However, the purchase costs are only one of the types of cost associated with pesticide use. Indeed, the spraying of these chemicals has an impact on the environment and health, with potentially serious financial consequences (Fig. 2.1). For instance, in a report published in 1990, the World Health Organization (WHO)

Fig. 2.1 Vietnamese farmer spraying pesticide on rice without protections in Hội An, Quảng Nam, Vietnam. A fair evaluation of the net benefits provided by pesticides requires a thorough estimation of their costs, including those associated with their impact on health and the environment. The purchase costs are only one of the types of cost associated with pesticide use. Indeed, the spraying of these chemicals has an impact on the environment and health, with potentially serious financial consequences. For instance, farmers take safety measures when handling and applying pesticides to their crops, to decrease or prevent direct exposure to these chemicals. The defensive expenditures taken into account include costs associated with precautions taken to reduce direct exposure to pesticides, such as masks, caps, shoes/boots, handkerchiefs, long-sleeved shirts/pants. Spraying is sometimes carried out without protection and even those farmers who do try to protect themselves generally limit this protection to the wearing of long-sleeved shirts and long pants. Low levels of income, awareness and education, the hot and humid climate, cultural taboos, fashion and discomfort are significant factors accounting for the lack of personal protection (Unmodified photography by Garycycles, under Creative Common License CC BY (https://creativecommons.org/licenses/by/2.0/))

indicated that there may be as many as one million unintentional severe acute poisoning incidents annually, resulting in 20,000 deaths (WHO 1990). These serious cases of poisoning account for a minute fraction of the overall impact of pesticides on health. On the basis of a survey of self-reported minor poisoning events in Asia, Jeyaratnam (1990) estimated that as many as 25 million agricultural workers in the developing world annually may suffer a poisoning incident.

A fair evaluation of the net benefits provided by pesticides requires a thorough estimation of their costs, including those associated with their impact on health and the environment. Donald J Epp and coworkers (1977) were probably the first to espouse this idea, with the description of a complete taxonomy of the negative impacts of pesticide use to be taken into account. However, they concluded that the state-of-the-art at the time at which they wrote their report was insufficiently advanced for a monetary evaluation of environmental impacts. David Pimentel performed such an evaluation few years later, providing the first overall estimate of the externalities induced by pesticide use. The articles he published from the late 1970s (Pimentel et al. 1979) onwards (most recently, Pimentel and Burgess 2014) focused on the United States. They inspired a few studies in other countries, but there has never been a synthetic analysis of these studies, their shortcomings, limitations and conclusions. Such a synthesis is essential for the current debate on the benefits and consequences of the use of these chemicals.

This review aims to (i) identify and categorize the various costs triggered by the use of chemical pesticides, (ii) provide a comprehensive overview of the articles estimating – in economic terms – these costs, whether at local, regional or national scale, for a single pesticide or for total pesticide use, (iii) report the costs estimated in these articles. These costs, in US$, have been updated to 2013 values, using annual inflation factors and the 2013 purchasing power parity (PPP) conversion factors obtained from the development indicators of the World Bank (http://data.world-bank.org/indicator/PA.NUS.PPP), (iv) identify the consequences for benefit-cost ratio analyses on pesticide use and (v) provide perspectives concerning the evaluation of these costs.

2.2   Types of Costs Generated by the Deleterious Consequences of Pesticide Use

Pesticides are designed to kill, repel, attract, regulate or stop the growth of living organisms considered to be pests (United States Environmental Protection Agency 2007). A pest is any type of living organism, e.g. mammals, birds, reptiles, fish, amphibians, mollusks, insects, nematodes, weeds and microbes (bacteria and viruses), that competes with our food crops or space, spreads disease or acts as a vector for disease and/or causes us discomfort.

Pesticides include chemicals, biopesticides and biological agents (United States Environmental Protection Agency 2007). We have decided to focus this review on

chemical pesticides, for several reasons. First, chemical pesticides account for the vast majority of pesticides used worldwide, e.g. more than 80 % in the United States (United States Environmental Protection Agency 2008). Second, chemical pesticides are probably the most harmful pesticides for the environment and human health. For instance, according to the Stockholm Convention on Persistent Organic Pollutants, nine of the 12 most dangerous and persistent organic pollutants are chemical pesticides (United Nations Environment Programme 2001).

We will also focus mostly on chemicals protecting plants from the damage caused by weeds, plant diseases or animals, notably insects. In fact, the term '*pesticide*' is often exclusively used to refer to plant protection products, although pesticides are also used for non-agricultural purposes. Chemical pesticides are of three main types – herbicides, insecticides and fungicides – but several other types of biocides, such as nematicides and rodenticides, are also used.

Pesticide use has been shown to have a marked positive effect on agriculture (Cooper and Dobson 2007; Gianessi 2009; Gianessi and Reigner 2005, 2007) and human health (Cooper and Dobson 2007). However, pesticides may also have deleterious effects on the environment and human health, generating several types of costs.

For the purposes of this review, we have defined four broad categories of costs (Table 2.1): regulatory costs, human health costs, environmental costs and defensive expenditures. Regulatory costs are all the costs entailed as part of private or public mandatory measures to remove pesticides, to protect the environment or human health from the potential damage caused by pesticides and/or to repair damage already inflicted. For instance, the monitoring and decontamination of tap water can be considered a regulatory cost. Human health costs, often referred to as cost-of-illness, are the expenses associated with acute or chronic pesticide poisoning. These costs are mostly incurred by the farmers applying pesticides, although all citizens can be exposed to pesticides and may, therefore, suffer chronic health effects, in particular. Environmental costs are the costs of both pesticide damage to animals, plants, algae and microorganisms and pest resistance to pesticides. These costs may be incurred by farmers or by society as a whole. Finally, defensive expenditures cover all expenses by farmers and society to prevent pesticide exposure, such as the purchase of organic food or bottled water consumption. These four broad categories of costs include both internal and external costs (Table 2.1).

The internal costs of pesticide use are the costs, to the farmer, of pesticide use within the agricultural production process. These costs are described as "internal" because they determine the price of the final product, i.e. they are internal to the market. We do not review here the "usual" internal costs of pesticide use such as market prices of pesticides, taxes on these products, costs of the application, transport and storage of pesticides, accounting costs, etc., but these costs are taken into account in the re-evaluation of overall costs and of the benefit-cost ratio of pesticide use (see Sect. 2.9). We were particularly interested in the "hidden" costs associated with the impact of pesticides on the environment and human health, regulatory measures and defensive behavior. These additional costs are "hidden" in the sense that farmers are not necessarily aware of them. This is the case for environmental

Table 2.1 Types and categories of costs generated by environmental and health impacts, regulatory actions and defensive behavior

Category of cost	Pesticide impact	Hidden costs			External costs	
		Decrease in benefits[a]	Increase in "usual" internal costs[b]	Generation of other internal costs	Private external costs	External costs *sensu stricto*
Regulatory costs	Public research, communication, expertise on pesticides					X
	Regulations, decrees and laws					X
	Mandatory pesticide handling and disposal			X		
Human health costs	Preventive medicine, annual check-ups			X	X	
	Health issues for farmers	X		X	X	
	Health issues for the public					X
Environmental costs	Pesticide resistance	X	X			X
	Soil degradation	X				X
	Pollination decrease	X				X
	Decrease in natural enemies	X	X			X
	Lower plant production due to herbicide application	X				
	Bee renting			X		
	Degradation of the farm environment				X	
	Livestock health issues				X	
	Degradation of the environment					X
	Domestic animal health issues					X
Defensive expenditure	Purchase of protective clothing, glasses and masks			X		
	Purchase of organic food and bottled water					X

[a]Due to lower yields

[b]Due to an increase in the amount of pesticide applied

impacts increasing pesticide requirements for the production process. These hidden costs increase the "usual" internal costs (Table 2.1). The environmental impact of pesticide use may also decrease production levels. Such "hidden" costs are paid through the achievement of a smaller benefit than would have been achieved by farmers in the absence of a deleterious impact of pesticide use (Table 2.1). Finally, pesticide use generates other internal costs, concerning the purchase of protective equipment, e.g. gloves and masks, the renting of bees for pollination, specific mandatory requirements for pesticide handling and disposal, preventive medicine and annual check-ups for farmers. In addition to the usual internal costs, farmers incur this third class of hidden costs directly (Table 2.1).

Environmental and human health impacts, regulatory actions and defensive behavior triggered by pesticide use also generate external costs (Table 2.1). These costs are described as "external" because they are not included in the farmers' production costs, i.e. they are external to the market. They are mostly paid by a third party, but some, such as those concerning the health of the farmer or degradation of the farm environment, may have a direct impact on farmers. Hence, external costs may be incurred by the farmers themselves ("external private costs", Table 2.1) or by other parties, e.g. consumers, public authorities, people living close to the farm ("external costs sensu stricto", Table 2.1).

Health issues for farmers generate both hidden internal costs and external private costs. The impairment of the farmer's health due to the use of pesticides for a specific type of production, such as crop production, may increase crop production costs, e.g. loss of working hours devoted to crop production, lower yields or the need to pay workers for a larger number of hours of work. Some of the costs of pesticide use relating to health are therefore internal. However, the impairment of farmers' health due to pesticide use may also have economic consequences relating to other types of production, such as livestock production, or lower levels of non-market goods, such as childcare or leisure time. Thus, some pesticide costs relating to health issues are external (Table 2.1). However, it is difficult to determine the proportions of health costs that should be considered internal and external. We will therefore consider all these costs as externalities in this review.

Here, we defined four categories of costs – regulatory costs, human health costs, environmental costs and defensive expenditures – that are commonly not included in the economic evaluation of pesticide use. These costs are either internal to the market, but hidden to the users, or external to the market and most often paid by a third party.

2.3   Literature Surveyed

We carried out a literature review as comprehensive as possible, using Google Scholar and the Web of Science, and screening the references cited by the articles identified relating to this topic. We excluded papers based on contingent valuation methods (see below), resulting in the identification of 61 relevant articles in total

(Table 2.2). These articles were published in peer-reviewed scientific journals (23), scientific journals without peer review (11), books (1), book chapters (10), conference proceedings (2), PhD theses (2) and reports (12) (Table 2.2). These 61 papers are based on only 30 independent datasets, because several papers were based on the same dataset (Table 2.2). These publications have differed in terms of their scientific impact. The 12 articles written by Pimentel and coworkers obtained more than 1500 citations in Google Scholar, the other 49 papers having about 2500 citations between them (Table 2.2). The costs estimated for the United States by David Pimentel et al. are the most widely known, and the corresponding dataset is often considered to be the key dataset when referring to the overall cost of pesticide use. Two other datasets have been widely cited: one relating to the externalities of pesticide use in the United Kingdom (Pretty et al. 2000, 2001, cited about 750 times in total) and the other concerning these externalities in the Philippines (Pingali et al. 1994, 1995; Rola and Pingali 1993, cited about 500 times in total).

The studies identified used different methodologies to estimate costs and these methodological differences partly reflect the heterogeneity of the types of cost considered. Some of the impacts of pesticide use have a value that can be directly estimated from market prices. For instance, mandatory governmental regulations concerning pesticide use may require particular activities, e.g. water monitoring (Pretty et al. 2000, 2001; Waibel et al. 1999), and equipment, e.g. water filters (Pimentel et al. 1992, 1993a, b; Pimentel and Greiner 1997; Pimentel and Hart 2001). Their costs can be determined from market values. Other effects, such as food contamination (e.g. Jungbluth 1996) or the loss of working days if the farmer is ill, have costs based on market price (e.g. Pimentel et al. 1980a, b) that can be evaluated by productivity function methods (Bowles and Webster 1995). The same is true for losses of agricultural production (see the series of papers by Pimentel et al.) due to lower pollination rates, livestock health issues, soil degradation or increases in pesticide use due to the selection of pesticide resistance (e.g. Tegtmeier and Duffy 2004). However, some of the goods affected by pesticide use are non-market goods. For instance, the increase in health risk associated with pesticide use has no directly observable price. In such cases, economists must use non-market evaluation techniques to monetize individual preferences. The monetary values obtained with these techniques reflect the individuals' willingness to pay for a reduction of the risk (Travisi et al. 2006). Revealed willingness to pay is an approach in which the monetary value of a change in risk is derived from individuals' purchasing decisions in existing markets. This approach is often used to estimate the costs of aversive behavior, e.g. wearing protection clothes, drinking bottled or purified water, eating organic food, designed to decrease the risk of human health impairment. Revealed willingness to pay can also be used to estimate the cost of wildlife loss. For instance, the cost linked to human activities, such as bird watching, can be used to estimate bird losses due to pesticides (Pimentel 2005). The contingent valuation method – also referred to as stated willingness to pay – is also often used for the market valuation of non-market goods (Venkatachalam 2004). This method is based on stated preferences in hypothetical market settings. We decided not to use estimates based on stated willingness to pay because the answers

Table 2.2 Characteristics of the papers providing at least one estimate of the external cost of pesticide use

Reference	Source	Language	Peer Reviewed?	Dataset	Category of cost considered[a]					Country of the study	No. of citations in Google Scholar
					Reg.	COI	Env.	DE			
Ajayi (2000)	Report	English	No	1		X				Ivory Coast	50
Ajayi et al. (2002)	Report	English	No	2	X	X	X	X		Mali	23
Athukorala et al. (2012)	Sci Journal	English	Yes	3		X		X		Sri Lanka	3
Atreya (2005)	Sci Journal	English	Yes	4		X		X		Nepal	17
Atreya (2007)	Report	English	No	5		X		X		Nepal	8
Atreya (2008)	Sci Journal	English	Yes	5		X		X		Nepal	33
Atreya et al. (2012)	Sci Journal	English	Yes	6		X		X		Nepal	6
Atreya et al. (2013)	Sci Journal	English	Yes	6		X		X		Nepal	0
Brenna (2001)	Sci Journal	Italian	Yes	7		X				Italy	0
Choi et al. (2012)	Sci Journal	English	Yes	8		X				South Korea	0
Cole and Mera-Orcés (2003)	Chapter book	Spanish	No	9		X				Ecuador	6
Cole et al. (2000)	Sci Journal	English	Yes	9		X				Ecuador	60
Corriols et al. (2008)	Sci Journal	English	Yes	10		X				Nicaragua	18
Crissman et al. (1994)	Sci Journal	English	Yes	9		X				Ecuador	129
Devi (2007)	Report	English	No	11		X				India	16
Dung (2007)	PhD thesis	English	No	12		X				Vietnam	2
Dung and Dung (1999)	Report	English	No	12		X				Vietnam	71
Fleischer (1999)	Conf Proc	English	No	13	X	X	X			Germany	5

(continued)

Table 2.2 (continued)

Reference	Source	Language	Peer Reviewed?	Dataset	Category of cost considered[a]				Country of the study	No. of citations in Google Scholar
					Reg.	COI	Env.	DE		
Houndekon and De Groote (1998)	Conf Proc	English	No	14		X	X		Niger	11
Houndekon et al. (2006)	Sci Journal	English	No	14	X	X	X		Niger	5
Huang et al. (2000)	Report	English	No	15		X			China	49
James (1995)	Sci Journal	English	Yes	16			X		Canada	11
Jansen et al. (1998)	Report	English	No	17		X			Costa Rica	4
Jungbluth (1996)	Report	English	No	18	X	X	X		Thailand	63
Khan et al. (2002)	Sci Journal	English	Yes	19	X	X	X		Pakistan	25
Maumbe and Swinton (2003)	Sci Journal	English	Yes	20		X			Zimbabwe	103
Ngowi et al. (2007)	Sci Journal	English	Yes	21		X			Tanzania	64
Pimentel (2005)	Sci Journal	English	Yes	22	X	X	X		United States	272
Pimentel (2009)	Book chapter	English	No	22	X	X	X		United States	b
Pimentel and Burgess (2014)	Book chapter	English	No	22	X	X	X		United States	1
Pimentel and Greiner (1997)	Book chapter	English	No	22	X	X	X		United States	6
Pimentel and Hart (2001)	Book chapter	English	No	22	X	X	X		United States	11
Pimentel et al. (1980a)	Sci Journal	English	Yes	22	X	X	X		United States	181
Pimentel et al. (1980b)	Book chapter	English	No	22	X	X	X		United States	11

										a
Pimentel et al. (1991a)	Book chapter	English	No	22	X	X	X	X	United States	136
Pimentel et al. (1991b)	Sci Journal	English	Yes	22	X	X	X	X	United States	632
Pimentel et al. (1992)	Sci Journal	English	Yes	22	X	X	X	X	United States	133
Pimentel et al. (1993a)	Book chapter	English	No	22	X	X	X	X	United States	93
Pimentel et al. (1993b)	Sci Journal	English	Yes	22	X	X	X	X	United States	
Pingali et al. (1994)	Sci Journal	English	Yes	23		X	X		Philippines	154
Pingali et al. (1995)	Book chapter	English	No	23		X	X		Philippines	31
Praneetvatakul et al. (2013)	Sci Journal	English	Yes	24	X	X	X	X	Thailand	7
Pretty et al. (2000)	Sci Journal	English	Yes	25	X	X	X	X	United Kingdom	537
Pretty et al. (2001)	Sci Journal	English	Yes	25	X	X	X	X	United Kingdom	225
Rola and Pingali (1993)	Book	English	No	23		X	X		Philippines	322
Soares and Porto (2009)	Sci Journal	English	Yes	26		X			Brazil	36
Soares and Porto (2012)	Sci Journal	English	Yes	26		X			Brazil	3
Soares et al. (2002)	Sci Journal	English	Yes	27		X		X	Brazil	14
Steiner et al. (1995)	Book chapter	English	No	28	X	X	X	X	United States	49
Tegtmeier and Duffy (2004)	Sci Journal	English	Yes	29	X	X	X	X	United States	179

(continued)

Table 2.2 (continued)

Reference	Source	Language	Peer Reviewed?	Dataset	Category of cost considered[a]				Country of the study	No. of citations in Google Scholar[a]
					Reg.	COI	Env.	DE		
Waibel and Fleischer (1998)	Report	German	No	13	X	X	X		Germany	[a]
Waibel et al. (1999)	Sci Journal	English	Yes	13	X	X	X		Germany	27
Wilson (1999a)	PhD thesis	English	No	30		X		X	Sri Lanka	25
Wilson (1999b)	Report	English	No	30				X	Sri Lanka	0
Wilson (2000a)	Sci Journal	English	Yes	30		X			Sri Lanka	33
Wilson (2000b)	Report	English	No	30		X		X	Sri Lanka	2
Wilson (2002a)	Sci Journal	English	Yes	30		X			Sri Lanka	8
Wilson (2002b)	Book chapter	English	No	30				X	Sri Lanka	15
Wilson (2003)	Sci Journal	English	Yes	30		X		X	Sri Lanka	11
Wilson (2005)	Sci Journal	English	Yes	30				X	Sri Lanka	5
Yanggen et al. (2003)	Report	English	No	9		X			Ecuador	8

[a]*Reg., Env.,* COI and DE correspond to regulatory costs, environmental costs, cost-of-illness and defensive expenditures, respectively
[b]Does not appear in Google Scholar

given by respondents may be highly dependent on the way in which contextual information is presented (see Florax et al. 2005).

The literature on hidden internal and external costs of pesticide use thus consists of 61 papers published between 1980 and 2014, these papers being based on 30 independent datasets. The costs were evaluated using both market and non-market methods. Among these latter we chose to exclude studies based on stated willingness to pay.

2.4 Regulatory Costs

Regulations concerning pesticide use are laid down by government bodies and concern (i) mandatory actions that must be undertaken by users and consumers, (ii) governmental actions to organize and check compliance with mandatory actions, and (iii) the activity of governmental agencies associated with pesticide use, such as research agencies. These regulations entail monetary costs. In general, these costs are not included in the market price of the pesticides. They must therefore be paid subsequently, as externalities, by public authorities (hence by consumers and citizens), producers or users.

2.4.1 A Small Number of Studies

Regulatory costs were taken into account in 24 articles (Table 2.2): eight book chapters (7 written by Pimentel and coworkers), four reports (2 from the Hannover Pesticide Policy project), one non-reviewed journal article and 11 articles published in peer-review scientific journals. However, the estimates given in several articles were partly or fully based on the same dataset. This was the case of all papers written by Pimentel and coworkers. It was also the case for Praneetvatakul et al. (2013), who actualized some of the costs originally estimated by Jungbluth (1996). We identified 15 different estimates, but only nine fully independent datasets (Table 2.3).

2.4.2 A High Diversity of Costs

Both external and internal costs are associated with the testing and registration, production, distribution – including importation, transport and sales – use and disposal of pesticides. The external costs are the economic burden to the public authorities responsible for organizing controls and checks on the compliance of stakeholders, e.g. public authorities, consumers, sellers and producers, with the regulations. The internal costs are the monetary subsidiaries paid by pesticide

Table 2.3 Economic costs due to regulations governing pesticide use

Reference	Country	Year	Fully independent dataset[a]	Overall costs (million US$ 2013 per year)
Houndekon and De Groote (1998); Houndekon et al. (2006)	Niger	1996	A	0.15
Ajayi et al. (2002)	Mali	1999	B	1.58
Khan et al. (2002)	Pakistan	2002	C	9.71
Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Germany	1996	D	168.26
Pretty et al. (2000, 2001)	United Kingdom	1996	E	318.51
Praneetvatakul et al. (2013)	Thailand	2010	F	357.28
Pimentel et al. (1980a, b)	United Sates	1980	G	491.96
Jungbluth (1996)	Thailand	1995	F	558.33
Pimentel et al. (1991a, b)	United States	1991	G	2372.34
Steiner et al. (1995)	United States	1991	H	3203.00
Pimentel and Hart (2001)	United States	2001	G	3451.19
Pimentel and Greiner (1997)	United States	1997	G	3751.06
Pimentel (2005, 2009); Pimentel and Burgess (2014)	United States	2005	G	4229.13
Pimentel et al. (1992, 1993a, b)	United States	1992	G	4319.01
Tegtmeier and Duffy (2004)	United States	2002	I	4988.69

[a]The same letter indicates a partial dependence of cost estimates

handlers, e.g. users, sellers and producers, when they have to comply with mandatory regulations (Ajayi et al. 2002).

The various types of regulatory costs considered in the 24 articles investigating those costs are given in Table 2.4. The sources of these costs were highly diverse, including campaigns to raise public awareness of the impact of pesticides, monitoring and control, and public research on pesticides. The considerable diversity of these items may go some way to explaining why none of the studies considered the entire set of costs and heterogeneity in the costs considered by the various studies. Several articles listed a large number of qualitatively different regulatory costs, but estimates were frequently lacking. For instance, Ajayi et al. (2002) mentioned extension services as one of the externalities of pesticide use, but they provided no estimate of the costs involved. Waibel et al. (1999) also considered several costs, including the costs of removing contaminated products from the market and the cost of administrative activities, e.g. laws and decrees, and researches, but these

Table 2.4 Types of regulatory costs for pesticide use

Type of cost	Ajayi et al. (2002)	Houndekon et al. (2006)	Jungbluth (1996)	Praneetvatakul et al. (2013)	Khan et al. (2002)	Steiner et al. (1995)	Tegtmeier and Duffy (2004)	Pimentel et al. (1980a, b)	Pimentel et al. (1991a, b)	Pimentel et al. (1992, 1993a, b)	Pimentel and Greiner (1997)	Pimentel and Hart (2001)	Pimentel (2005); Pimentel and Burgess (2014)	Pretty et al. (2000, 2001)	Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)
Pesticide registration, regulation and market monitoring	X		X	X		X									X
Public awareness campaigns on pesticide impact				X										X	X
Disposal of obsolete and leftover pesticides	X	X				X									
Farm work safety						X									
Control & monitoring															
Crop and/or food										X	X	X	X	X	X
Water (surface, underground and/or wells)					X	X			X	X	X	X	X	X	X
Livestock														X	
Wildlife								X	X						
Undefined			X	X				X	X	X	X	X	X		
Water decontamination				X						X	X	X	X	X	X
Public research on pesticides			X	X											
Extension services			X												X
Economic shortfall															
Crop			X	X	X			X	X	X	X	X	X		
Water	X														X
Livestock								X	X						
Milk								X	X						
Fishing								X	X						

costs were not quantified. Differences in public regulations between countries also underlie the considerable differences in the items considered between papers. For instance, Khan et al. (2002) pointed out that there was no monitoring program in Pakistan in 2002.

Most papers took into account the economic shortfall of crops exceeding the maximum residue limit or the costs of controls and monitoring (Table 2.4). Water decontamination, the regulation of pesticide registration and market monitoring costs were estimated in a small number of papers (Table 2.4) (Fig. 2.2). Other costs, such as those associated with governmental public information campaigns, economic shortfalls for water exceeding the maximum residue limit and public research on pesticides, were considered and estimated even less frequently (Table 2.4). However, these costs may account for a large proportion of the external costs of pesticides. For instance, public information campaigns accounted for about 10 % of the total external costs estimated by Khan et al. (2002) in the Pakistan, and public research costs were estimated at about 10 % of the total external costs by Praneetvatakul et al. (2013) in Thailand.

Finally, some costs, such as the time and money spent establishing regulations, have never been estimated. This is unfortunate, because it has been acknowledged that such costs may be high, due to the need for research and development, expert advice and a number of official tests (Ajayi et al. 2002; Waibel et al. 1999).

2.4.3   Estimated Costs

Estimates of total annual regulatory costs vary considerably, from US$150,000 (2013) in Niger (Houndekon and De Groote 1998; Houndekon et al. 2006) to US$5 billion (2013) in the United States (Tegtmeier and Duffy 2004) (Table 2.3). We did not carry out a meta-analysis to find the cause of this variation. However, as a first approximation, we can consider this variation to be due to the differences in the categories of costs considered, the detailed composition of each category and the geographic scale of the study. The costs of commonly considered categories were particularly variable and depended strongly on the subcategories included. For instance, monitoring and control costs were frequently considered, but different aspects of these costs were covered. The estimates obtained thus differed considerably between papers, depending, in particular, on whether or not they considered the control of underground water. For instance, Pimentel and coworkers began to consider the costs of monitoring underground water and wells in their papers published in 1991. The consideration of these costs led to an immediate increase in their estimates of the overall cost of pesticide regulations of more than 300 %, with these costs accounting for 90 % of total regulatory costs for pesticide use (Pimentel et al. 1991a, b). Water decontamination and economic shortfalls due to crop contamination have

Fig. 2.2 Peace River Manasota Regional Water Supply Authority Water Treatment Plant facility. Water decontamination corresponds to one of the several regulatory costs induced by pesticide use. Estimates of regulatory costs differed considerably between studies, depending, in particular, on whether or not they considered the control of underground water. For instance, in the United States, Pimentel and coworkers began to consider the costs of monitoring underground water and wells in their papers published in 1991. The consideration of these costs led to an immediate increase in their estimates of the overall cost of pesticide regulations of more than 300 %, with these costs accounting for 90 % of total regulatory costs for pesticide use (Pimentel et al. 1991a, b). Moreover Pimentel (2005, 2009) and Pimentel and Burgess (2014) estimated that the current monitoring of wells in the United States (about US$2 billion per year) would have reached US$17 billion per year if all the wells in the United States were monitored (Unmodified photography by Florida Water Daily, under Creative Common License CC BY (https://creativecommons.org/licenses/by/2.0/))

been taken into account by Pimentel et al. since 1992. These costs accounted for about 40 % of the externalities associated with pesticide use.

2.4.4 Actual Versus Theoretical Costs

Most estimates of regulatory costs were based on the actual expenditure of various stakeholders, including public authorities, manufacturers, distributors, sellers and farmers. No attempt was made to estimate non-monetary values. Due to the '*regulatory*' nature of these costs, estimates were generally based on the official budget reports of public agencies.

However, current costs may be much lower than the theoretical value. For instance, Pimentel (2005, 2009) and Pimentel and Burgess (2014) estimated the current monitoring of wells in the United States at about US$2 billion per year, but indicated that this cost would have reached US$17 billion per year if all the wells in the United States were monitored. Including these theoretical costs made a large difference, increasing the overall regulatory costs estimated by Pimentel (2005, 2009) and Pimentel and Burgess (2014) from US$4.2 billion to almost US$22 billion. Similarly, Jungbluth (1996) noted that costs related to pesticide residues in food in Thailand were difficult to estimate and were based on hypothetical scenarios rather than on real situations. In the absence of pesticide residue control for most food products, Jungbluth (1996) had to extrapolate the proportion of products exceeding the maximum residue limit from scarce data. Assuming that 10 % of all fruits and vegetables were above the maximum residue limit and assuming that these products would be unsaleable according to regulations, Jungbluth (1996) obtained a cost of about five billion Baht in 1996. He considered this value – corresponding to almost 90 % of the regulatory costs – as an upper limit for the costs truly paid by the corresponding stakeholders. Conversely, Jungbluth (1996) noted that if the maximum residue limit was not reached, then only the cost of control and monitoring should be taken into account, corresponding to 48.5 million Baht in 1996. This value should be taken as the lower limit of estimates. Along the same lines, Khan et al. (2002) distinguished between actual and potential costs. The potential costs they considered included the cost of establishing laboratories for pesticide residue analyses, residue monitoring programs, and training programs on the safe use of pesticides. These costs were largely theoretical, because there were no such activities in the region covered by their study in 2002, like in many developing countries (Ecobichon 1999). They reported the existence of regulations, but a lack of enforcement. They pointed out, in particular, that there was no comprehensive national monitoring system, and this may remain the case.

2.4.5   Conclusions

Regulatory costs, in particular, have been underestimated. We will see that this is also true for the other categories of "hidden" and external costs, but this underestimation may be particularly marked for regulatory costs. First, only 24 of the 61 articles assessing the external cost of pesticides included regulatory costs, and these 24 articles were actually based on only nine fully independent datasets. Second, each of these articles considered only a small number of regulatory costs. Finally, current costs are probably much lower than the costs that would have to be paid if the complete control, monitoring and decontamination of pesticide residues were to be undertaken and if all products exceeding the legal maximum residue limit had to be withdrawn from the market.

Although underestimated, regulatory costs could reach very large values such as US$4 billion (2013) yearly in the United States in the 2000s. Our analysis shows

that if all regulations were respected, these costs would have jumped to US$22 billion (2013).

2.5 Human Health Costs

Despite strict regulations on the registration and use of pesticides, there are major concerns about their direct impact on human health following occupational exposure and the indirect exposure of non-occupationally exposed populations. Agricultural workers in fields and greenhouses are often occupationally exposed to pesticides, as they are responsible for preparing, mixing and loading pesticide preparations, spraying pesticides, sowing pesticide-treated seeds, harvesting sprayed crops, and cleaning and disposing of pesticide containers. Similarly, workers in the pesticide industry are also likely to experience occupational exposure. The families of farmers and other people living in rural areas in which pesticides are intensively used may also be indirectly exposed to these chemicals, through off-target pesticide drift from agricultural applications in particular (Lee et al. 2011) (Fig. 2.3). Finally, the overall population is also indirectly exposed to pesticides, through the consumption of food and drinking water contaminated with pesticide residues. Many pesticides can damage human health (Damalas and Eleftherohorinos 2011) and, for this reason, high doses over short periods (acute poisoning) and lower doses over longer periods of time (chronic exposure) may have an impact on human health. Karabelas et al. (2009) found that 84 of the 276 active substances authorized as plant protection products in Europe at the end of 2008 – 32 of the 76 fungicides, 25 of the 87 herbicides and 24 of the 66 insecticides – had at least one deleterious effect on health following acute and/or chronic exposure. These effects included acute toxicity, carcinogenicity, reproductive and neurodevelopmental disorders and endocrine disruption. Worldwide, pesticide use has resulted in thousands of cases of acute and chronic poisoning, with effects of varying severity on human health, from mild effects to death. In this section, we review the studies providing estimates of the economic consequences of human health impairment, from benign health damage to death, due to pesticide use.

2.5.1 Several Studies Based on a Limited Number of Datasets

We identified 57 articles providing monetary costs of the impact on health of pesticide exposure. These studies were published in diverse forms, including articles in scientific peer-reviewed journals (e.g. Choi et al. 2012), book chapters (e.g. Cole and Mera-Orcés 2003), PhD dissertations (e.g. Dung 2007), conference proceedings (e.g. Yanggen et al. 2003) and specific reports (e.g. Devi 2007). Some datasets were used as the basis of several publications. For instance, the dataset from the pioneering study by David Pimentel in the United States has been used in several

Fig. 2.3 Pesticides are sprayed in crop fields to protect them against agricultural pests. During these spray applications, these chemicals may disperse by drifting. They may therefore reach non-target crops in neighbouring fields, weakening these plants and reducing yields. Such crop injuries have been reported, in particular, for aerial applications of glyphosate (e.g. Ding et al. 2011; Reddy et al. 2010). Families of farmers and other people living in rural areas in which pesticides are intensively used may also be indirectly exposed to these pesticides, through this off-target pesticide drift from agricultural applications. After spraying, pesticides can also seep into the soil (Gil and Sinfort 2005; Pimentel 1995). Once in the soil, some soluble pesticides may be washed out in runoff water and during soil erosion, resulting in leaching into rivers and lakes (Chopra et al. 2011) (Unmodified photography by Santiago Nicolau, under Creative Common License CC BY-SA (https://creativecommons.org/licenses/by-sa/2.0/))

publications reporting either the same estimates (Pimentel and Greiner 1997; Pimentel and Hart 2001) or providing new estimates (Pimentel et al. 1992; Pimentel and Greiner 1997; Pimentel 2005, 2009) but describing the same types of cost. Similarly, the original dataset of Clevo Wilson (1999a) has been used in several articles in scientific journals and in several book chapters (e.g. Wilson 1999b, 2000a, b, 2002a, b, 2003, 2005). These 57 articles thus actually correspond to 29 independent cost-of-illness studies, starting with two papers by Pimentel et al. published in 1980 and ending with a book chapter written by Pimentel and Burgess and published in 2014 (Table 2.5). All 29 datasets involved cost-of-illness analyses, but they were produced by different methodologies (Table 2.5). Some focused on occupational exposures, notably those of the individuals spraying pesticides, whereas others focused on the pesticide exposure of the whole population. Some authors

provided direct estimates of the various health costs, whereas other inferred health costs indirectly, by complex statistical modeling (Table 2.5).

2.5.2   Estimated Costs

The economic impact on human health has been evaluated per case, per farmer (or household), per rural establishment and at regional or national levels. The detailed costs reported in the 29 independent studies are shown in Table 2.6.

The costs of pesticide poisoning were evaluated at between about US$30 in Thailand and US$600 in Costa Rica (2013) per case, with each farmer/household using pesticides incurring annual costs of US$3 in China to US$187 in Sri-Lanka (2013) per year. In Central America, several authors have reported annual costs of US$32 to US$100 (2013) (see Vaughan (1993) and Villagrán (1976) cited by García (1998) and Castillo and Appel (1990) and Alvarado et al. (1998) cited by Cole et al. (2000)). These costs may be as high as US$850 (2013) per year for a rural establishment. At national level, health costs due to pesticide exposure have been estimated at US$1.1 million in Italy to about US$1.5 billion in the United States (2013) (Table 2.6).

These costs cannot be considered comparable, because they are influenced by several parameters, e.g. the type of pesticide used, the number of treatments applied, the degree to which farm staff spraying pesticides are protected etc., that may differ considerably between countries, with particularly marked differences between developed and developing countries. Moreover, in any given country, these costs have probably decreased over time, for two reasons. First, farmers have certainly become more aware of the effects of pesticide use on health and, therefore, probably protect themselves better against pesticide drifts. Second, some of the most dangerous pesticides have been withdrawn in many countries. Hence, on the one hand, costs actualized to 2013 values in US$ could easily be considered overestimates of current costs. On the other hand, human health costs were probably greatly underestimated at the time at which these reports were published, for three reasons. First, the frequencies of illness and death triggered by chronic exposure to pesticides have rarely been evaluated (see Sect. 2.5.5). Second, acute poisoning events generate various types of costs, and none of the studies performed to date has taken all these costs fully into account (see Sect. 2.5.3). Third, not all pesticide-poisoning events are recorded in databases or reported by farmers, particularly in developing countries (e.g. Lekei et al. 2014; Shetty et al. 2011). Indeed, some of the individuals carrying out pesticide spraying consider the symptoms of poisoning to be 'normal' and do not, therefore, pay much attention to them.

Table 2.5 Cost-of-illness studies on pesticide exposure

Reference	Country	Year	Method[a]	Type Acute	Chronic	Who?	Estimates from Interviews or surveys	Databases	Type of costs Treatment	Loss of work time	Death
Ajayi (2000)	Ivory Coast	1996/1997	DC	X		Farmers	X		X	X	
Ajayi et al. (2002)	Mali	2000	DC	X		Overall	X	X	X	X	X
All Pimentel and Pimentel et al. papers	United States	1980–2014	DC	X	X	Overall		X	X	X	X
Athukorala et al. (2012)	Sri Lanka	2007/2008	DC	X		Farmers	X		X	X	
Atreya (2005)	Nepal	2004	DC	X		Farmers	X		X	X	
Atreya (2007, 2008)	Nepal	2005	SI	X		Farmers	X		X	X	
Atreya et al. (2012, 2013)	Nepal	2008	SI	X		Farmers	X		X	X	
Brenna (2001)	Italy	1999	DC	X		Overall		X	X		
Choi et al. (2012)	South Korea	2009	DC	X		Overall		X	X	X	X
Crissman et al. (1994); Cole et al. (2000); Cole and Mera-Orcés (2003); Yanggen et al. (2003)	Ecuador	1991/1992	DC	X		Overall	X	X	X	X	
Corriols et al. (2008)	Nicaragua	2001	DC	X		Overall	X		X		

Devi (2007)	India	2004/2005	SI	X		Farmers	X		X	X	
Dung and Dung (1999); Dung (2007)	Vietnam	1996/1997	SI	X		Farmers	X		X	X	
Houndekon and De Groote (1998); Houndekon et al. (2006)	Niger	1996	SI	X	X	Farmers	X		X	X	
Huang et al. (2000)	China	2000	SI	X		Farmers	X		X	X	
Jansen et al. (1998)	Costa Rica	1993	DC	X		Farmers		X	X	X	
Jungbluth (1996)	Thailand	1995/1996	DC	X		Farmers		X	X	X	
Khan et al. (2002)	Pakistan	2002	DC	X		Farmers and industrial workers		X	X	X	X
Maumbe and Swinton (2003)	Zimbabwe	1998/1999	DC	X		Farmers	X		X	X	
Ngowi et al. (2007)	Tanzania	2005	DC	X		Farmers	X		X	X	
Pingali et al. (1994, 1995); Rola and Pingali (1993)	Philippines	1989–1991	SI	X	X	Farmers	X		X	X	
Praneetvatakul et al. (2013)	Thailand	2010	DC	X		Overall		X	X	X	
Pretty et al. (2000, 2001)	United Kingdom	1996	DC	X		Farmers	X		X	X	
Soares et al. (2002)	Brazil	1991–2000	Si	X		Rural workers	X		X	X	
Soares and Porto (2009, 2012)	Brazil	1998/1999	SI	X		Farmers		X	X	X	

(continued)

Table 2.5 (continued)

Reference	Country	Year	Method[a]	Type		Who?	Estimates from		Type of costs		
				Acute	Chronic		Interviews or surveys	Databases	Treatment	Loss of work time	Death
Steiner et al. (1995)	United States	1988	DC	X	X	Overall		X			
Tegtmeier and Duffy (2004)	United States	2002	DC	X	X	Overall		X	X	X	X
Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Germany	1996	DC	X		Overall		X	X	X	X
Wilson (1999a, 2000a, b, 2002a, 2003)	Sri Lanka	1995/1996	DC	X	X	Farmers	X	X	X	X	

[a]*DC* direct cost and *SI* statistically inferred

Table 2.6 Estimated costs of the impact of pesticide exposure on health

Estimate	Country	Year	US$ 2013 (per year)	Reference
Per case	Ecuador	1992	44.71	Crissman et al. (1994); Cole et al. (2000); Cole and Mera-Orcés (2003); Yanggen et al. (2003)
	Thailand	1995	32.30	Jungbluth (1996)
	Nicaragua	2001	134.93	Corriols et al. (2008)
	Tanzania	2005	<1 to 400	Ngowi et al. (2007)
	Costa Rica	1993	591.18	Jansen et al. (1998)
Per farmer/household per year	China	2000	2.63	Huang et al. (2000)
	Nepal	2005	3.10	Atreya (2007, 2008)
	Ivory Coast	1996	11.88	Ajayi (2000)
	Nepal	2008	16.24	Atreya et al. (2012, 2013)
	Vietnam	1996	25.53	Dung and Dung (1999); Dung (2007)
	Sri Lanka	2007	15.66	Athukorala et al. (2012)
	Nepal	2004	35.06	Atreya (2005)
	Philippines	1989	82.37	Pingali et al. (1994, 1995); Rola and Pingali (1993)
	India	2004	104.52	Devi (2007)
	Brazil	2000	168.37	Soares et al. (2002)
	Sri Lanka	1995	187.36	Wilson (1999a, 2000a, b, 2002a, 2003)
	Zimbabwe	1998	9.42	Maumbe and Swinton (2003)
Per rural establishment per year	Brazil	1998	5.05–1072.09	Soares and Porto (2009, 2012)

Table 2.6 (continued)

Estimate	Country	Year	US$ 2013 (per year)	Reference
At national level per year	Italy	1999	1,100,000	Brenna (2001)
	Thailand	1995	1,260,000	Jungbluth (1996)
	United Kingdom	1996	2,297,000	Pretty et al. (2000, 2001)
	Mali	1999	3,714,000	Ajayi et al. (2002)
	Thailand	2010	2,990,000	Praneetvatakul et al. (2013)
	Niger	1996	4,397,000	Houndekon and De Groote (1998); Houndekon et al. (2006)
	Nicaragua	2001	4,058,000	Corriols et al. (2008)
	South Korea	2009	7,792,000	Choi et al. (2012)
	Germany	1996	17,986,000	Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)
	Sri Lanka	2007	35,885,000	Athukorala et al. (2012)
	Pakistan	2002	78,064,000	Khan et al. (2002)
	Sri Lanka	1995	21,636,000–130,044,000	Wilson (1999a, 2000a, b, 2002a, 2003)
	United States	1988	246,290,000–486,510,000	Steiner et al. (1995)
	United States	1991	439,320,000	Pimentel et al. (1991a, b)
	United States	1980	581,180,000	Pimentel et al. (1980a, b)
	United States	2002	1,308,890,000	Tegtmeier and Duffy (2004)
	United States	1992	1,326,450,000	Pimentel et al. (1992, 1993a, b)
	United States	1997	1,367,040,000	Pimentel and Greiner (1997); Pimentel and Hart (2001)
	United States	2005	1,492,960,000	Pimentel (2005, 2009); Pimentel and Burgess (2014)

2.5.3   Non-fatal Cases of Acute Poisoning

Acute poisoning, leading to respiratory, gastrointestinal, allergic, and neurologic disorders, is commonly reported by farmers, and particularly by those carrying out pesticide applications (e.g. Hudson et al. 2014; Kishi et al. 1995). For instance, in a broad survey performed in 2010, Lee et al. (2012) found that 25 % of South Korean male farmers had suffered acute occupational pesticide poisoning, suggesting that there may be more than 200,000 cases per year across South Korea. About 12 % of these pesticide-poisoning cases led to the consultation of a medical doctor or hospitalization (Lee et al. 2012). In the United States, the incidence of pesticide poisoning events requiring medical care among the 3,380,000 agricultural workers is thought to be between 10 and 600/100,000 (Calvert et al. 2008 and references therein), corresponding to about 300–20,000 cases annually.

All the cost-of-illness studies took acute poisoning events into account, but they considered very different types of costs associated with such poisoning events. Both indirect and direct costs were incurred. Direct costs are paid either by the farmers themselves or by the society, if, for example, hospital admission is free of charge. Indirect costs correspond to the working time lost by poisoned individuals and their families during and after the poisoning event. This time, which many farmers may not have considered – 90 % in the study by Athukorala et al. (2012) –, can be converted into wage loss and, therefore, into a monetary cost. All cost-of-illness studies took the cost of hospitalization and/or doctor fees into account (Table 2.7). By contrast, the costs of medication and of transport to and from hospital visits and medical consultation were explicitly included in only two thirds and one third, respectively, of the studies (Table 2.7). The economic burden due to the number of days taken off work to recover from poisoning events is the indirect cost classically identified in cost-of-illness studies. Almost all studies included this cost, paid by farmers, and some found that it outweighed, by far, the direct cost of acute poisoning (e.g. Wilson 1999a, 2000a, b, 2003)

However, absence from work to recover from illness is only one of the various indirect costs associated with pesticide poisoning. Indeed, Wilson (1999a, 2000a, b, 2002a, 2003), who generated what is probably the most comprehensive and complete list of indirect costs to date, also identified (i) a decrease in productivity for farmers not taking time off from work to recover and just after their return to work, (ii) impaired decision-making and (iii) a loss of leisure time (Table 2.7). However, he recognized that it would be difficult to estimate the number of leisure hours lost and the decrease in working efficiency. Leisure hours were defined as '*any time spent at home after work, such as time spent reading a newspaper, watching television, listening to the radio, playing a game or practicing a hobby, or time spent with the family*'. As suggested by Becker (1965), Wilson evaluated leisure time costs on the basis of the hourly wage, given that any loss of leisure time would be likely to affect productivity at work.

Decreases in productivity at work and in decision-making abilities were estimated in a few other cost-of-illness studies (Table 2.7). However, none of these

Table 2.7 Types of costs taken into account for acute pesticide poisoning in cost-of-illness studies

Type of costs	Atreya (2005)	Atreya (2007, 2008)	Atreya et al. (2012, 2013)	Wilson (1999a, 2000a, b, 2002a, 2003)	Ajayi (2000)	Crissman et al. (1994); Cole et al. (2000); Cole and Mera-Orcés (2003); Yanggen et al. (2003)	Choi et al. (2012)	Devi (2007)	Ngowi et al. (2007)	Ajayi et al. (2002)	Dung and Dung (1999); Dung (2007)	Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Houndekon and De Groote (1998); Houndekon et al. (2006)	Jansen et al. (1998)	Athukorala et al. (2012)	Pingali et al. (1994, 1995); Rola and Pingali (1993)	Maumbe and Swinton (2003)	Khan et al. (2002)	All Pimentel and Pimentel et al. articles	Tegtmeier and Duffy (2004)	Jungbluth (1996)	Praneetvatakul et al. (2013)	Pretty et al. (2000, 2001)	Soares et al. (2002)	Soares and Porto (2009, 2012)	Brenna (2001)	Corriols et al. (2008)	Huang et al. (2000)	Steiner et al. (1995)
Direct costs																													
Hospitalization and/or doctors' fees	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	c	d	e
Treatment (drugs, local healing, local cure)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X												
Transport to and from hospital/doctor	X	X	X	X	X	X	X	X	X									X											
Indirect costs																													
Loss of working days whilst recovering	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	a	X	X	X	X	X	X	X	X				

Cost category													
Lower productivity if remaining at work and on return to work	X	X	X	X	X							X	X
Impairment of decision-making abilities	X	X	X	X									
Time taken by family members to nurse the victim of illness	X	X	X				X						
Childcare											X		
Loss of leisure time			X										
Cost of fatal poisoning incidents													
Loss of work time/productivity					X		X						
Cost of life										[b]	X	X	X

[a] These costs were estimated in terms of the number of days lost, but the corresponding economic value was not estimated

[b] These authors took the cost of deaths into account. However, they did not specify which costs they took into account. They merely wrote '*Substantial amounts of 24 million in terms of treatment, 18 million for work loss during ailments and 224 million rupees for accidental deaths are estimated as the externality costs*'

[c] These authors provide the mean out-of-pocket costs (without details) for a single acute pesticide poisoning episode – they also indicate that poisoned farmers loss about 20 days of productive working time, without quantifying this cost

[d] No details are given, except that they took into account only the treatment costs for visible impairments (excluding chronic poisoning). However, it is not clear whether their costs include the loss of work. However, they clearly do not include the cost of life

[e] Unknown, because no details are provided about the different type of costs included in the estimates

other studies evaluated the loss of leisure time as in the study by Wilson. However, Wilson did not estimate all the indirect costs due to pesticide poisoning and recognized that '*the costs to the family were not taken into account*'. These costs, including the time taken by family members to nurse the victim of illness, were investigated in cost-of-illness studies performed in Nepal (Atreya 2005, 2007, 2008; Atreya et al. 2012, 2013) and Ecuador (Cole et al. 2000; Cole and Mera-Orcés 2003; Crissman et al. 1994; Yanggen et al. 2003). The cost of childcare, which was estimated by Fleischer and coworkers (Table 2.7), is another indirect cost that was not considered by Wilson. Finally, an additional indirect cost, identified but not estimated by Devi (2007), is the time spent traveling to seek medical help. Thus, none of the cost-of-illness studies performed to date fully took into account all the various costs associated with acute pesticide poisoning.

2.5.4   Fatal Cases of Acute Poisoning

Suicide accounts for most of the fatal cases of acute poisoning. Gunnell et al. (2007) estimated that 250,000 people die from voluntary pesticide ingestion each year, accounting for 30 % of all suicides. The costs associated with such deaths cannot be considered an externality of pesticide use. Nevertheless, accidental pesticide poisoning, mostly in the occupational setting, may be fatal in some cases and the costs associated with such deaths can be treated as external costs. Fatal accidents due to occupational pesticide poisoning are very rare in some countries, such as the United States (1 case recorded from 1998 to 2005, Calvert et al. 2008), but may concern several tens or hundreds of workers per year in other countries with higher levels of pesticide use or in which workers are less well equipped with personal protection equipment (Fig. 2.1). For instance, Santana et al. (2013) reported that 2052 deaths, excluding homicides and suicides, were recorded as due to pesticide poisoning in Brazil, between 2000 and 2009. Half of these deaths concerned agricultural workers and most of them were caused by poisoning with organophosphate and carbamate pesticides.

The cost of fatal cases of accidental poisoning was estimated in only six sets of cost-of-illness studies: Ajayi et al. (2002), Choi et al. (2012), Khan et al. (2002), Tegtmeier and Duffy (2004), Pimentel and coworkers and Fleischer and coworkers (Table 2.7). Fatal cases have generally been ignored, mostly due to the type of cost-of-illness studies performed. Indeed, several of these studies involved interviews with a sample of farmers about the costs they incurred during pesticide poisoning incidents (Table 2.5). By definition, studies of this type cannot take deaths into account and, therefore, did not assess the cost of fatal poisoning events.

Two studies estimated the cost of these deaths, by evaluating the corresponding loss of work time. Ajayi et al. (2002) economically quantified the loss of life as the decrease in agricultural gross domestic product per habitant during the mean duration of an economically active life in agriculture set, in their study, at 50 % of 30 years. Similarly, Choi et al. (2012) estimated the loss of productivity loss due to

premature death. Age- and sex-specific mean wages and employment rates were used as surrogates for per capita productivity for each sex and age group. Like Ajayi et al. (2002), Khan et al. (2002) included fatal injuries in their overall estimate of health costs. They attributed an overall cost of 224 million Rupees (US\$15.1 million (2013)) to such injuries, but provided no details about how this cost was estimated.

David Pimentel and coworkers also considered the cost of fatal cases of pesticide poisoning. They used different sources for their estimates, based on the reasoning that no-one can place a precise monetary value on a human life. In their first estimate, Pimentel et al. (1980a, b) estimated the value of an individual human life at about US\$1 million (about US\$3.2 million (2013)). This value was considered to be the amount of money that industry and government might reasonably spend to prevent a death, but Pimentel et al. (1980a, b) wrote that '*obviously it is much less than the true value of a human life*'. In their article published in 1992, Pimentel et al. used the monetary ranges computed by the insurance industry and used an estimate of US\$2 million (about US\$3.4 million (2013)), which they considered to be conservative. Pimentel and Greiner (1997) and Pimentel and Hart (2001) used an estimate of US\$2.2 million (about US\$3.2 million (2013)) per human life, corresponding to the mean value of the damages paid to the surviving spouses of slain policemen in New York City, which they again considered to be a conservative estimate. Finally, Pimentel (2005, 2009) and Pimentel and Burgess (2014), in their most recent re-evaluation of pesticide externalities, used the United States Environmental Protection Agency standard of US\$3.7 million (about US\$4.7 million (2013)) per human life. Finally, Fleischer and coworkers estimated the cost of acute fatal poisoning events in Germany, using the estimate of US\$2 million per life taken by Pimentel et al. (1993a) (see Waibel and Fleischer 1998).

2.5.5   The (Almost) Uncounted Costs of Chronic Exposure

The most striking feature of cost-of-illness studies on pesticide use is the lack of data concerning the long-term effects of chronic exposure. Several studies have highlighted the possible occurrence of severe health impairment, e.g. cancers, diabetes, depression, neurological deficits, respiratory diseases, fertility problems, cutaneous effects, effects on the unborn embryo, blindness, polyneuropathy, associated with chronic exposure to these chemicals. However, only six estimated the monetary costs of such impairment (Table 2.5). The other studies mostly stated that it was not possible to estimate costs due to chronic exposure because the corresponding illnesses, such as cancers, are multifactorial, making it difficult to estimate the number of cases directly due to pesticide exposure.

The six studies including the costs of health impairment due to chronic exposure provided very rough and incomplete estimates. Steiner et al. (1995) merely considered the cost of chronic illnesses to be as high as that associated with acute poisoning. Pimentel and coworkers based their estimates of the costs of chronic pesticide

exposure on a rough estimate of the number of cancers per year. This number varied from 0.5 % of all cancers (Pimentel et al. 1980a, b, 1991a) to 6000 (Pimentel et al. 1991b), <10,000 (Pimentel et al. 1992, 1993a, b), <12,000 (Pimentel and Greiner 1997), 10,000 (Pimentel and Hart 2001) and between 10,000 and 15,000 cases (Pimentel 2005, 2009; Pimentel and Burgess 2014). All but one of these estimates were based on a personal communication from David Schottenfeld indicating that '*US cases of cancer associated with pesticides in human are less than 1 % of the nation's total cancer cases*' (see Pimentel et al. 1980a, 1992). Tegtmeier and Duffy (2004) did not provide another estimate for the United States: they incorporated the estimate of Pimentel et al. (1992) into their overall externalities of pesticide use. Houndekon and De Groot (1998) and Houndekon et al. (2006) took chronic exposure into account to some extent in their estimates, but it is impossible to determine to what extent. Indeed, they asked farmers how much money they spent on medication and medical consultations and how many working days per year they lost to illness, without specifying the type of health effect (acute or chronic and, for chronic effects, the illnesses concerned). Similarly, Pingali et al. (1994, 1995) and Rola and Pingali (1993) performed medical tests, providing an assessment of the ailments of each farmer or respondent and their seriousness. Such ailments may or may not be related to chronic exposure to pesticides. Finally, Wilson (1999a, 2000a, b, 2003) considered long-term illness diagnosed by a physician as arising from pesticide exposure. Given the small number of farmers examined ($n = 203$), long-term illnesses were probably underdetected.

   This lack of counts is certainly the major flaw of all cost-of-illness studies performed to date. Indeed, there are good reasons to think that the costs of chronic exposure may be not only as high as those of acute poisoning, as stated by Steiner et al. (1995), but probably higher. One reason for this is that sufferers of irreversible illnesses, e.g. blindness, not only undergo short-term treatments, but may also incur long-term costs over a number of years, sometimes until they die. In their most recent re-evaluation of externalities, Pimentel (2005, 2009) and Pimentel and Burgess (2014) estimated the costs of chronic exposure to pesticides, restricted to cancers, reached US$1 billion, a value four times that estimated for the cost of acute poisoning events. However, this estimate did not include the loss of working days and the cost of death. By taking a death rate of 20 % for people suffering from cancers (Siegel et al. 2014) and a rather conservative estimated 3 months of absence from work for cancer treatment and recovery, and using the same costs of death as for acute poisoning, the costs of chronic exposure estimated by Pimentel and coworkers would have reached US$10.2 billion per year in 2005, 45 times the cost of acute poisoning.

2.5.6 Conclusions

The cost-of-illness studies reviewed here clearly show that the external costs relating to human health associated with pesticide use have always been strongly underestimated. First, most studies considered only the costs associated with short-term effects following acute poisoning events. This resulted in a considerably lower estimate of the overall costs, because severe illnesses, e.g. cancers, diabetes, depression, blindness, potentially triggered by chronic pesticide exposure are probably associated with much higher costs than acute poisoning incidents. The few studies to have taken serious illnesses into account yielded only partial and very crude estimates, for only one of the multiple possible illnesses, cancers, and only some of the costs concerned. Moreover, the cost-of-illness studies generally ignored several direct and indirect costs due to acute poisoning.

Another major flaw in cost estimates to date is the lack of consideration of fatal cases of pesticide exposure. Pesticide exposure-related deaths have sometimes been counted for assessments of accidental acute poisoning incidents, but deaths due to chronic pesticide exposure have been completely ignored. Indeed, even though some authors, such as Pimentel et al. estimated the number of cancers, they did not estimate the corresponding number of deaths. In addition, the value of life has probably been underestimated in the past. Pimentel and coworkers increased the estimate of this cost from US$1 to 3.7 million between 1980 and 2005, but, surprisingly, they retained this value (the value provided by the United States Environmental Protection Agency in the early 2000s) in their reassessments published in 2009 (Pimentel 2009) and 2014 (Pimentel and Burgess 2014). There is no standard concept or tool for placing a precise monetary value on a human life, but the reviews and meta-analyses of Kniesner et al. (2012), Lindhjem et al. (2011), Viscusi and Aldy (2003), and Viscusi et al. (2014) converged on a mean of US$9 to 10 million in 2013, which would correspond to a value of US$7.4 million in 2005. The human health costs estimated by Pimentel (2005, 2009) and Pimentel and Burgess (2014) should therefore be re-evaluated. If we use the re-evaluation of the estimated cost of chronic pesticide exposure of Pimentel (2005) proposed above, then overall human health costs in the article published by Pimentel in 2005 would have reached US$15.65 billion (2005), rather than US$1.23 billion (2005) as originally estimated.

Our review shows that health costs studies generally did not take into account fatal cases due to chronic exposure such as fatal outcomes of cancers. Doing so would increase those health costs by up to tenfold, e.g. US$15 billion instead of US$1.5 billion (2013) in the United States in 2005.

2.6   Environmental Costs

We found 26 articles providing 15 different monetary estimates of environmental impacts of pesticide use (Table 2.8). These studies, based on 11 fully independent datasets, either focused on a particular impact or attempted to provide a complete valuation of these impacts. Not only are there only a limited number of studies on this topic, but most were carried out in the 1990s. We found only five studies based on data recorded after 2000 and only one article published since 2006 (Table 2.8).

Table 2.8 Costs of the environmental impact of pesticide use

Reference	Country	Year	Fully independent dataset[a]	Overall costs (million US$ 2013 per year)
James (1995)	Canada	1993	A	0.27–30.73
Houndekon and De Groote (1998); Houndekon et al. (2006)	Niger	1996	B	0.89
Jungbluth (1996)	Thailand	1995	C	5.58
Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Germany	1996	D	9.31
Praneetvatakul et al. (2013)	Thailand	2010	E	16.88
Ajayi et al. (2002)	Mali	1999	F	38.11
Pretty et al. (2000, 2001)	United Kingdom	1996	G	62.74
Steiner et al. (1995)	United States	1991	H/J	203.85–4029.46
Khan et al. (2002)	Pakistan	2002	I	815.12
Pimentel et al. (1991a, b)	United States	1991	J	948.94
Tegtmeier and Duffy (2004)	United States	2002	K/J	1469.74–1507.62
Pimentel et al. (1980a, b)	United States	1980	J	1621.17
Pimentel (2005, 2009); Pimentel and Burgess (2014)	United States	2005	J	5973.50
Pimentel and Greiner (1997); Pimentel and Hart (2001)	United States	1997	J	6993.99
Pimentel et al. (1992, 1993a, b)	United States	1992	J	7967.84

[a]The same letter indicates a partial dependence of cost estimates

2.6.1 Various Types of Environmental Impact

Several types of environmental impact have been considered, but there have been few attempts to classify these impacts into a particular framework (but see Khan et al. 2002). In addition, the costs of these environmental impacts were poorly differentiated from regulatory costs. For instance, several authors considered water monitoring costs and the costs of water decontamination to be costs associated with environmental impact (Pimentel et al. 1980a, b, 1991a, b, 1992, 1993a, b; Pimentel and Greiner 1997; Pimentel and Hart 2001; Pimentel 2005, 2009; Pimentel and Burgess 2014). In this review, we have considered the impact of pesticide use on surface and underground waters as regulatory costs, because these controls and decontamination processes are, in most countries, mandatory. Similarly, the costs of crops and livestock (meat, milk, eggs etc.) contaminated with pesticides to levels exceeding the maximum residue limit, resulting in their mandatory withdrawal from the market and destruction, are considered here as regulatory rather than environmental costs. Finally, we found that environmental impacts could be classified into two main categories: (i) damage to animals (vertebrates and invertebrates), plants, algae and microorganisms and (ii) pest resistance to pesticides (Table 2.9).

2.6.1.1 Damage to Animals, Plants, Algae and Microorganisms

The main environmental impact of pesticides is probably the direct or indirect damage they cause to animals, plants and microorganisms, varying from minor injuries to death. This impact is not restricted to the area in and around fields. Indeed, during applications, pesticides drift away in the air and seep into the soil (Gil and Sinfort 2005; Pimentel 1995). Once in the soil, some soluble pesticides may be washed out in runoff water and during soil erosion, resulting in leaching into rivers and lakes (Chopra et al. 2011).

Damage to Vertebrates

Pesticide use has two main unintentional effects on vertebrate (mammals, birds, fish, reptiles and amphibians) wildlife: (i) deaths due to direct or indirect, e.g. feeding on contaminated plants and/or prey, exposure to high doses and (ii) poorer survival, growth and reproduction due to exposure to sublethal doses and a decline in or the elimination of habitats and food sources due to pesticides (Gibbons et al. 2014; Guitart et al. 2010; Sánchez-Bayo 2011).

Pesticides have a particularly strong impact on birds (Mitra et al. 2011), through direct deaths and the reduction or elimination of habitats and food sources. The indirect effects of insecticides, herbicides and fungicides have been identified as one of the main factors contributing to the decline of farmland birds in several European countries (Geiger et al. 2010). For example, herbicides and insecticides, together

Table 2.9 Environmental impacts of pesticide use and associated costs

Environmental impact	Associated economic costs	Pimentel et al. (1980a, b)	Pimentel et al. (1991a, b)	Pimentel et al. (1992, 1993a, b)	Pimentel and Greiner (1997); Pimentel and Hart (2001)	Pimentel (2005, 2009); Pimentel and Burgess (2014)	Tegtmeier and Duffy (2004)	Steiner et al. (1995)	Khan et al. (2002)	Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Pretty et al. (2000, 2001)	Ajayi et al. (2002)	Jungbluth (1996)	Praneetvatakul et al. (2013)	Houndekon and De Groote (1998); Houndekon et al. (2006)	James (1995)
Damage to animals, plants and microorganisms																
Crops/cultivated plants/trees	Decrease in yields	X	X	X	X	X										
	Insurance of the person applying the pesticide	X	X	X	X	X										
Wild plants (other than weeds)	Loss of biodiversity									X						
Domestic animals and livestock	Illnesses due to poisoning (veterinary costs)	X	X	X	X	X			X							
	Mortality due to poisoning	X		X	X	X			X							
	Loss of productivity in weakened animals								X							
Fish	Loss for fish	X		X	X	X	X	X							X	
	Decrease in recreational value for fishermen	X	X													

Birds	Loss of birds (average cost per bird)					X	X	X	X	X				
	Loss of pest control ensured by birds													X
	Monitoring of birds	X	X											
	Re-establishment programs	X	X	X	X	X								
Arthropods (other than bees)	Increase in pesticide use due to a loss of natural enemies	X	X	X	X	X	X			X	X			
	Decrease in yields due to the resurgence of pests or the emergence of secondary pests		X	X	X	X								
Microorganisms	Increase in pesticide use due to a loss of natural enemies		X	X	X	X								
Bees	Colony losses	X	X	X	X	X	X	X	X					
	Honey and wax losses	X	X	X	X	X	X	X		X				
	Loss of potential honey production	X	X	X	X	X	X							
	Pollination losses	X	X	X	X	X	X							
	Bee rental for pollination	X	X	X	X	X								
Pest resistance to pesticides	Increase in insecticide use	X	X	X	X	X				X	X			
	Increase in pesticide (other than insecticide) use		X	X	X	X								
	Yield losses due to incomplete control of resistant pests		X	X	X	X								

with certain agricultural practices, decrease levels of cereal grains, weed seeds and arthropods, thereby potentially contributing to the decline of bird species dependent on these resources for survival, e.g. Wilson et al. (1999) for granivorous birds and Hallmann et al. (2014) for insectivorous birds. In North America, the decline of several grassland birds, including songbirds in particular, is thought to be mostly due to a direct impact of insecticides (Mineau (2002) and Mineau et al. (2005) for Canada; Mineau and Whiteside (2006, 2013) for the United States). Birds are particularly susceptible to cholinesterase-inhibiting pesticides, e.g. organophosphates and carbamates, mostly because, unlike mammals, they have low levels of anticholinesterase detoxifying enzymes (Walker 1983). The extensive use of carbofuran, a carbamate, through a granular form resembling plant grains in North America has been reported to lead to the death of millions of birds annually (Mineau et al. 2012) (Fig. 2.4). Other birds, such as those predating on rodents, e.g. owls and other birds of prey, are also directly or indirectly poisoned by rodenticides in many developed countries (Christensen et al. 2012; Elliott et al. 2014; Langford et al. 2013; Thomas et al. 2011).

Fig. 2.4 The extensive use of carbofuran, a carbamate, through a granular form resembling plant grains in North America has been reported to lead to the death of millions of birds – like the horned lark *Eremophila alpestris* – annually (Mineau et al. 2012). The ban on these granular formulations of carbofuran introduced in 1991 (Heier 1991) and effective by 1994, in particular, probably had a considerable beneficial effect on bird survival in farmland. The estimate of 17–91 million birds killed per year during the 1980s was therefore almost certainly, as stated by Mineau (2005), the "worst-case" impact of pesticides on birds in an agricultural setting'. The current impact of pesticide use on birds is probably much lower (Unmodified photography by Kelly Colgan Azar, under Creative Common License CC BY-ND (https://creativecommons.org/licenses/by-nd/2.0/))

Many studies have documented direct and indirect effects of both high and sublethal doses of pesticides on several wild vertebrates other than birds. Herbicide treatments can be lethal for amphibians. For instance, one of the surfactants added to glyphosate, the most widely used herbicide worldwide, has been shown to be highly toxic to several species of amphibians in North America (Relyea 2005). Recent reviews and meta-analyses have confirmed that several pesticides decrease amphibian survival (Baker et al. 2013; Egea-Serrano et al. 2012). It has also been shown that pesticides have indirect and sublethal effects on this class of vertebrates, reducing their growth (Baker et al. 2013; Egea-Serrano et al. 2012) and increasing the frequency of abnormalities (Egea-Serrano et al. 2012). For instance, the herbicide atrazine, one of the most commonly used pesticides worldwide, adversely affects amphibians by disrupting metamorphosis, reducing antipredator behavior, decreasing immune function and increasing the frequency of infection (Rohr and McCoy 2010). The endocrine disruptor activities of atrazine, which decreases both time to metamorphosis and size at metamorphosis, can be enhanced by the presence of insecticides and fungicides. The effects of such mixtures of pesticides have probably played a major role in the global decline of amphibians (Hayes et al. 2006). Atrazine also disrupts several life history traits in fish (Rohr and McCoy 2010). Several pesticides, including atrazine, have been shown to have immunotoxic effects (Dunier and Siwicki 1993) and to cause oxidative stress (Slaninova et al. 2009) in fish, and these compounds can also interfere with olfaction in these organisms (Tierney et al. 2010).

Finally, pesticides also injure wild and domestic mammals. Rodenticides, particularly second–generation compounds, kill not only target pests, but many nontarget rodent species (Elliott et al. 2014; Fournier-Chambrillon et al. 2004). Species abundance and diversity in rodent communities can also be altered by herbicides, particularly in situations in which these chemicals are used to convert bushwood to grassland (Freemark and Boutin 1995). Pesticides can also poison several domestic mammals (Wang et al. 2007; Berny et al. 2010). In the United States, and probably also in many European countries, the incidence of poisoning is highest in cats and dogs (Berny et al. 2010). These animals often wander freely around homes and farms. They are therefore much more likely to come into contact with pesticides than other domesticated animals. The presence of sprayed chemicals on fodder or of pesticide residues in feed for livestock may lead to fatal poisoning events in domestic farm animals, particularly in developing countries (Ajayi et al. 2002).

Damage to Invertebrates

Insecticide treatments controlling pests also have damaging effects on many nontarget terrestrial arthropods in agroecosystems, including the natural enemies (predators, parasites and parasitoids) of agricultural pests (Croft and Brown 1975). Damage to these species may be greater than initially thought, because such damage can occur even at low non-lethal doses of insecticides (Desneux et al. 2007). For instance, sublethal doses of neonicotinoids (a new generation of insecticides) have

clearly been shown to affect the foraging success, survival, colony growth, and queen production of honey and bumble bees (Henry et al. 2012; Schneider et al. 2012; Whitehorn et al. 2012) (Fig. 2.5). Beneficial arthropods are also affected by herbicides. This impact may be direct (Norris and Kogan 2000), but it is generally indirect. By killing weeds and non-target plants, herbicides reduce the fitness of many of the arthropods developing or resting on weeds, thereby decreasing the growth of their populations (Freemark and Boutin 1995; Norris and Kogan 2005). Even if herbicides do not actually kill non-target plants, they may still suppress flower formation in some species (Schmitz et al. 2014a), or markedly delay flowering time and decrease flower production in many other species (Boutin et al. 2014). As a consequence, herbicide treatments may indirectly decrease the fitness of pollinating insects in non-crop habitats during periods in which crop plants are unavailable for pollination. Egan et al. (2014) showed that changes in the structure and function of arthropod communities depend on species composition, crop rotation patterns and the timing of herbicide exposure.

Pesticides can also have an impact on aquatic invertebrates (Rasmussen et al. 2013), particularly during pulses of contamination triggered by surface runoff and

Fig. 2.5 Honey bee on apple blossom in Bedfordshire, United Kingdom. Damage to non-target terrestrial arthropods in agroecosystems may be greater than initially thought, because such damage can occur even at low non-lethal doses of insecticides (Desneux et al. 2007). Sublethal doses of neonicotinoids (a new generation of insecticides) have clearly been shown to affect the foraging success, survival, colony growth, and queen production of honey and bumble bees (Henry et al. 2012; Schneider et al. 2012; Whitehorn et al. 2012) (Unmodified photography by Orangeaurochs, under Creative Common License CC BY (https://creativecommons.org/licenses/by/2.0/))

through tile drains during heavy rain. Invertebrates may also be injured during short pulses of contamination due to pesticide desorption from suspended solids or sediment particles. Finally, they can be poisoned via the ingestion of "polluted" particles. Several studies have found associations between pesticide concentrations and decreases in the numbers and abundances of taxa and changes to invertebrate community structure (e.g. Friberg et al. 2003; Liess and von der Ohe 2005; Schäfer et al. 2007, 2011, 2012). These studies were performed at many sites in Europe, Siberia and Australia, and the authors concluded that there was little doubt that pesticides were responsible for the observed changes in aquatic invertebrate communities. Liess and von der Ohe (2005) and Schäfer et al. (2007) showed that the number and abundance of aquatic invertebrate taxa could be compensated, probably through recolonization from undisturbed sections of the stream. Nevertheless, Beketov et al. (2013) found that pesticides had significant effects on regional species and family richness in Germany, France and Australia, with up to 42 % of the taxa from the recorded taxonomic pools lost. Furthermore, in Europe, effects were detected at concentrations considered environmentally benign in current legislation (Beketov et al. 2013).

Damage to Plants, Algae and Corals

Pesticides can accidentally injure crops. First, the crops protected by the pesticide may be damaged by it. In particular, some pesticides may disrupt photosynthesis, thereby decreasing both growth and yield. Such an effect has been shown for several fungicides, on many crops (Petit et al. 2012), and for some herbicides, on cotton (Reddy et al. 1990) and soybean (Hagood et al. 1980). Similarly, insecticide treatments may also lower yields when applied to lettuce (Toscano et al. 1982) and cotton (Youngman et al. 1990). Second, pesticides may disperse by drifting during spray applications. They may reach non-target crops in neighboring fields, weakening these plants and reducing yields. Such crop injuries have been reported, in particular, for aerial applications of glyphosate (e.g. Ding et al. 2011; Reddy et al. 2010). Third, as some herbicides persist in the soil, other crops (notably vegetables) in the rotation may be affected and display lower yields (e.g. Felix et al. 2007; Mahmoudi et al. 2011). These carryover injuries may be accentuated in fields previously treated with several herbicides. For instance, the addition of atrazine to mesotrione treatments in the year before planting has been shown to increase injury rates by 3–55 % in broccoli, carrot, cucumber, onion, and potato (Robinson 2008).

In some agroecosystems, field margins and boundaries (e.g. hedgerows, woodlots, etc.) are the only remaining habitats for many wild plant species, some of which are beneficial, considered of heritage value or protected (Türe and Böcük 2008). The long-term maintenance of their populations, particularly close to edges of crop fields, may be jeopardized by the drift of herbicide treatments. Several studies have shown that non-target plants are affected by herbicides (e.g. Freemark and Boutin 1995; Gove et al. 2007; Schmitz et al. 2014a), leading to short- and long-term changes in the richness and/or structure of plant communities (e.g. Egan et al.

2014; Gove et al. 2007; Schmitz et al. 2014b). Changes also occur among weed communities within crop fields (e.g. Andreasen and Streibig 2011). These changes in the composition of weed plant communities may reflect lower rates of reproduction in the species most affected by herbicides, as demonstrated by Boutin et al. (2014).

Aquatic plants, algae and coral species may also be affected by pesticide use. The large distances between sprayed fields and bodies of fresh and inshore waters should theoretically provide some protection, through the adsorption of some of the drift by bank vegetation and, probably, also through the dilution of the herbicides in water. In some ecosystems, aquatic and algal species are, indeed, considered to be not necessarily at risk (e.g. Cedergreen and Streibig 2005). However, there may be a major impact on aquatic species in bodies of water subject to intense agricultural runoff (Fabricius 2005). A textbook example is provided by the inshore waters of the Australian Great Barrier Reef. This lagoon has World Heritage status, but is widely contaminated with insecticides and herbicides (Haynes et al. 2000; Lewis et al. 2009; Packett et al. 2009). Kroon et al. (2012) estimated that >30,000 kg of herbicides enter the Great Barrier Reef lagoon each year. Despite their dilution in the water, concentrations exceeding 1 μg L⁻1 have been reported for some herbicides within the lagoon (Lewis et al. 2009). These concentrations may be high enough (Lewis et al. 2012) to have deleterious effects on corals (Cantin et al. 2007; Jones et al. 2003; Negri et al. 2011), seagrasses (Flores et al. 2013), foraminifera (van Dam et al. 2012), benthic microalgae (Magnusson et al. 2008, 2010, 2012) and coralline algae (Negri et al. 2011). The Great Barrier Reef is probably the most widely studied ecosystem threatened by pesticides, but other species in several other coastal water systems are also threatened by the effects of pesticide runoff. The ecosystems concerned include Chesapeake Bay in the United States (Hartwell 2011), the Seto Inland Sea (Balakrishnan et al. 2012) and two lagoons (Yamamuro 2012) in Japan.

Damage to the Soil Community

The effects of pesticides on earthworms (Yasmin and D'Souza 2010), microarthropods (Adamski et al. 2009), nematodes (Zhao et al. 2013), fungi (Morjan et al. 2002) and microorganisms (viruses, protozoa and bacteria) (Imfeld and Vuilleumier 2012; Lo 2010) within the soil may have major environmental consequences. The soil community plays a critical role in crop production and crop protection (Barrios 2007). These small organisms are essential to the functioning of all ecosystems, because they break down waste, thereby recycling the chemical elements required for life. Bacteria and fungi make nitrogen and other elements available to plants (Bonfante and Anca 2009) and, like nematodes, some soil-borne fungi are natural enemies of pest insects (Kaya and Gaugler 1993; Klingen and Haukeland 2006). Earthworms, which are widely recognized as 'ecosystem engineers', contribute to several ecosystem services through pedogenesis, the development of soil structure, water regulation, nutrient cycling, primary production, climate regulation, the remediation of pollution and cultural services (Blouin et al. 2013).

Damage Due to Interactions Between Species and Between Stressors

Species are not isolated from their environment or from other interconnected species. Pesticide exposure may, therefore, have indirect effects on biotic interactions, such as host-parasite relationships (Köhler and Triebskorn 2013). For instance, Rohr et al. (2008) showed that atrazine use was the best predictor of the abundance of larval trematodes (parasitic flatworms) in the declining northern leopard frog *Rana pipiens*. Pesticides can also increase the frequency of deformities associated with trematode infection in amphibians (Kiesecker 2002). More generally, interactions between pesticides and other environmental stressors may play a key role in the decline of amphibian populations (Mann et al. 2009). Synergistic effects of pesticides and natural stressors, such as heat, desiccation, oxygen depletion and pathogens, have already been documented in many other classes of animals (Holmstrup et al. 2010). Pesticides can also affect food webs and competition between species (Köhler and Triebskorn 2013). For instance, benomyl, a widely used fungicide, suppresses populations of arbuscular mycorrhizal fungi in grasslands, altering floral display at the patch level. Such changes have been shown to induce a shift in the community of floral visitors, from large-bodied bees to small-bodied bees and flies, and to decrease the total number of visits to flowers (Cahill et al. 2008).

2.6.1.2 Pest Resistance to Pesticides

The second main environmental consequence of pesticide use is the selection of pesticide resistance. The impact of such resistance is well documented, for all classes of pests targeted and for almost all types of insecticides, herbicides and fungicides (REX Consortium 2013). More than 10,000 cases of resistance to 300 insecticide compounds have been reported in about 600 species of arthropods (Arthropod Pesticide Resistance Database; www.pesticideresistance.com). Similarly, 300 cases of field resistance to 30 fungicides have been reported in 250 species of phytopathogenic fungi (Fungicide Resistance Action Committee database; http://www.frac.info). The International Survey of Herbicide-Resistant Weeds (http://www.weedscience.com) has suggested that there are currently about 429 biotypes resistant to 153 herbicides in 234 weed species.

2.6.2 Economic Consequences Considered to Date

The environmental impacts described above are obviously costly, in many ways. The various economic consequences considered in the 15 sets of studies are shown in Table 2.9.

Pimentel et al. (1980a, b, 1991a, b, 1992, 1993a, b), Pimentel and Greiner (1997), Pimentel and Hart (2001), Pimentel (2005), Pimentel and Burgess (2014), followed by Steiner et al. (1995), Khan et al. (2002) and Tegtmeier and Duffy (2004), tried to carry out a complete evaluation of the economic consequences of pesticide exposure

in bees (Table 2.9). They evaluated colony losses, but also considered (i) losses of honey and wax due to bee colonies being either seriously weakened by pesticides or suffering losses when moved by beekeepers to minimize the risk of pesticide damage, (ii) losses of potential honey production because heavy pesticide applications on some crops may result in beekeepers being excluded from sites otherwise suitable for beekeeping, (iii) the lack of pollination due to losses of bee colonies and (iv) bee rental to compensate for this lack of pollination. Pollination losses were the greatest loss by far, accounting for more than 60 % of the total economic impact of pesticide exposure in bees.

A thorough analysis, such as that performed for bees, has never been undertaken for plants, microorganisms or animals other than bees. Considerations of the economic consequence of arthropod and microorganism depletion have focused on the loss of natural enemies of agricultural pests (Table 2.9). This loss of beneficial arthropods, fungi, bacteria and viruses increases pest pressure on crops. First, such losses allow the primary pests themselves to occur at higher densities. Several outbreaks of primary pests have been accounted for by the depletion of their natural enemies by pesticides (Bommarco et al. 2011; Hardin et al. 1995; Wilson et al. 1998). Second, many secondary pests, i.e. species that were once minor or unimportant crop pests, may become major pests if no longer controlled by their natural enemies (Hardin et al. 1995; Eveleens et al. 1973). Primary and secondary pest outbreaks due to the depletion of natural enemies have two main economic consequences: they increase pesticide use and decrease yields.

Pesticide resistance increases the amount of pesticide used, because higher doses are required to kill resistant pests. The use of alternative pesticides to which the resistant pests are still susceptible, or of a mixture of pesticides, which may be more expensive, may prove necessary. Resistance also decreases yields, because some pests become so resistant that they can no longer be fully controlled by pesticides or because the larger amounts of pesticides required to control resistant pests damage the crops treated.

The annual cost of mortality in birds and fish has been evaluated by multiplying the number of individuals actually killed due to direct or indirect exposure to pesticides by the estimated mean price of the individuals concerned. For birds, two additional types of environmental costs have been considered: the monitoring of species threatened by pesticide exposure and the re-establishment of endangered species, e.g. the bald eagle, *Haliaeetus leucocephalus*, affected by pesticides (Table 2.9).

Three economic consequences have been associated with damage to domesticated animals: the cost of illness, e.g. veterinary fees, the cost of dead livestock and the loss of productivity of animals weakened by poisoning, with affected individuals producing less milk, meat or eggs, for example (Table 2.9).

Yield loss is the principal economic consequence of accidental injury to crops from pesticide use. Contractors applying pesticides can be sued for damage to the crop during or after treatment. In many states of the United States, contractors applying pesticides must provide evidence of financial responsibility before spraying. Most are insured, to protect themselves against expensive lawsuits, and this increases the environmental cost of pesticide use.

2.6.3   Counting Environmental Costs: From Specific to Overall Costs

Some studies have focused on a particular impact. For instance, James (1995) specifically estimated the cost of bird losses in Canada. Some studies have been devoted to a specific crop in a specific area, such as the Punjabi cotton zones in Pakistan (Khan et al. 2002). Others have focused on externalities *sensu stricto*: Steiner et al. (1995) and Tegtmeier and Duffy (2004) in the United States and Pretty et al. (2000, 2001) for the United Kingdom. Steiner et al. (1995) therefore chose to ignore the costs associated with pesticide resistances and the loss of natural enemies, because these costs are mostly met by users (see also Pearce and Tinch 1998). Finally, Pimentel et al. (1980a, b, 1991a, b, 1992, 1993a, b), Pimentel and Greiner (1997), Pimentel and Hart (2001), Pimentel (2005) and Pimentel and Burgess (2014) in the United States, Jungbluth (1996) in Thailand and Ajayi et al. (2002) in Mali assessed the total environmental costs associated with pesticide use on all crops at the national level.

Estimates of economic costs due to environmental damages are therefore highly variable, from US$270,000 (2013) for the birds killed in Canada (James 1995) to about US$8 billion (2013) for total environmental impact in the United Sates (Pimentel et al. 1992, 1993a, b) (Table 2.8).

The two main environmental costs considered stemmed from the increase of pesticide use due to pest resistance and the number of birds killed by pesticide exposure. In the study by Pimentel et al. (1992), these two categories accounted for 35 % and 40 % of the total environmental costs. However, it is particularly difficult to assess the costs associated with bird losses. Pimentel et al. (1992), Pimentel and Greiner (1997), Pimentel (2005) and Pimentel and Burgess (2014) reported that the cost of a bird's life in the United States could be estimated at $0.40, $216 or $800. As pointed out by Bowles and Webster (1995), the techniques used to evaluate this cost were not described. In fact, these values correspond, to the cost per bird for bird watching, bird hunting and for rearing and releasing a bird of an affected species in the wild, respectively. In 1992, Pimentel et al. decided to take an average cost of $30 per bird (Table 2.10). Surprisingly, this cost of $30 was never updated and has remained constant in all the papers since published by Pimentel and coworkers. This resulted in a decrease in the estimated annual cost of bird losses from US$3.37 billion (2013) in Pimentel et al. (1992) to US$2.55 billion (2013) in Pimentel and Burgess (2014) (Table 2.10). Based on the estimate of Pimentel et al. (1992), Tegtmeier and Duffy (2004) decided to take the lowest monetary value assigned per bird (US$0.40 in 1992 – re-evaluated to US$0.51 in 2004). This resulted in estimated costs of US$45 million (2013) per year, almost two orders of magnitude lower than the estimates provided by Pimentel et al. in 1992 (Table 2.10). Finally, Steiner et al. (1995) indicated that the cost of a bird may vary between the lower limit of US$0.40 to the mean value of US$30 chosen by Pimentel and coworkers, resulting in annual costs of about US$47 million to US$3.5 billion (2013) (Table 2.10).

Table 2.10 Annual costs for fish and birds killed by pesticides in the United States

Wildlife	Reference	Year of evaluation	Cost per individual (US$)	Killed individuals per year (in million)	Total cost per year (million US$)	Updated total cost per year (million US$ 2013)
Fish	Pimentel et al. (1980a, b)	1980	0.40	2.00	0.80	2.53
	Pimentel et al. (1991a, b)	ND[a]	–	–	–	–
	Pimentel et al. (1992, 1993a, b)	1992	1.70	6–14	10.00–24.00	16.86–40.47
	Pimentel and Greiner (1997); Pimentel and Hart (2001)	1997	4.00	6–14	24.00–56.00	35.15–82.02
	Pimentel (2005)	2005	10.00	6–14	60.00–140.00	72.92–170.14
	Tegtmeier and Duffy (2004)	2002	3.65	6–14	21.90–51.10	28.41–66.29
	Steiner et al. (1995)	1991	1.70–10	1.74–2.10	2.89–21.00	5.08–36.90
Birds	Pimentel et al. (1980a, b)	NQ[b]	–	–	–	–
	Pimentel et al. (1991a, b)	NQ[b]	–	–	–	–
	Pimentel et al. (1992, 1993a, b)	1992	30.00	67.00	2000.00	3372.92
	Pimentel and Greiner (1997); Pimentel and Hart (2001)	1997	30.00	67.00	2000.00	2929.38
	Pimentel (2005, 2009); Pimentel and Burgess (2014)	2005	30.00	72.00	2100.00	2552.06
	Tegtmeier and Duffy (2004)	2002	0.51	67.00	34.50	44.75
	Steiner et al. (1995)	1991	0.40–30	67.00	27.00–2000.00	47.45–3514.58

[a]ND no details (they provide an overall cost for fishery losses using a re-evaluation of the 1980 figures without providing any details)
[b]NQ not quantified

Similar variations were observed in estimates of fish losses in the United States. The cost of a fish varied from US$0.40 to US$10 between papers, resulting in estimates of the annual cost of fishery losses of between US$2.53 million (2013) in 1980 and US$170 million (2013) in 2005, 2009 and 2014, in studies by the same authors (Table 2.10).

2.6.4   Underestimated and Uncounted Costs

The costs provided by these studies are probably far from the actual costs. There are, indeed, several reasons for thinking that the counted costs were underestimated. In addition, several types of environmental damage have yet to be assessed.

2.6.4.1   Most Costs Were Probably Underestimated

Pimentel et al. (1980a) considered their estimate of the cost of domesticated animal poisoning to be low because it was based only on poisoning cases reported to veterinary surgeons. They indicated that in cases of poisoning in which little can be done for the animal, veterinary surgeons are rarely called.

They also considered their estimates of fish deaths to be low, for many reasons. They indicated that 20 % of the reported fish kills gave no estimate of the number of fish killed and that fish kills often cannot be investigated quickly enough to determine whether they result from pesticide exposure. Furthermore, the fast-moving water in rivers dilutes pollutants, making it difficult to identify the chemical involved, and washes away the poisoned fish. Finally, many dead fish sink to the bottom or are eaten by other fish and therefore cannot be counted. Perhaps most importantly, unlike direct kills, few, if any, of the widespread, low-level pesticide poisoning events result in dramatic manifestations and these events are, therefore, not recognized or reported.

The total numbers of birds killed by pesticides is difficult to determine because, like most vertebrate species, they are often secretive, camouflaged, highly mobile and, as pointed by Pimentel et al. (1980a), they do not conspicuously '*float to the surface*' as fish do. They often live in dense grass, shrubs, and trees. Dead birds disappear quickly, well before they can be found and counted (Mineau and Collins 1988). Scavengers have been shown to remove >90 % of bird carcasses placed in farmland overnight (Prosser et al. 2008). Furthermore, field studies seldom account for birds dying outside the treated areas, but birds often hide and die in inconspicuous locations. Estimates of bird mortality do not include birds that die due to the death of one of their parents or the deaths of the nestlings. They do not include nestlings killed because they were fed contaminated arthropods and other foods either. Mineau (2005) considered the estimate of Pimentel et al. (1992) – a mortality of 67 million birds per year in the United States – to be too conservative. Indeed, he estimated that, at the start of the 1980s, 17–91 million songbirds were dying annually

in the United States Corn Belt, purely due to the use of a granular formulation of carbofuran in corn. However, Mineau (2005) felt that this figure was still too conservative, because it did not include birds dying in other crops treated with granular carbofuran, such as soybean, sorghum, groundnut, tobacco, cotton or sunflower, or the lethal impact of all the other pesticides, including rodenticides, on birds. Based on the analysis of Mineau (2005), there were probably more than 100 million birds lost annually in the United States between 1978 and 1985.

Crop losses due to pesticides are also probably underestimated because, for many losses, the parties involved come to an out-of-court settlement, and the losses are therefore never reported to the state and federal agencies (Pimentel et al. 1993a). In addition, pesticide damage to target crops due to the application of larger doses to kill pesticide-resistant pests, has probably been underestimated.

2.6.4.2   Several Costs Have Never Been Evaluated

Production and storage sites may be particularly polluted (Elfvendahl et al. 2004; Jit et al. 2010), but this pollution has never been taken into account. Half a million tons of obsolete pesticides are stored throughout the developing world (Food and Agriculture Organization 2011a), often outdoors, in leaky containers, resulting in particularly high levels of pollution of the surrounding soil and water (Ahad et al. 2010; Dvorská et al. 2012). Similarly, the sites at which pesticides are prepared and loaded into sprayers and at which tractors and sprayers are washed may be highly polluted (Helweg et al. 2002). Some costs are covered by the chemical companies themselves. However, this pollution generates externalities *sensu stricto*, through decreases in the price of land, houses and recreational activities close to the sites concerned (Epp et al. 1977).

The cost of damage to wildlife has been counted only for birds and fishes. However, as indicated in Sect. 2.6.1.1, many other non-human vertebrates are also damaged by pesticide use. Similarly, the monetary cost of pesticide impact on aquatic invertebrates, plants, algae and the soil community has never been estimated.

The direct costs of bird and fish losses have been estimated, but several indirect costs associated with these losses have yet to be analyzed. Indeed, birds and fish provide several ecosystem services. Birds make a significant contribution to the four principal types of ecosystem services defined by the United Nations Millennium Ecosystem Assessment: provisioning, regulating, cultural and supporting services. In agricultural ecosystems, they control pests, by eating arthropods, rodents and weeds (Whelan et al. 2008). Interestingly, James (1995) estimated the cost of bird losses in Canada, by setting the cost of an individual bird at the cost of achieving the same level of insect control with insecticides, if the birds were absent. This clearly corresponds to only part of the economic advantage birds provide to humans. Indeed, in addition to their contribution to pest control, birds also play significant roles in pollination, seed dispersal, and scavenging (Whelan et al. 2008).

Arthropods also provide substantial ecosystem services. However, the studies performed to date have considered only the lack of pest control provided by natural enemies killed by pesticides. However, like bees, '*wild*' insects provide other services in addition to pest control, including pollination, dung burial and food for wildlife (Losey and Vaughan 2006).

2.6.5   Conclusions

The cost of the environmental impact of pesticides has been poorly investigated to date. Only 15 sets of studies have evaluated these costs, and these studies were actually based on only 11 independent datasets. Only six studies provided an overall cost assessment at national level. The pioneering work of David Pimentel in the United States remains the key reference, but this work dates from the 1980s and 1990s, with a partial update published in 2005, 2009 and 2014. Although Pimentel and coworkers provided the most complete evaluation of environmental impairment available, we have shown that this assessment was probably highly incomplete, with a strong underestimation of costs.

It should be borne in mind that the current environmental impact of pesticide use is probably very different from that during the 1980s and 1990s (see Sect. 2.8.4). In North American and European countries, the most dangerous and persistent pesticides (e.g. DDT, carbofuran) have been banned and partly replaced by less toxic and less persistent compounds, strongly decreasing the impact on birds and fish. However, other countries, such as India and China, are still producing, exporting and using DDT (van den Berg et al. 2012). Moreover, pesticide resistance has steadily increased over the last 30 years (Rex Consortium 2013). The doses of pesticides applied to many crops are, therefore, almost certainly higher than in the past, resulting in a greater impact on the environment.

To conclude on environmental costs of pesticide use, we show that they suffered large underestimation and most of them were never considered in the literature. They were nevertheless estimated to up to US$8 billion (2013) in the United States in 1992.

2.7   Defensive Expenditures

The aversive behavior approach estimates the amount that someone is willing to pay to reduce their environmental exposure to hazardous chemicals, such as pesticides (Dickie 2003). This expenditure can be seen as an investment, to protect against both short- and long-term illnesses. As for the cost-of-illness approach, different names have been given to the costs due to aversive behavior: averting costs, precautionary costs, mitigating costs, revealed willingness to pay for safety and defensive

expenditures (Wilson 1999a). In this review, we will use the term "defensive expenditures". Defensive expenditures can be either private if incurred by the farmers themselves or external if incurred by consumers (Pearce and Tinch 1998) (Table 2.1). Defensive expenditures may be incurred due to several types of aversive behavior, such as wearing protective clothes when applying pesticides for farmers, monitoring and removing pesticides from drinking water for consumers, and eating organic food to avoid, or at least reduce the levels of pesticide residues on food for consumers.

2.7.1 Defensive Expenditures for Pesticide Handling and Spraying

Farmers take safety measures when handling and applying pesticides to their crops, to decrease or prevent direct exposure to these chemicals. The defensive expenditures taken into account include costs associated with precautions taken to reduce direct exposure to pesticides, such as masks, caps, shoes/boots, handkerchiefs, long-sleeved shirts/pants (Table 2.11). These products may have multiple uses, but only products purchased specifically for the use and handling of pesticides are considered and their costs are generally annualized according to the expected lifespan of the product (e.g. Atreya 2008). Wilson (1999a, 2000b, 2003, 2005) considered the hiring of personnel to spray pesticides as a defensive activity, and therefore included this expense as defensive expenditures.

Only 13 articles have estimated the cost of defensive expenditures, and these estimates were based on only seven independent datasets (Table 2.1). This small number of studies considering defensive expenditures may be accounted for by defensive expenditures not being an externality *sensu stricto*. These costs are paid by farmers, which accounts for their lack of inclusion in studies focusing on the external costs of pesticide use such as those performed by Pimentel and coworkers.

Two groups of authors, in particular, have explored the defensive expenditures of farmers: Clevo Wilson (Wilson 1999a, b, 2000b, 2002b, 2003, 2005) and Athukorala et al. (2012) in Sri Lanka and Kishor Atreya (Atreya 2005, 2007, 2008 and Atreya et al. 2012, 2013) in Nepal. We were able to identify only one other studying exploring defensive expenditures, by Ajayi et al. (2002), in Mali.

In Nepal and Sri Lanka, farmers were found to spend a mean of between US$6 and US$32 (2013) per year on defensive expenditures (Table 2.11). Ajayi et al. (2002) estimated that farmers in Mali would need to spend US$30 to US$60 (2013) per year on equipment to ensure that they were protected against pesticide exposure. Wilson (Wilson 1999a, 2000b, 2003, 2005) and Athukorala et al. (2012) used data obtained directly from farmers to estimate the annual cost for the whole of Sri Lanka. They estimated these costs at between US$1 million (2013) if only 5 % of the farmers used pesticides and US$10 million (2013) if 20 % of the farmers used pesticides (Table 2.11).

Table 2.11 Costs of aversive protection (defensive expenditures) against pesticide use

	Atreya et al. (2012)	Atreya et al. (2012, 2013)	Atreya (2005)	Atreya (2007, 2008)	Athukorala et al. (2012)	Wilson (1999a, b, 2000b, 2002b, 2003, 2005)	Ajayi et al. (2002)
Country	Nepal	Nepal	Nepal	Nepal	Sri Lanka	Sri Lanka	Mali
Year	2008	2008	2004	2005	2007	1995	1999
Items							
Boots/Closed shoes	X	X	X	X	X	X	X
Shawls/Face shield	X	X	X	X			X
Gloves	X	X	X	X	X	X	X
Mask	X	X	X	X	X	X	X
Cap/Hat	X	X	X	X			X
Handkerchief	X	X	X	X			
Long-sleeved shirts	X	X	X	X	X	X	X
Full-length pants	X	X	X	X	X	X	X
Spectacles	X	X	X	X			
Building of special storage units						X	
Hired labor						X	
Cost per year (US$ 2013)							
Average cost per farmer	7.09	11.67	6.75	4.88	16.52	34.1	39.26–78.85
Overall cost at national level	–	–	–	–	971,116–3,937,820	1,728,000–10,379,000	–

In Nepal, defensive expenditures accounted for about 15 % of the total cost of pesticide use and 27 % of pesticide expenditure, i.e. the amount spent on purchasing pesticides in a year. Defensive expenditures were slightly higher (Atreya 2008) or slightly lower (Atreya et al. 2012, 2013) than the cost-of-illness, but essentially of a similar magnitude. In Sri Lanka, Athukorala et al. (2012) found these costs to be one quarter those for medical expenditure and one seventh the loss of earnings; cost-of-illness was thus 11 times higher than defensive expenditures (Wilson 1999a). Nevertheless, in this country, annual defensive expenditures corresponded to 12 % of the monthly income of a farmer (Athukorala et al. 2012; Wilson 1999a, 2000b, 2003, 2005). These costs, although low, could be a significant burden to farmers, whose incomes fluctuate greatly, due to adverse biotic, e.g. pest and disease damage, and abiotic, e.g. weather conditions, crop price fluctuations, conditions.

Several types of defensive expenditures have not been considered, probably due to data, time and financial constraints. The elements not analyzed include the purchase of more expensive sprayers less likely to malfunction and place the user at risk of exposure. They also include the time spent purchasing, cleaning and fixing defensive/protective equipment, and reading '*warnings and instructions*'. Precautionary drug treatment to protect against pesticide exposure and leisure time given up in favor of aversive behavior should also be taken into account. The estimates to date therefore almost certainly constitute the lower limit of the range of actual defensive expenditures paid by farmers to reduce their exposure to pesticides.

Moreover, in developing countries, these costs could probably be increased to levels much higher than those currently observed, as pesticide users often adopt few protective measures (Food and Agriculture Organization 2011b). Spraying is sometimes carried out without protection and even those farmers who do try to protect themselves generally limit this protection to the wearing of long-sleeved shirts and long pants. Low levels of income, awareness and education, the hot and humid climate, cultural taboos, fashion and discomfort are significant factors accounting for the lack of personal protection (Atreya et al. 2013) (Fig. 2.1).

Sivayoganathan et al. (1995) reported that some Sri Lankan farmers were keen to use protective measures but did not do so due to cultural taboos, such as wearing shoes in the field. The field is seen as a sort of "temple" because the land within it produces food. Another cultural taboo mentioned concerned the wearing of long pants during pesticide applications, which many farmers, especially the elderly, were reluctant to do, due to their low socioeconomic status.

Finally, not only might farmers be unable to afford adequate precautionary/defensive measures, but the protective gear required may be unavailable as it may not be sold by any shop to which the farmer has access. Hence, defensive expenditures have never been correctly counted, both because the actual expenses were not fully estimated and because they could potentially be much higher than they currently are, particularly in developing countries.

2.7.2 Defensive Expenditures for Safe Drinking Water

The presence of pesticides in tap water may be one of the key reasons for consumers buying bottled water or drinking purified or filtered water. These sources of water are much more expensive for the consumer than tap water. The excess costs of purified or bottled water over tap water could be considered as both a private cost borne by farmers if they drink such water and as an external costs to non-farming consumers buying such water. The production and transportation of bottled water also require the consumption of massive amounts of fossil fuels (Gleick and Cooley 2009). Finally, the bottles degrade slowly, and their incineration can produce toxic byproducts. Bottled water thus has an environmental impact between 90 and 1000 times greater than that of tap water (Jungbluth 2005). The resulting pollution can be considered as a negative externality for society as a whole. However, if the production, transportation and purchase of bottled water and all devices for water purification or filtration are to be considered as defensive expenditures, and hence as external costs, these expenditures should be made specifically to protect against pesticide residues. This relationship is anything but simple.

Consumers choose to drink bottled, purified or filtered water for two main reasons: because they think this water tastes better and/or is safer than tap water (Doria 2006; Doria et al. 2009; Dupont et al. 2010). Several factors are known to influence the public perception of drinking water quality: organoleptic properties, risk perception, attitude towards water chemicals, past problems attributed to water quality, trust in water companies, information from the mass media and family members (Doria 2010). Hence, the presence of pesticides, whether real or imagined, in tap water may be only one of a number of factors pushing people to buy bottled water and/or to drink purified or filtered water. Unfortunately, we were able to identify no study specifically exploring this question. Studies on factors influencing drinking behavior have considered chemical pollutants either as a general entity, i.e. with no specification of the type of chemical substance (e.g. Auslander and Langlois 1993), or have concentrated on lead, chlorine and/or water hardness, e.g. the survey of Statistics Canada (2009), which specifically mentioned chlorine. Pesticides, like other chemical substances including fluoride, nitrates, heavy metals and industrial chemicals, are sometimes specified, but, according to Doria (2010), their relevance to the perception of drinking water safety appears to be very limited or restricted to specific locations.

No specific data are available for pesticides, but several studies have explored the influence of chemicals on the water-drinking behavior of consumers, notably in Canada. In Toronto, 73 % of those questioned felt that tap water contained "some" or "a lot" of chemical pollutants, but half the households overall rated this source of water as "good" or "very good" (Auslander and Langlois 1993). In a more recent national survey of a representative sample of 1633 Canadians, 62 % felt that tap water posed no problem for health (Dupont et al. 2010). Only 12 % and 3 % believed that this source of water posed moderate or serious problems for health, respectively. In their study focused in one Canadian province, McLeod

et al. (2014) also found that no more than 12 % of the 2000 respondents believed tap water to be unsafe to drink. Noteworthy, those respondents who believed tap water to be unsafe appeared more likely to choose bottled water (McLeod et al. 2014). In other countries with reliable supplies, surveys generally indicate that most people perceive the risk associated with drinking tap water to be small (Doria 2006). In low- and medium-income countries, in which tap water quality is often poorer, surveys of the motives for choosing bottled water over tap water have not been performed. However, in such countries, the average per capita consumption of bottled water is low.

In conclusion, the extra cost of drinking bottled, purified and filtered waters, rather than tap water, cannot be firmly attributed to the presence of pesticides. Of course, consumers indirectly pay for the monitoring and elimination of pesticides from the tap water they use, as these costs are passed on by water companies, through the billing process. We decided to count these costs as regulatory rather than as defensive expenditures because, as indicated in Sect. 2.4, monitoring and decontamination processes are mandatory in most countries: see the United States Safe Drinking Water Act (http://water.epa.gov/lawsregs/rulesregs/sdwa/index.cfm), for example.

Pesticides trigger defensive expenditures when they are detected in tap water at levels beyond the threshold considered acceptable, thus causing a decrease in quality. The monitoring of private wells, which are generally not regulated by public authorities, and the use of filtering/purifying devices for detecting and eliminating pesticides from these wells can also be considered as defensive expenditures.

Water quality violations may trigger aversive behavior, such as the purchase of bottled water. When such violations are due to pesticide contamination (e.g. Zaki et al. 1982), the increased in the purchase of bottled water in the area concerned may be considered defensive expenditures. Zivin et al. (2011) estimated that, in 2005, United States citizens spent US$47.15 million (2005) in response to element/chemical violations of water quality. They indicated that this estimate probably constituted the lower limit of the cost of defensive expenditures, because they only considered bottled water consumption and did not include other responses to violations, such as purchasing alternative beverages, e.g. juice, other actions people may have taken, e.g. boiling water, and more permanent responses, e.g. installing water filters. Zivin et al. (2011) did not provide details of the elements/chemicals responsible for the quality violations. We know only that they did not include nitrate, which was counted separately. It is therefore difficult to determine what proportion of the costs corresponded to pesticide contamination. Similarly, Dupont and Jahan (2012) estimated that Canadian households spent almost US$600 (2010) per year on tap water substitutes (purchase of bottled water and devices for filtering/purifying tap water), to decrease the perceived health risks associated with tap water consumption. Unfortunately, the influence of pesticides on this perception was not investigated.

The second type of defensive expenditures concerns the monitoring and decontamination of private wells and small-scale public systems. As indicated above, in the United States, state and federal authorities do not generally regulate these sources of drinking water. The householders concerned therefore pay for the detec-

tion of pesticides in these wells and their elimination. In the United States, 15 million households regularly obtain drinking water from their own private wells (United States Environmental Protection Agency 2002) and the groundwater in those wells may be contaminated with pesticides, particularly in rural areas (Toccalino et al. 2014). Pesticides, such as atrazine, deethylatrazine, simazine, metolachlor, and prometon are, indeed, regularly detected in groundwater and wells (Goss et al. 1998; Hallberg 1989; Ritter 1990, 2001; Toccalino et al. 2014). However, pesticide concentrations in North American domestic wells were found to be generally low. In Ontario, for instance, only six of the 1292 water-wells surveyed contained pesticide residues at concentrations above the maximum acceptable value (Goss et al. 1998). Similar findings were reported for the United States: for the 1993–2011 period, pesticide concentrations exceeded human-health benchmarks in only 1.8 % of the 2541 samples collected from 1271 wells in well networks distributed nationwide (Toccalino et al. 2014). However, pesticide contamination rates and concentration may reach higher values in some countries. In the Netherlands, several pesticides were detected in 27 % of groundwater samples taken from 771 monitoring wells. In 11 % of these samples, the concentration exceeded the upper regulatory limit (Schipper et al. 2008).

Worldwide, the most important contaminant of groundwater and private wells, in terms of health concerns, is arsenic (Nordstrom 2002). Arsenic contamination may have diverse sources, some of which are entirely natural, as in Bangladesh (Nickson et al. 1998). However, arsenic contamination may also result from local anthropogenic activities, such as mining (Mukherjee et al. 2006). In Canada and the United States, significant amounts of arsenic contamination result from the use of arsenic-based pesticides (Smedley and Kinniburgh 2002; Wang and Mulligan 2006).

According to the Massachusetts Department of Environmental Protection, testing a well for arsenic costs US$15 to US$30. Treatment systems for removing arsenic (reverse osmosis, activated alumina) cost at least US$400 per year (Sargent-Michaud et al. 2006). In addition to the costs of monitoring and testing, the presence of arsenic may also increase the consumption of bottled water (Jakus et al. 2009). As arsenic comes from diverse sources, which may vary over space and time, it is not easy to evaluate defensive expenditures due to arsenic-based pesticides. However, in the United States, where 15 million households regularly obtain drinking water from their own private wells, this cost might reach several hundred million US$ per year.

2.7.3 Defensive Expenditures to Avoid Pesticide Residues in Food: The Purchase of Organic Food

Consumers choose to purchase organic food for several reasons, some of which are linked to the externalities of pesticides and to a demand for pesticide-free food (Fotopoulos and Krystallis 2002; Misra et al. 1991; Squires et al. 2001; Tsakiridou et al. 2008; Williams and Hammitt 2001) (Fig. 2.6). Most consumers of organic

Fig. 2.6 Consumers choose to purchase organic food for several reasons, but partly as a consequence of the perceived negative risk of pesticides to the environment and to the consumer. The world market for organic food has grown considerably over the last 15 years: it almost tripled between 2000 and 2008 and continued to grow thereafter, from US$50 billion in 2008 to US$64 billion in 2012 (Sahota 2014). Assuming that prices in this market are 20 % higher than those of conventional food and that about 50 % of the reasons for consumers choosing organic food are directly linked to the avoidance of pesticide risk (e.g. Schifferstein and Oude Ophuis 1998), then the added cost of pesticide use may be about US$6.4 billion (2013) worldwide (Unmodified USDA photography courtesy of Sam Jones-Ellard, under creative common license CC BY (https://creativecommons.org/licenses/by/2.0/))

food declare that the main reasons for this choice are connected to personal health and the avoidance of environmental damage (e.g. Huang 1996; Hughner et al. 2007; Magnusson et al. 2003; Saba and Messina 2003; Schifferstein and Oude Ophuis 1998; Schlegelmilch et al. 1996; Squires et al. 2001; Tregear et al. 1994; Wier et al. 2008). In Greece, about 90 % of general consumers consider organic food to be healthier than conventionally farmed food, and 75 % think that it is better for the environment; even higher percentages were recorded among the consumers of organic food (Tsakiridou et al. 2008). Animal well-being, taste or simply fashion are other factors less frequently proposed by consumers to explain their choices (Pearson et al. 2011). Parents of young children and babies are among those most likely to consume organic food, as a proactive measure, to prevent health problems (Pearson et al. 2011). Another reason cited for buying organic food is also linked to

health, with some ill individuals choosing to buy organic food because they hope that it will help them to recover more rapidly (Pearson et al. 2011). Health is thus a key motive behind organic food consumption. Another reason often given for purchasing organic food is that it decreases damage to the environment, and this idea is generally supported by scientific evidence (e.g. Mäder et al. 2002; Gomiero et al. 2011). Buying organic food is thus partly a consequence of the perceived negative risk of pesticides to the environment and to the consumer.

Organic food consumption can thus be considered, at least in part, as an externality of pesticide use if organic food is more expensive than non-organic, conventional foods. Comparisons of the organic and conventional food markets show that organic food is generally more expensive than conventionally produced food (e.g. Bonti-Ankomah and Yiridoe 2006). The excess cost of organic food varies considerably between countries and products (Bonti-Ankomah and Yiridoe 2006) and is dependent on several factors. However, according to several studies, the lower limit for this price premium would lie somewhere between 10 % and 20 % (e.g. Bonti-Ankomah and Yiridoe 2006; Rodríguez et al. 2008), although price premiums of between 50 % and more than 100 % were reported in the United States in 2013 for fruits and vegetables, respectively (see the web page on Organic prices of the United States Department of Agriculture Economic Research Service: http://www.ers.usda.gov/data-products/organic-prices.aspx#.VAmF0mTV_sk). This price premium, paid by the consumers of organic food thus corresponds, at least in part, to the consumers' willingness to pay for avoiding pesticide risks (Onozaka et al. 2006) and, more precisely, to the hedonic estimation of willingness to pay for a reduction of the presence of pesticides in food. The range of values for the mean price premium of organic food has been confirmed by studies of the willingness to pay for organic food carried out with the contingent valuation technique. Consumers were asked to set a value on the premium they would be prepared to pay for organic food rather than conventionally produced food. These studies also highlighted considerably variability in the responses obtained (e.g. Zehnder et al. 2003; reviewed by Bonti-Ankomah and Yiridoe 2006), but they frequently suggested that the minimum value was about 10–20 % (e.g. Bonti-Ankomah and Yiridoe 2006; Gil et al. 2000; Onozaka et al. 2006; Rodríguez et al. 2008).

The worldwide organic food market was of the order of US$64 billion in 2012 (Sahota 2014), equally split between Europe (US$29 billion) (Schaack et al. 2014) and the United States (US$29 billion) (Fitch Haumann 2014). In Europe, the organic food market in 2012 represented about US$9 billion in Germany, US$5 billion in France and US$2.5 billion in the United Kingdom (Schaack et al. 2014). The world market for organic food has grown considerably over the last 15 years: it almost tripled between 2000 and 2008 and continued to grow thereafter, from US$50 billion in 2008 to US$64 billion in 2012 (Sahota 2014).

If we assume that prices in this market are 20 % higher than those of conventional food and that about 50 % of the reasons for consumers choosing organic food are directly linked to the avoidance of pesticide risk (e.g. Schifferstein and Oude Ophuis 1998), then the added cost of pesticide use is about 10 % of the total market value of organic food. This amounts to US$2.9 billion for the United States and

Europe, and about US\$0.9 billion for Germany, US\$0.5 billion for France, and US\$0.25 billion for the United Kingdom. Griffith and Nesheim (2008) used hedonic prices and purchase quantities for 2003 and 2004 in the United Kingdom to estimate the aggregate lower limit of willingness to pay for organic products. They obtained a value of about 22 % of the annual expenditure on organic products, corresponding to about US\$0.55 billion, based on the figures obtained for the organic market in the United Kingdom in 2012. Griffith and Nesheim (2008) estimated that about 20 % of the lower limit of the willingness to pay was directly linked to health and environmental concerns – about US\$110 million, corresponding to 44 % of our estimate of US\$0.25 billion.

2.7.4   Conclusion

Defensive expenditures have rarely been considered among the external and "hidden" costs of pesticide use. For instance, we found no study considering the defensive expenditures of both farmers and consumers. In particular, the consumption of organic food as a defensive action against pesticide residues has never been fully considered as a negative externality of pesticide use. Indeed, all studies to date on the economics and rationale of organic food consumption have been completely disconnected from studies analyzing the benefit-cost ratio of pesticide use.

In general, aversive actions have been little studied and, when considered, they have generally been restricted to the protection of the body and respiratory system by farmers handling or applying pesticides. However, these costs are only part of the costs directly borne by farmers.

Furthermore, aversive actions could be carried out on a much wider scale than is currently the case. This is certainly true for protective clothing, which is rarely worn by farmers in most developing countries, and for the monitoring and decontamination of drinking water. If all owners of private wells carried out monitoring and were equipped with a filter/purifier, or if the consumption of bottled water continues to grow, then defensive expenditures to avoid residues in drinking water could rise exponentially. However, it should be borne in mind that these costs are somewhat linked to cost-of-illness. If tap water contains pesticide residues at levels that may injure human health, then an increase in defensive expenditures should lead to a decrease in cost-of-illness. Put another way, some of the current cost-of-illness could be due to a lack of aversive action. Alternatively, an increase in defensive expenditures might decrease the overall cost of pesticide use if these additional defensive expenditures are overcompensated by the decrease in cost-of-illness they trigger. Similarly, an increase in the consumption of organic food might decrease the cost-of-illness by reducing chronic illness although the relationship between exposure to low pesticide doses and chronic illnesses remains very difficult to quantify.

Here, we show that defensive expenditures have rarely been considered in the literature of pesticide use cost. These costs include at least the extra cost of organic food consumption due to aversive behavior linked to pesticide use. This cost reached more than US\$6.4 billion worldwide in 2012.

2.8   Overall Hidden and External Costs

Pesticide use has a marked positive impact on agriculture (Cooper and Dobson 2007; Gianessi 2009; Gianessi and Reigner 2005, 2007) and human health (Cooper and Dobson 2007). However, as highlighted above, it also has a significant negative impact on the environment and on human health, and entails economic costs linked to regulations and defensive actions. It is therefore worthwhile estimating the global cost of pesticide use, for comparison with the economic benefits, with a view to re-evaluating the overall economic balance of pesticide use (see Sects. 2.9.1 and 2.9.2). This is a prerequisite for the evaluation of public policies concerning pesticide use, including the reduction of pesticide use (e.g. Barzman and Dachbrodt-Saaydeh 2011; Löfstedt 2003). Unfortunately, several current policies relating to the reduction of pesticide use are based on estimates that do not consider the global cost of pesticide use, including external costs, but only the benefits in terms of agricultural production, e.g. the Ecophyto 2018 plan of the French government, which aims to halve pesticide use over a 10-year period (Jacquet et al. 2011). In evaluations of the consequences of regulations aiming to decrease pesticide use, very different conclusions may be reached depending on whether the global costs of pesticide use are (Pimentel et al. 1993b; Pimentel 2005; Pimentel and Burgess 2014) or are not (Gianessi 2009; Gianessi and Reigner 2005, 2007; Jacquet et al. 2011) taken into account. This section reviews the few studies that have tried to estimate the overall hidden and external costs at national level. We will see that such costs are underestimated and that the available estimates are out-of-date. By comparing different datasets and estimating the specific costs that were not estimated in previous studies, we tried to perform a more complete evaluation of the hidden and external costs of pesticide use in the United States at the beginning of the 1990s.

2.8.1   A Small Numbers of Estimates

We found only ten independent groups of papers combining estimates of regulatory, environmental and human health costs at the national level. These groups of studies are those of Ajayi et al. (2002) for Mali, Houndekon and De Groot (1998) and Houndekon et al. (2006) for Niger, Jungbluth (1996) and Praneetvatakul et al. (2013) for Thailand, Khan et al. (2002) for Pakistan, Pimentel and coworkers (Pimentel et al. 1980a, b, 1991a, b, 1992, 1993a, b; Pimentel and Greiner 1997; Pimentel and Hart 2001; Pimentel 2005; Pimentel and Burgess 2014), Steiner et al. (1995) and Tegtmeier and Duffy (2004) for the United States, Pretty et al. (2000, 2001) for the United Kingdom, and Fleischer and coworkers (Fleischer 1999; Waibel and Fleischer 1998; Waibel et al. 1999) for Germany.

These articles revealed considerable heterogeneity for overall hidden and external costs, which ranged from US$5.4 million (2013) in Niger in 1996 (Houndekon and De Groote 1998; Houndekon et al. 2006) to US$13.6 billion (2013) in the

United States in 1992 (Pimentel et al. 1992, 1993a, b) (Table 2.12). For the United States, the estimates of Pimentel and coworkers also varied over time. They reported overall hidden and external costs of US\$2.7, 3.7, 13.6, 11.8 to 12.1 and 11.7 billion (2013) in 1980, 1991, 1992, 1997 and 2005, respectively (Table 2.12). These differences mostly reflected differences in the types of costs taken into account. Hence, from 1991, Pimentel and coworkers included the cost of monitoring wells and groundwater, accounting for 55 % of the external costs. From 1992, they also estimated the cost of bird losses, accounting for 25 % of the external costs, and re-evaluated the cost of pesticide resistance from about 7–17 % of the external costs.

2.8.2   Overall Costs Are Underestimated

The overall hidden and external costs reported above are underestimated for two reasons. First, none of the available estimates include defensive expenditures (Table 2.13). Second, as shown above, they did not take into account some, or even in some cases most of the specific costs within the other three cost categories, i.e. environmental impact, human health and regulatory actions (Table 2.13). For instance, losses of reptiles, amphibians, soil and aquatic communities and wild vertebrates other than birds and fish have never been evaluated (Table 2.13). Similarly, the costs of the human health impact of pesticide use have not been fully explored. Pimentel et al. estimated the costs of cancer treatment, but they did not calculate the cost of deaths due to these cancers (Table 2.13). Finally, none of the estimates took into account major environmental disasters associated with pesticide production and disposal sites. The dramatic pesticide industry accidents at Bhopal in India (Mishra et al. 2009) (Fig. 2.7) and Seveso in Italy (Consonni et al. 2008), together with less severe incidences, such as the James River kepone disaster in the United States (Huggett and Bender 1980), caused thousands of deaths and long-term disorders in humans, together with damage to the soil, animals and plants that could probably be estimated at several billion of US\$.

   This bias towards an underestimation of external costs is not related to a lack of rigor on the part of the authors conducting these studies. Instead, it results principally from the difficulties involved in estimation of the economic costs of the unintentional impacts of pesticide use, particularly for goods without market values. Indeed, Pimentel and Greiner (1997) pointed out that the scarcity of data made their assessments of the external costs inaccurate, such that the costs themselves had to be considered incomplete. Hence, as indicated by Waibel et al. (1999), most estimates of external costs performed to date must be considered as minimum costs.

Table 2.12 Overall hidden and external costs of pesticide use. DE corresponds to defensive expenditures

Reference	Country	Year	Costs in million US$ 2013 (per year)				Overall hidden and external costs
			Human health impacts	Regulatory actions	Environmental impacts	DE	
Ajayi et al. (2002)	Mali	1999	3.71	1.58	38.11	NE[a]	43.40
Houndekon and De Groote (1998); Houndekon et al. (2006)	Niger	1996	4.40	0.15	0.89	NE	5.44
Jungbluth (1996)	Thailand	1995	1.26	558.33	5.58	NE	565.17
Khan et al. (2002)	Pakistan	2002	78.06	9.71	815.12	NE	902.89
Pimentel et al. (1980a, b)	United States	1980	581.18	491.96	1621.17	NE	2694.31
Pimentel et al. (1991a, b)	United States	1991	439.32	2372.34	948.94	NE	3760.60
Pimentel et al. (1992, 1993a, b)	United States	1992	1326.45	4319.01	7967.84	NE	13,613.30
Pimentel and Greiner (1997); Pimentel and Hart (2001)	United States	1997	1367.04	3451.19–3751.06	6993.99	NE	11,812.22–12,112.09
Pimentel (2005, 2009); Pimentel and Burgess (2014)	United States	2005	1492.96	4229.13	5973.50	NE	11,695.59
Praneetvatakul et al. (2013)	Thailand	2010	2.99	357.28	16.88	NE	377.15
Pretty et al. (2000, 2001)	United Kingdom	1996	2.30	318.51	62.74	NE	383.55
Steiner et al. (1995)	United States	1986	246.29–486.51	3203.00	203.85–4029.46	NE	3653.14–7718.97
Tegtmeier and Duffy (2004)	United States	2002	1308.89	4988.69	1469.74–1507.62	NE	7767.32–7805.20
Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Germany	1996	17.99	168.26	9.31	NE	195.56

[a]NE not estimated

Table 2.13 Cost taken (green) or not (red) into account in the estimates of the overall cost of pesticide use

Column headers (left to right):
- Ajayi et al. 2002
- Houndekon and De Groot 1998; Houndekon et al. 2006 (a)
- Jungbluth 1996
- Khan et al. 2002
- Pimentel et al. 1980a, 1980b
- Pimentel et al. 1991a, 1991b
- Pimentel et al. 1992, 1993a, 1993b
- Pimentel and Greiner 1997; Pimentel and Hart 2001
- Pimentel 2005, 2009; Pimentel and Burgess 2014
- Praneetvatakul et al. 2013
- Pretty et al. 2000, 2001
- Steiner et al. 1995
- Tegtmeier and Duffy 2004
- Fleischer 1999; Waibel and Fleischer 1998; Waibel et al. 1999

Category of cost

Regulatory actions
- *Pesticide registration, regulation and market monitoring*
- *Public awareness campaigns on pesticide impact*
- *Disposal of obsolete and leftover pesticides*
- *Farm work, mandatory safety*
- *Control & monitoring*
  - Crop and/or food
  - Water (surface, underground and/or wells)
  - Livestock
  - Wildlife
  - Undefined
- *Water decontamination*
- *Public research on pesticides*
- *Extension services*
- *Economic shortfall*
  - Crop
  - Water
  - Livestock
  - Milk
  - Fishing

Human health impacts
- *Acute poisoning*
  - Medical care
  - Loss of work
  - Other indirect costs
  - Cost of fatal cases
- *Chronic poisoning*
  - Medical care
  - Loss of work
  - Other indirect costs
  - Cost of fatal cases

Environmental impact
- *Damage to animals, plants, algae and microorganisms*
  - Crops/cultivated plants/trees
  - Wild plants (other than weeds)
  - Domestic animals and livestock
  - Fish
  - Birds
  - Wild vertebrates (other than birds and fish)
  - Bees
  - Natural enemies
  - Invertebrates (other than bees and natural enemies)
  - Soil community
  - Aquatic communities (other than fish)
- *Pest resistance to pesticides*

Defensive expenditures (DE)
- *DE for pesticide handling and spraying*
- *DE for safe drinking water*
- *Purchase of organic food*

[a]Houndekon et al. (2006) took chronic poisoning partly into account in their estimates, but it is impossible to know the extent to which this was done. Indeed, they merely asked farmers to state how much money they spent on medication, consultations and loss of working days during the year, without specifying the type of health effect (acute or chronic, and, for chronic effects, the corresponding illnesses)

Fig. 2.7 The Abandoned Union Carbide Pesticide Plant, Bhopal, India. This production site gave probably the most dramatic pesticide industry accidents of the history (Mishra et al. 2009). This disaster led to the death of several thousands of people and induced long-term disorders in humans, together with damage to the soil, animals and plants that could probably be estimated at several billion of US$ (Unmodified photography by Bhopal Medical Appeal, under Creative Common License CC BY-SA (https://creativecommons.org/licenses/by-sa/2.0/))

2.8.3   A Re-evaluation of the Overall Costs for the United States at the Start of the 1990s

As authors sometimes evaluate different impacts, we felt that it would be possible to perform a more complete evaluation of the external cost of pesticide use in the United States at the start of the 1990s (Table 2.14). For this purpose, we used the estimates of Pimentel et al. (1992), but we (i) actualized some external costs already estimated by these authors, e.g. honeybee and pollination losses, (ii) corrected some of their costs by taking additional data into account, e.g. bird losses, (iii) included several costs that were not evaluated by Pimentel et al. (1992), e.g. deaths due to chronic poisoning, the purchase of organic food, and (iv) removed costs that were theoretical rather than actual, e.g. wells and groundwater monitoring and decontamination, economic shortfall due to crop contamination and the disposal of contaminated crops. We ended up with a cost of US$35.2 billion (2013) (Table 2.14), a value 2.5 times higher than the original value of US$13.6 billion (2013) estimated by Pimentel et al. in 1992 (Table 2.12).

This new estimate is more complete, but it remains conservative because a number of costs, e.g. the loss of reptiles, amphibians, soil and aquatic communities and

wild vertebrates other than birds and fish, the costs of acute and chronic poisoning, the purchase of bottled water and purifying devices to protect consumers against pesticide exposure, are still not included. In addition, we decided to remove from the overall sum the costs of monitoring and decontaminating wells and groundwater, and the economic shortfall due to crop contamination (Table 2.14). We removed these economic shortfalls due to crop contamination because they were conditional on the absolute respect of United States regulations, which would be unrealistic (Pimentel 2005). Pimentel et al. (1992) calculated the cost of monitoring and decontaminating all wells and groundwater, even though these activities were not actually carried out. As indicated above, it should be borne in mind that some of the human health costs to society would disappear if all wells and groundwater were effectively cleaned. If we take some of these costs into account, the overall costs would probably have been between US\$35.2 billion and US\$39.5 billion (2013) at the end of the 1980s/start of the 1990s.

2.8.4   Most, If Not All Overall Costs Are Out-of-Date

The articles reviewed here were retrieved from more than 30 years of studies on the costs of pesticide use. Over this period, there has been a massive, rapid change in pesticide use, as a consequence of changes in governmental legislation, i.e. the establishment of higher standards for pesticide registration, and efficiency issues, i.e. due to the exponential increase in pesticide resistance within pest and pathogen populations. This has led to a change in the panel of active ingredients used, which is currently very different from that employed 10, 20 or 30 years ago. DDT, one of the most noxious pesticides ever used, was one of the first agents to be banned, initially in the United States in 1972, and then in most other countries. In Europe, as in the United States, older pesticides are being reassessed one-by-one, to ensure that they meet the new regulatory standards (Damalas and Eleftherohorinos 2011). This re-registration process has already resulted in a substantial decrease in the number of pesticides available on the market: in an 8 year period (2001–2008), 704 pesticides were banned in Europe, 26 % of which were insecticides, 23 % herbicides and 17 % fungicides (Karabelas et al. 2009). Of the 276 pesticides authorized for use in Europe in 2009, 194 existed before 1993 and 82 had been released onto the market in the last 20 years (Karabelas et al. 2009). However, two factors may limit the benefits expected from prohibition of the most dangerous active ingredients. First, resistance to pesticides has resulted in the need for higher doses to be applied. Second, pesticides are sometimes used after they are banned (Shetty et al. 2011).

In any case, the current impact of pesticides is necessarily different from that in the past. Hence, while reporting the impact of insecticide use on the decline of many grassland birds in the United States, Mineau and Whiteside (2013) wrote that their '*analysis considered bird trends from 1980 to 2003*' and that '*there is evidence that the acute lethal risk to birds was already dropping during the second half of that period*'. Indeed, Mineau and Whiteside (2006) noted that '*the lethal risk to birds*

Table 2.14 Re-evaluation of the overall hidden and external costs of pesticide use in the United States

	In million US$			
	Original estimate	Year of estimate	Updated estimates (2013)	Reference
Cost				
Human health				
Acute health effect (treatment plus loss of work)	61	1988	123	Steiner et al. (1995)[a]
Chronic (treatment of cancer)	707	1992	1192	Pimentel et al. (1992)
(loss of work for the person with cancer)	–	–	87	Own calculations[b]
Death due to acute poisoning	–	–	405	Own calculations[c]
Death due to chronic poisoning	–	–	18,000	Own calculations[d]
Environmental impact				
Domestic animal and livestock death	30	1992	51	Pimentel et al. (1992)
Increase in pesticide use due to the destruction of natural enemies	260	1992	439	Pimentel (2005)
Crop losses due to pesticide resistance	1400	1992	2361	Pimentel et al. (1992)
Colony losses due to pesticides	13	1992	22	Pimentel et al. (1992)
Honey and wax losses	25	1992	43	Pimentel et al. (1992)
Loss of potential honey production	27	1992	46	Pimentel et al. (1992)
Bee rental for pollination	4	1992	7	Pimentel et al. (1992)
Pollination losses	200	1992	337	Pimentel et al. (1992)
Crop losses due to pesticide injury	136	1992	229	Pimentel et al. (1992)
Crop losses due to the destruction of natural enemies	260	1992	439	Pimentel (2005)
Insurance of the person applying the pesticide	245	1992	413	Pimentel et al. (1992)
Fishery losses	100	2005	122	Pimentel (2005)
Bird losses	–	–	5903	Own calculations[e]
Re-establishement of endangered birds	102	1992	172	Pimentel et al. (1992)
Regulatory actions				
Monitoring and decontamination of pesticide-polluted groundwater	1800	1992	3036	Pimentel et al. (1992)[f]

(continued)

Table 2.14 (continued)

	In million US$			
	Original estimate	Year of estimate	Updated estimates (2013)	Reference
Pesticide registration, certification, cancellation, training and farm work safety	757	1991	1330	Steiner et al. (1995)[g]
Government funds for monitoring the pesticide contamination of fruits, vegetables, grains, meat, milk, water, and other items	400	2005	486	Pimentel (2005)
Pesticide monitoring in wildlife	5	1980	16	Pimentel et al. (1980a, b)
Economic shortfalls				
Crops	1000	2005	1215	Pimentel (2005)[h]
Livestock	3	1980	9	Pimentel et al. (1980a, b)
Milk	<1	1980	1	Pimentel et al. (1980a, b)
Fish	5	1980	15	Pimentel et al. (1980a, b)
Defensive expenditure				
Purchase of organic food	2900	2012	2961	Own calculations[i]
Overall cost			35,208	

[a]Cost for 1988, see Table 10.3 of Steiner et al. (1995). For the cost in 2013, we considered the lower limit of 61 million dollars in 1988

[b]Based on 10,000 cases of cancer per year (Pimentel 2005) and 3 months (90 days) of recuperation per person with a cost per day of recuperation = $80 in 2005 (Pimentel 2005)

[c]Based on 45 deaths per year (Pimentel 2005) and a cost of 9 million US$ per life in 2013 (Viscusi et al. 2014)

[d]Based on 10,000 cancers per year (Pimentel 2005), a mortality rate of 20 % amongst individuals with cancer (Siegel et al. 2014) and a cost of life of US$9 million per life in 2013 (Viscusi et al. 2014)

[e]Based on 100 million bird deaths annually (see Mineau 2005), with a cost of 30 dollars per bird (Pimentel et al. 1992). This price relates purely to recreational value. We can add a value of 5 dollars for the protection against insects provided by the birds lost (see James 1995). Hence, the cost in 1992 would be $100 \times 35 = US3.5 billion

[f]Assuming that monitoring and decontamination were actually carried out. Theoretical rather than actual cost. Not included in the overall cost

[g]The original estimate is for 1991, but expressed in 1986 US$ (see Table 10.1 in Steiner et al. 1995)

[h]Assuming that all the crops and crop products exceeding the regulatory thresholds were disposed of. Theoretical rather than actual cost. Not included in the overall cost

[i]Considering that the United States organic food market represented US$29 billion in 2012 and assuming that prices in this market are 20 % higher than the price of conventional food and that about 50 % of the incentives of consumers to buy organic food are directly linked to pesticide risk avoidance (e.g. Schifferstein and Oude Ophuis 1998). See Sect. 2.7.3

has generally declined over the last decade in most crops /…/ The reasons for this improvement vary from crop to crop, but usually entail the replacement of older more hazardous products with newer ones with lower acute toxicity to birds'. The ban on granular formulations of carbofuran introduced in 1991 (Heier 1991) and effective by 1994, in particular, probably had a considerable beneficial effect on bird survival in farmland. The estimate of 17–91 million birds killed per year during the 1980s was therefore almost certainly, as stated by Mineau (2005), the ''*worst-case' impact of pesticides on birds in an agricultural setting*'. The current impact of pesticide use on birds is probably much lower.

The cost of the impact of pesticide use on human health may not have decreased in recent years. The trend towards the use of less dangerous chemicals may have decreased the frequency and severity of acute poisoning events. However, the illnesses resulting from chronic exposure, such as cancers in particular, may take years to appear. As an example, Cohn et al. (2007) showed that DDT exposure in young women during the period of peak DDT use in the United States predicts breast cancer later in their life (Cohn et al. 2007). Cohn et al. (2015) also showed that a larger exposition to DDT in utero is associated with an increased risk of breast cancer in adult women. As the authors stated, these findings are relevant "*even in countries in which DDT is not currently used*". This delayed effect is reinforced by the fact that "*DDT remains a global environmental contaminant, even in places where it has been banned, due to its environmental persistence and semivolatility*". Illnesses due to chronic exposures may therefore occur long after the chemicals that played an active role in triggering them have been banned. This time lag effect may have resulted in such illnesses being more frequent and, thus, more costly now than they were in the past. Similarly, most of the benefits to human health of the current process of pesticide re-registration may not appear for some time.

Our synthesis shows that overall hidden and external costs ranged from US$5.4 million (2013) in Niger in 1996 to US$13.6 billion (2013) in the United States in 1992 and were strongly underestimated. Performing an updated and more complete evaluation of these costs in the United States at the start of the 1990s, we show that overall hidden and external costs probably reached the value of US$39.5 billion (2013) per year.

2.9   Conclusions and Perspectives

2.9.1   Benefit-Cost Ratio Analysis of Pesticide Use: A Necessary…

The use of pesticides is economically justified if the benefit-cost ratio of pesticide use is greater than 1, indicating that the benefits are greater than the costs. The issue of how to measure pesticide productivity has been addressed in a large number of articles within the field of agricultural economics, although most did not consider

the externalities of pesticide use. Fernandez-Cornejo et al. (1998) reviewed the estimates of the marginal product of pesticide use (the product obtained from one additional unit of pesticide use expressed in $/$ pesticide expenditure). These estimates, obtained between 1963 and 1991, were highly variable, ranging from less than 1 to more than 10 and tending to decrease over time, with a mean value, since the 1980s, of about 4. All the papers by Pimentel and coworkers were based on these estimates (those of Headley 1968) and took into account a benefit-cost ratio of 4. This value has become the most widely cited benefit-cost ratio for pesticide use. Yancy (2005) proposed a benefit-cost ratio of about 3 for herbicide use. In their highly cited paper published in *Science*, Zilberman et al. (1991) noted that '*a $1 increase in aggregate pesticide expenditures has been estimated to raise gross agricultural output from $3 to $6.50*'. Based on the estimated benefits of pesticide use calculated by Gianessi (2009) and Gianessi and Reigner (2005, 2007), Popp (2011) proposed a benefit-cost ratio of about 6.5.

However, this ratio did not include the external and hidden internal costs of pesticide use reviewed above. Any fair calculation of this ratio must include not only the usual internal costs to farmers (pesticide market costs and application costs), but also the external costs and hidden internal costs corresponding to the "other internal costs" defined in Sect. 2.2 (see also Table 2.1). However, it should exclude the hidden internal costs resulting in either an increase in the usual internal costs, such as costs linked to pesticide resistance, or a decrease in benefits, such as a reduced pollination. Indeed, these last two types of cost are already accounted for in estimates of the usual internal cost of pesticides or the gross value of agricultural production.

Some of the papers estimating the overall costs of pesticide use also provided estimates of the benefits of pesticide use (Khan et al. 2002; Pimentel et al. 1980a, b, 1992, 1993a; Pimentel and Greiner 1997; Pimentel and Hart 2001; Pimentel 2005; Pimentel and Burgess 2014; Waibel and Fleischer 1998). This enabled us to re-evaluate the benefit-cost ratio of pesticide use, by calculating the overall costs to be included in this ratio as the sum of the usual internal costs, the hidden internal costs generating "other internal costs" and external costs. The resulting ratios are given in Table 2.15.

In most cases, the ratio was higher than 1 (Table 2.15), but some of the ratios obtained were close to 1 (Waibel and Fleischer (1998) for Germany, and Pimentel et al. (1992, 1993a) for the United States) and one was below 1 (Khan et al. (2002) for Pakistan), indicating that overall costs have sometimes outweighed the benefits of pesticide use in agriculture. Hence, Pingali et al. (1994) concluded that '*When health costs are explicitly considered for a risk-neutral farmer, the net benefits of insecticides applied are negative. In other words, the positive production benefits of applying insecticides are exceeded by the increased health costs*'. This may have been the case, even in developed countries. Based on our re-evaluation of the overall costs of pesticide use for the United States in Sect. 2.8.3, the benefit-cost ratio in this country at the start of the 1990s was 0.70 (Table 2.15). In 1992, Pimentel *et al.* concluded '*complete long-term cost/benefit analysis of pesticide use would reduce the*

Table 2.15 Benefits versus costs of pesticide use

| Reference | Country | Year | Costs or benefits in million US$ (2013) per year | | | | Benefit/cost ratio |
			"Usual" internal costs	Other hidden costs and external costs	Total cost	Benefits[a]	
Khan et al. (2002)	Pakistan	2002	532.67	186.38	719.05	610.19	0.85
Fleischer (1999); Waibel and Fleischer (1998); Waibel et al. (1999)	Germany	1996	1309.38	196.46	1505.84	2198.74	1.46
Pimentel et al. (1980a, b)	United States	1980	8855.42	1534.96	10,390.38	34,473.00	3.32
Pimentel et al. (1992, 1993a, b)	United States	1992	6914.00	10,038.01	16,952.01	26,983.00	1.59
Pimentel and Greiner (1997); Pimentel and Hart (2001)	United States	1997	9520.47	8707.08–9006.95	18,227.52–18,527.42	38,080.00	2.06–2.09
Pimentel (2005, 2009); Pimentel and Burgess (2014)	United States	2005	12,152.66	8985.55	21,138.21	48,610.65	2.30
Pimentel et al. (1992) and our calculations[b]	United States	1992	6914.00	31,404.00	38,318.00	26,983.00	0.70

[a]Estimates of the benefits provided in the papers of Pimentel et al. come from a 1:4 ratio of direct costs: benefits (Pimentel et al. 1978; Headley 1968)

[b]The values for "Usual" internal costs and benefits are from Pimentel et al. (1992). The values for the other hidden costs and external costs are from our own calculations (see Table 2.14)

perceived profitability of pesticides'. The re-analysis of their data shows that the profitability of pesticides has, indeed, undoubtedly been overestimated in the past. Hence, pesticide use, at the doses applied, may have entailed costs exceeding the profits generated.

2.9.2 … Yet Difficult Approach

When estimating the benefit-cost ratio of pesticide use, we need to bear in mind the alternative farming system to which pesticide use may be compared. Only benefit or cost items differing between the two types of agriculture should then be considered. For instance, conventional food production with pesticide use is often compared with organic farming, as pesticide use is lower in organic systems. In this review, we decided to include the purchase of organic food in the external cost of pesticide use (see Sect. 2.7.3) because (i) the alternative mode of production is not necessarily organic farming, e.g. it could be farming based on genetically modified crops, and (ii) the price premium of organic food would probably decrease considerably in a totally organic farming system.

The estimates of the benefits used to determine the benefit-cost ratio in the previous section were restricted to internal benefits, i.e. agricultural production. They did not include external benefits, such as reduced morbidity and mortality or a decrease in biological invasions (Felsot 2011). The estimation of external benefits is a difficult task that has been attempted by few authors (but see Felsot 2011). One of the difficulties is that the list of external benefits may, like that of external costs, be very long. For instance, conventional agriculture based on chemical pesticides has a positive effect on the activity of research laboratories in chemistry, the chemical industry, chemical sellers, agricultural advisors specializing in chemical usage, chemical waste disposal and treatment. It even has a positive effect on research into the cost of pesticide use, e.g. such as the analyses on which this review is based and this review itself.

As for costs, the most meaningful way to describe the external benefits of pesticide use is to compare conventional agriculture involving pesticide use with an alternative farming system. Only the benefit items differing between the two types of farming considered should then be compared. For instance, when comparing pesticide use as a tool for integrated pest management or organic farming, food production is often considered to be constant between strategies and is not considered as an adjustment variable. Thus, the external benefits, such as positive health effects linked to sufficient food production, are also common to the different strategies considered. However, other external benefits, such as the positive effects on health of a high sanitary quality of food, side effects on invasion biology, and the positive economic consequences of a developed pesticide industry compared to the developed work force in the field may differ between modes of agricultural production.

2.9.3   Chronic Exposure, Severe Illnesses and Death: The Cornerstones of Externalities

Our literature review provided evidence to suggest that hidden and external costs have been underestimated. The key parameter is probably the cost of illnesses and deaths due to pesticide use, notably due to chronic exposure. The benefit-cost ratio may easily fall below 1 if the costs of chronic illness and acute fatal poisoning events due to pesticide use are taken into account, because human life is clearly of great value. Our re-analysis of the data of Pimentel et al. suggested that each percent of cancers attributable to pesticides was associated with a cost of about 20 billion dollars annually.

Unfortunately, it is very difficult to estimate the cost of chronic diseases. A relationship has been found between exposure to some pesticides over a number of years and several severe illnesses (see Baldi et al. 2013). Several reviews and/or meta-analyses of case-control and/or long-term epidemiological surveys have shown that (i) occupational exposure is associated with an increase in the frequencies of Parkinson's disease (Van Maele-Fabry et al. 2012), amyotrophic lateral sclerosis (Malek et al. 2012), non-Hodgkin lymphoma (Schinasi and Leon 2014), the impairment of several neurobehavioral functions (Mackenzie-Ross et al. 2013), disorders of the reproductive system (notably low sperm concentration and quality) (Martenies and Perry 2013; Mehrpour et al. 2014) and several cancers (Alavanja and Bonner 2012; Alavanja et al. 2013) and (ii) the risks of brain cancer, leukemia and lymphoma in childhood are also significantly associated with parental exposure to pesticides (Vinson et al. 2011; Van Maele-Fabry et al. 2010, 2013).

However, the development of most illnesses, including cancers in particular, is generally multifactorial. Hence, despite the significant association between pesticide exposure and such illnesses, it is difficult to prove a causal effect of pesticides. According to Andersson et al. (2014), the conclusion of Dich et al. (1997) warning that '*few, if any of the associations* (between pesticide exposure and cancers) *can be considered established and causal*' still holds in 2014, for most, if not all long-term human disorders. Even if certain pesticides were clearly proved to be involved in these disorders, their contribution relative to other factors would still be difficult to determine. There may also be a general reluctance of the epidemiologists to compute and publish the health burden attributable to specific factors. Doing so 'takes epidemiologists as impartial scientists and thrusts them more clearly into the political arena of public health' (Steenland and Armstrong 2006). This, together with more technical causes, probably explains why we found no study providing scientifically based estimate of the number of cancers and other severe illnesses that could actually be attributed to pesticide exposure, not only among farmers, but also for the whole population.

In March 2015, the International Agency for Research on Cancer held a meeting in Lyon. This World Health Organization agency concluded that the herbicide glyphosate (Fig. 2.8), the insecticides malathion and diazinon were probably

Fig. 2.8 In March 2015, the herbicide glyphosate – contained in the widely known Roundup herbicide by Monsanto – has been classified "probably carcinogenic to humans" by the International Agency for Research on Cancer (Unmodified photography by Mike Mozart, under Creative Common License CC BY (https://creativecommons.org/licenses/by/2.0/))

carcinogenic to humans and that the insecticides tetrachlorvinphos and parathion were classified as possibly carcinogenic to humans (Guyton et al. 2015).

Based on the increasing body of evidence suggesting a tight association between some cancers and pesticide exposure, attributable risk estimates may be proposed soon. This would make it possible to revise, either upward or downward, the estimate of 0.5–1 % used by David Pimentel and coworkers as the basis of their estimations over the last 35 years. In any case, such data would bring us closer to the actual overall costs of pesticide use and would provide policy makers with tangible elements to guide their decisions.

Meanwhile, our re-evaluation of past benefit-cost ratio of pesticide use in various countries reveals that the costs of pesticide use might have outreached its benefits in the past, e.g. in the United States at the start of the 1990s. We finally advocate that the key impact to be evaluated is the illnesses and deaths due to chronic exposure to pesticides. Taking into account the costs they generate could drastically decrease the benefit-cost ratio of pesticide use. The quantification of this key cost is therefore urgently required for a more accurate evaluation of pesticide use and for regulatory purposes.

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Chapter 3
Cocoa in Monoculture and Dynamic Agroforestry

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Abstract The growing demand for cocoa beans and products worldwide has been met by expanding the area under cocoa production while productivity per hectare has stagnated at a low level of around 450 kg/ha per year in the last decade. Throughout the tropics cocoa has increasingly been cultivated in full-sun monocultures in order to maximize short-term productivity and profitability, which has been associated with soil erosion and degradation, biodiversity loss, as well as increased susceptibility to climate change impacts and pests and diseases. Dynamic agroforestry systems are an alternative production method which has long been practiced in Latin American countries such as Bolivia. Through mimicking natural forests, these systems offer multiple benefits such as soil fertility enhancement, reduction in pest and disease pressure, erosion control, and revenue diversification. In Côte d'Ivoire, where most cocoa is still produced in monocultures, dynamic agroforestry systems were recently introduced on a small scale.

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Here we use different research projects conducted in Bolivia and Côte d'Ivoire as case studies to review productivity, soil fertility as well as pests and diseases in dynamic agroforestry systems and monocultures, and outline factors influencing the adoption of dynamic agroforestry systems from the farmers' perspective. We found productivity under agroforestry systems to be either similar or higher compared to monocultures. We recorded 161 % higher total system yields in an on-station field trial and an on-farm study in Bolivia, and in an on-farm study in Côte d'Ivoire. Cocoa yields were 12–46 % higher in agroforestry systems compared to monocultures. In addition, cocoa in dynamic agroforestry systems exhibited significantly less incidences of witches' broom, *Moniliophthora perniciosa*, compared to monocultures in Bolivia.

Farmers in Bolivia and Côte d'Ivoire observed more soil-related problems and incidences of pests and diseases in monocultures than in agroforestry systems, and they showed high interest to learn dynamic agroforestry management practices. However, adoption was strongly limited to project areas where dynamic agroforestry plots had been installed with farmers' participation. This highlights the importance of local organizations such as Ecotop, Ecosaf, El Ceibo and Biopartenaire Ltd., who implement such interventions on the ground. However, we found that there is space for improvement in the way organizations interact with farmers, especially in Côte d'Ivoire. Interactive knowledge sharing methods such as farmer field schools may help to stimulate farmers' protagonism and give scientists and external consultants the role of facilitators who integrate different forms of knowledge and make them visible to different stakeholders. Such a social learning process requires transdisciplinary research for the development of decision support tools which facilitate the determination of both optimal planting densities and shade levels, as well as adequate combinations of trees and accompanying species in order to achieve effective regulation of pests and diseases while ensuring favourable growing conditions.

Keywords Cocoa • Bolivia • Côte d'Ivoire • Dynamic agroforestry systems • Pests and diseases • Resilience • Participatory on-farm research • Transdisciplinary research

3.1 Introduction

3.1.1 Cocoa: Origin, Productivity, and Different Production Systems

The world produced 4.5 million tonnes of cocoa (*Theobroma cacao*) beans in 2013. Two-thirds were produced in Africa, especially in West African countries such as Côte d'Ivoire, Ghana, Nigeria, and Cameroon. The Americas and Asia each produced about one-sixth. The world's biggest producer country by far is Côte d'Ivoire:

it produced some 1.5 million tonnes, or one-third of the world's production. However, many countries only produce a small amount: Bolivia, for instance, only produced some 5,000 tonnes or 0.3 % of Côte d'Ivoire's production in 2013 (FAOSTAT 2015).

Not only the scale of production but also the methods differ vastly between Côte d'Ivoire and Bolivia. Cocoa originates from the lower strata of the Amazonian forests, and was traditionally grown beneath shade tree canopies of primary or secondary forest (Purseglove 1968; Rice and Greenberg 2000; Wood and Lass 2001). Today, growing cocoa in full-sun monocultures is widespread throughout the tropics, despite numerous problems associated with these systems (Tscharntke et al. 2011). In Côte d'Ivoire, most farmers produce cocoa in monocultures, while in Bolivia, shaded agroforestry systems are common. Cocoa production in monocultures often focuses on the use of agrochemicals and improved genetic material specifically developed and optimized for these systems. By contrast, in agroforestry systems producers often aim at substituting external inputs by the use of systems-inherent resources, e.g. nutrient cycling through pruning of shade trees (Vaast and Somarriba 2014).

Growing worldwide demand for cocoa beans and products has been met by expanding the area under cocoa production by almost 3 % in the last decade (data 2003–2013). In the same period, productivity per ha declined by 0.6 %, stagnating at a low level of around 450 kg ha^{-1} per year (FAOSTAT 2015). Smallholders produce almost 90 % of the world's cocoa (ICCO 2012) but will be unable to meet the rise in demand without suitable technical and land-tenure-related innovations (Vaast and Somarriba 2014). Hence, pressure to intensify cocoa production is likely to increase in the near future, which may lead to more monocultures being installed in currently forested areas (Schroth and Harvey 2007). In the following, we review results from three studies on cocoa in agroforestry and monocultures in Bolivia and Cote D'Ivoire. We justify the choice of these two contrasting countries by the differences and similarities in the main features of cocoa production outlined in Table 3.1, as well as by the different parameters discussed in detail in the following two subchapters. In addition, Bolivia is interesting because of its long-term experience with dynamic agroforestry systems, and Cote D'Ivoire because of the significant challenges in monocultures as well as a new dynamic agroforestry systems movement initiated by South-South cooperation between the two countries.

3.1.2   Cocoa Production in Bolivia

Most of Bolivia's cocoa supply comes from the Alto Beni region in the eastern foothills of the Andes. Since colonization by Franciscan monks in the eighteenth century, a wide range of cocoa landraces were probably traditionally collected and cultivated along with introduced varieties. In the last decades, cocoa has been promoted as an alternative to the production of coca (*Erythroxylum* spp.) in Alto Beni and other parts of Bolivia, e.g. the Pilon Lajas Biosphere Reserve or the Chapare

Table 3.1 Comparison of main features of cocoa production in Bolivia and Côte d'Ivoire

Country	Total cocoa production in 2013 [t][a]	Total area cultivated with cocoa in 2013 [ha][a]	Productivity in 2013 [kg ha^{-1}][a]	No. of cocoa producing families[b, c]	Average size of cocoa farms [ha][b, c]	Main pests and diseases
Bolivia	4,950	8,856	559	8,420	12–15 (cocoa plots <5), plus wild cocoa collection areas	Cocoa mirid (*Monalonion dissimulatum*), witches' broom (*Moniliophthora perniciosa*), black pod rot (*Phytophthora* spp.), frosty pod rot (*Moniliophthora roreri*)
Côte d'Ivoire	1,448,992	2,500,000	580	700,000	2–5	Cocoa mirids (*Sahlbergella singularis*, *Distantiella theobroma*) black pod rot (*Phytophthora* spp.), Cocoa Swollen Shoot Virus Disease (CSSVD)

Sources: [a]FAOSTAT (2015). Available: http://faostat.fao.org/
[b]Bazoberry and Salazar (2008)
[c]Smith Dumont et al. (2014), and own research

region. With markets for speciality cocoa developing, there is now an increase in the collection of cocoa from landraces in more remote areas of the Beni and Pando departments (PNUD 2008).

Cocoa productivity per hectare in Bolivia lies below 600 kg ha^{-1} per year (FAOSTAT 2015). In Alto Beni, the farmers' organization El Ceibo and local consultancy Ecotop have played a pioneering role in promoting the production of cocoa under certified organic agroforestry systems since the 1980s. Founded in 1977, El Ceibo was the first organization worldwide to market certified organic cocoa beans according to USDA and EU requirements. El Ceibo's foundation "Programme of Implementation of Agroecological and Forestry initiatives; PIAF-El Ceibo" (PIAF) provides extension and organizes the internal control system needed for organic certification, while El Ceibo carries out the processing and trade at the national and international levels. Today, agroforestry systems are among the common cocoa production systems in the region, and farmers associated with El Ceibo receive a 42 %

higher price for their cocoa than others in the region, partly due to organic and Fair Trade premiums (Jacobi et al. 2015).

Cocoa in Bolivia is cultivated on the margins of the highly diverse sub-humid rainforests in the foothills of the Andes. Plots are usually clear-cut to install a cocoa plantation, and the shade trees are either planted or result from natural regeneration. Bolivian cocoa farmers use among many others the popular agroforestry shade trees *Gliricidia* spp., *Erythrina* spp., and *Inga* spp., which double up as hedgerows or fodder trees for livestock, or are used in the preparation of natural remedies. A study on tree diversity found 105 tree species from 38 families on cocoa agroforestry plantations in Alto Beni (Jacobi et al. 2014). The most frequent tree species were *Leucaena leucocephala*, an N-fixing species, *Amburana cearensis*, a high-value timber species, *Attalea phalerata*, a native palm tree of which all parts have a traditional use from construction materials to natural remedies, *Inga* spp., an N-fixing fruit tree, and *Swietenia macrophylla*, a high-value timber tree. Farmers mentioned the main advantages of agroforestry systems to be income from timber, better water balance and soil quality, and the positive effect of shade on cocoa trees and working conditions (Jacobi et al. 2014).

The worldwide trend of intensifying production through simplifying cocoa production systems (Vaast and Somarriba 2014; Tscharntke et al. 2011) is also observable in Bolivia. Today, 40–50 % of the cocoa plantations are monocultures (El Ceibo, personal communication). However, young cocoa trees are usually associated with bananas or plantains (*Musa* spp.) for temporal shade during the first years of the establishment of a cocoa plantation, but are later eliminated. The resulting full-sun systems are sometimes framed by fruit trees. Previous research indicated that Bolivian farmers who were not associated with a local farmers' organization, and who regarded cocoa as a short- to medium-term investment rather than a long-term livelihood strategy, cultivated cocoa in monocultures more often (Jacobi et al. 2013).

Climate change will affect Bolivia more serverely in the near future than it already does (World Bank 2009; Mc Dowell and Hess 2012; Seiler et al. 2013). A study on agroecosystem resilience of cocoa farms found that local farmers described the plantations of Alto Beni as highly susceptible to climate change and mentioned heat waves, droughts, floods, and disease outbreaks related to climatic variability as the main problems (Jacobi et al. 2013).

The main pests and diseases affecting cocoa production in Bolivia are the cocoa mirid (*Monalonion dissimulatum*), witches' broom (*Moniliophthora perniciosa*), and black pod rot (*Phytophthora palmivora*) (July 2008). Witches' broom has arguably been the biggest problem with reported yield losses of up to 100 % (Milz 2006). In recent years, the devastating fungal disease frosty pod rot (*Moniliophthora roreri*) appeared for the first time in Bolivia, severely affecting cocoa production in Alto Beni, also with yield losses of up to 100 % and many farming families abandoning cocoa production (El Ceibo, personal communication).

3.1.3   Cocoa Production in Côte d'Ivoire

Cocoa is the dominant crop in the economy of Côte d'Ivoire, accounting for 15 % of the country's GDP and representing 38 % of exports (Kouamé 2010; DBR 2014; CCC 2015). Small-scale farmers with an average farm size between 2 and 5 ha produce 95 % of Côte d'Ivoire's cocoa (Kouamé 2010), and the cocoa sector employs a total of more than four million of the country's 22 million inhabitants (Hatloy et al. 2012).

Historical large-scale expansion of cocoa production in Côte d'Ivoire started after World War I. At this time, cocoa was cultivated under primary forest trees, and later under naturally regrown forests (N'Goran 1998). Most cocoa farmers did not cut down the biggest forest trees, or at least not all of them. The undergrowth was cut and burnt, while some of the largest trees were maintained to form the upper canopy of cocoa agroforests. In the 1960s, the government started promoting intensive full-sun production systems in order to maximize short-term yields (Ruf and Schroth 2004; Asare 2005; Koko et al. 2013; N'Goran 1998). The programmes encouraged complete forest clearance (Ruf and Zadi 1998), advising farmers to remove native forest trees from their plots for a number of antagonistic reasons such as pest and disease relationships, allellopatic behaviour, or low shade quality because of their dense or low canopy (Smith Dumont et al. 2014; FIRCA 2008).

Cocoa production in Côte d'Ivoire expanded from East to West, with the Eastern and Central regions under traditional management with trees, while the Western region was planted more recently with monocultures or low shade systems (Asare 2005). During plantation establishment, temporal shade for young cocoa trees is usually provided by crops such as plantains (*Musa × paradisiaca*) or yams (*Dioscorea* spp.) which are later eliminated, leading gradually to a full-sun system (Petithuguenin 1998).

Even though much of Côte d'Ivoire's cocoa is grown in monocultures, input use and productivity remain low. Over a decade ago, Ruf (2001) already predicted an expected yield decrease in the near future. Today, low yields of 269–560 kg ha^{-1} per year which are further declining challenge Côte d'Ivoire's entire cocoa value chain, as most plantations are monoculture systems with low soil fertility and high pest and disease pressures (Ruf 2011; Tscharntke et al. 2011; Assiri et al. 2009; Gyau et al. 2014). According to Ruf and Zadi (1998), two to three generations of full-sun cocoa production caused considerably more environmental damage than shaded cocoa farming would have. Milz (2012) described the current challenges in cocoa production in Côte d'Ivoire as a function of full-sun production systems, pests and diseases, and a lack of management. In addition, farming families are challenged by increasing food insecurity due to yield declines of food crops such as yams, manioc, corn, peanuts which compete for land with cocoa and other perennial and annual cash crops (Smith Dumont et al. 2014).

Main pests and diseases affecting Ivorian cocoa are cocoa mirids (*Sahlbergella singularis*, *Distantiella theobroma*) and black pod rot (*Phytophthora* spp.), causing yield losses estimated at 15–30 % and 10–15 %, respectively (Petithuguenin 1998).

In addition, a disease eradicated at the end of the 1950s – Cocoa Swollen Shoot Virus Disease (CSSVD) – was rediscovered in 2003 in the Central-West region of Côte d'Ivoire (N'Guessan et al. 2013). CSSVD is now one of the major limitations to cocoa productivity in West Africa. In Ghana, for instance, a government eradication programme has cut down more than 200 million infected cocoa trees (Dzahini-Obiatey et al. 2010).

Although full-sun or low-shade smallholder production is dominant, shaded cocoa farms still exist in Côte d'Ivoire (Daniels 2006). The shade trees on Ivorian farms are either native, i.e. naturally regenerated and therefore randomly distributed, or planted. Farmers also use their trees for firewood such as *Cola nitida, Funtumia africana, Mangifera indica, Musanga cecropioides*; food in terms of fruits, leaves, flowers, palm wine among others e.g. *Persea americana, Citrus reticulate, Spondias mombin, Elaeis guineensis*; timber for local construction, e.g. *Funtumia africana, Cola cordifolia, Celtis mildbraedii*; and for the preparation of natural remedies, e.g. *Cola nitida, Alstonia congensis, Spathodea campanulata* (Smith Dumont et al. 2014; Gyau et al. 2014; Herzog 1994). Smith Dumont et al. (2014) showed that farmers favour the integration of trees in their production systems, as they believe that shade trees (i) protect the cocoa trees from heat stress during the dry season, (ii) enhance soil fertility, and (iii) control soil erosion.

In 2010 the Ivorian government started supporting the establishment of cocoa agroforestry through the reintroduction of shade trees (Gyau et al. 2014), which was reinforeced by launching a new law which transferred tree rights from the state to individual farmers or village collectives (MEF 2014). Research and development initiatives as well as various certification schemes have recently begun to encourage the planting of trees. They advise planting native trees to improve the provision of ecosystem services (TCC 2010; Matissek et al. 2012). The Ivorian national extension service (ANADER) has also started to promote agroforestry in cooperation with certification bodies. In both countries, total cocoa production increased between 1993 and 2013. In Côte d'Ivoire it increased by 79 %, from 800,000 tonnes to 1.5 million tonnes. In Bolivia it increased by 33 %, from 3,710 tonnes to 4,950 tonnes. However, this was achieved by expanding the area under production by +5 % in Côte d'Ivoire, and by +157 % in Bolivia, while productivity per hectare remained below 600 kg ha^{-1} per year in both countries (FAOSTAT 2015). Farmers in both countries mention erratic rainfall distribution as one of the main problems of cocoa production (Milz 2012; Jacobi et al. 2013). The use of agrochemicals is more common in Côte d'Ivoire than in Bolivia.

3.1.4 Agroforestry Systems: An Alternative?

In agroforestry systems, farmers can produce timber, fruits, fodder, firewood, construction material, ornamentals, and plants used in medicine and rituals along with cocoa and other marketable food crops (Cerda et al. 2014; Jagoret et al. 2014; Somarriba et al. 2014; Sonwa et al. 2014). These systems can therefore make an

Table 3.2 Benefits provided by cocoa agroforestry systems reported in the literature

Benefit	Study
Improved pollination	De Beenhouwer et al. (2013)
Long-term stable cocoa yields	Rice and Greenberg (2000); Obiri et al. (2007); Bisseleua et al. (2013)
Longer lifespan of cocoa plantations	Obiri et al. (2007); Ruf and Zadi (1998)
Control of pests and diseases, erosion control	Tscharntke et al. (2011); Smith Dumont et al. (2014); Bieng et al. (2013); Gidoin et al. (2014); Sperber et al. (2004); Lin (2011)
Biodiversity conservation and enhancement	Rice and Greenberg (2000); Clough et al. (2009b); Fonte and Six (2010); Sonwa et al. (2007)
Climate change mitigation through C sequestration	Schroth et al. (2013); Somarriba et al. (2013); Somarriba et al. (2014); Jacobi et al. (2014); Fonte et al. (2010b); Verchot et al. (2007); Saj et al. (2013); Clough et al. (2010)
Nutrient cycling	Buresh et al. (2004); Gama-Rodrigues (2011)
Soil fertility maintenance or enhancement	Fonte et al. (2010a); Isaac et al. (2007); Tscharntke et al. (2011); Mbow et al. (2014)
Watershed protection	Garrity (2004)
Reduction of deforestation	Asare (2006); Clough et al. (2011); Tscharntke et al. (2012)

important contribution to the livelihoods and food security of smallholders by decreasing their vulnerability towards changing external factors such as food price fluctuations on global markets or pest and disease outbreaks (Tscharntke et al. 2011; Duguma et al. 2001; Bentley et al. 2004; Cerda et al. 2014; Schroth et al. 2000; Bos et al. 2007; Rice and Greenberg 2000; Sonwa et al. 2007). In addition, agroforestry systems provide multiple benefits and contribute to a wide array of ecosystem services as outlined in Table 3.2.

While there is ample evidence for the high ecological and social potential of agroforestry systems (Clough et al. 2009a; Tscharntke et al. 2011; Jacobi et al. 2014), recent literature suggests that under current market conditions they are often not economically viable in the short term compared to monocultures (Vaast and Somarriba 2014). Consequently, the improvement of market conditions for agroforestry systems is a key factor for their implementation at a larger scale. Furthermore, it is necessary to understand the dynamics of economic benefits at farm level in agroforestry systems and monocultures (Schneider et al. under review).

3.1.5 Dynamic Agroforestry: Principles and Examples

"Dynamic", "successional" and "analog" agroforestry systems are cross-cutting concepts based on principles of plant density and diversity (Analog Forestry Network RIFA 2012; Schulz 2011; Milz 2012). Dynamic agroforestry systems are based on the understanding of the succession and structure of natural ecosystems. The main features of dynamic agroforestry systems are (i) high planting densities

rice, corn, beans, pumpkin

cassava, pineapple, ginger, banana, papaya, maracuya

orange, peach palm, pepper, coffee

bread fruit, cocoa, avocado, mahogany

Year 1 Year 1-3 Year 3-15 > 15 years
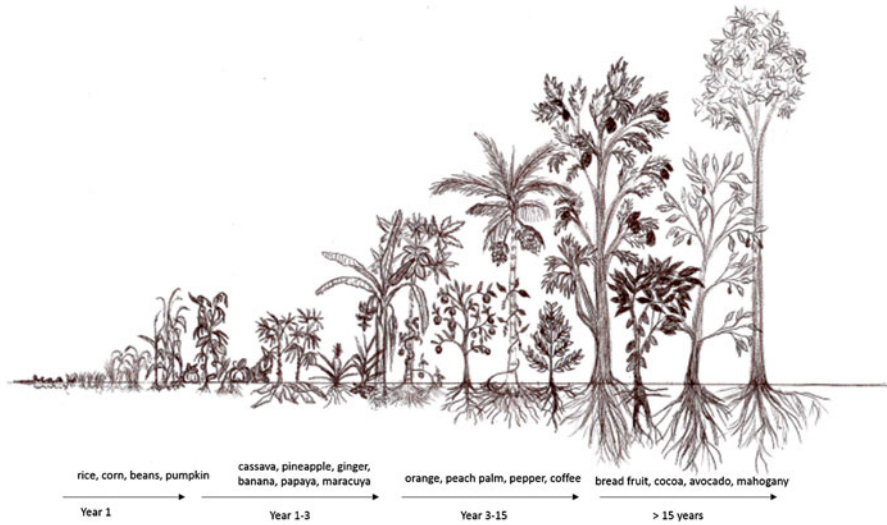
Fig. 3.1 Example of crops in a dynamic agroforestry system in the humid tropics of Bolivia. Note: In the majority of cases, all species are planted/sown at the same time, and also non-crop species are integrated e.g. to accumulate biomass (Source: figure based on own research)

and diversity, stratification, and a high energy flow usually without the use of external inputs; (ii) management practices such as different types of pruning interventions, e.g. rehabilitation, formative, maintenance pruning, selective weeding or grafting, and selection of healthy, productive planting material.

Like in natural species sucession, crops are grouped according to their lifespan into pioneer, secondary, and primary species. All the species are planted or sown at the same time, leading to a "crop succession" (Fig. 3.1) enriched by the regeneration of native plants (Götsch 1994). Pioneer species include rice, cassava, or pigeon peas. These are subsequently replaced until the system is characterized by secondary species such as pineapple, papaya, and banana, as well as slower growing secondary and primary tree species which simultaneously develop in their shade. The cocoa tree is a primary species with a potential life span of more than 100 years (Wood and Lass 2001). After about 10–15 years, the secondary species dominate the system, and are eventually replaced by the primary species. Plants which have completed their life cycle are either harvested or cut down, chopped up, and left to decompose in the plots to help maintain soil fertility.

The higher, emergent to canopy strata of the system may be occupied by rubber and timber trees, the middle, low canopy to understory strata by fruit trees including oil palm, and the lower, understory to forest floor strata, by cocoa trees. This stratification and the planting of tree species with complementary root systems aims at minimizing the competition for light, water, and nutrients, by assuring that different ecological niches are occupied (Götsch 1994). This way, synergies between the subsequent successional phases are enhanced, and each successional phase creates the necessary conditions for the plant species of the next successional phase.

In a dynamic agroforestry system, every plant is potentially useful. Ernst Götsch (1994) described for the Brazilian context how invasive pioneer plants can be highly beneficial for the system, as they may play a vital role in enhancing soil fertility. Götsch (1994) observed more vigorous plant growth and higher productivity when crops were introduced during an adequate successional phase of the overall system. The author further described how secondary species developed well under pioneer species, but not vice versa, and concluded that productivity depends on sucessional dynamics of the system which can be stimulated by pruning (Götsch 1994). Schulz et al. (1994) described that dynamic agroforestry farmers oberved a kind of allelopathic effect of maturing plants, reducing the growth of their neighbouring plants at the end of their life cycle, and a growth stimulating effect of young plants, increasing the vegetative growth of their neighbouring plants.

A major advantage of dynamic agroforestry systems is that the high crop diversity allows for harvests and income already during the first year of plantation establishment. This allows dynamic agroforestry farmers to avoid the "hunger gap" that occurs in cocoa monocultures, which only start to produce after 4–6 years. The continuous addition of organic material from pruning maintains soil fertility, and the complexity of the system may lead to a natural self-regulation of pests and diseases.

The few empirical studies on dynamic agroforestry systems conducted to date compared dynamic agroforestry plots to monocultures in Bolivia, and showed higher productivity and soil fertility in dynamic agroforestry systems. Todt et al. (2010, 2009) found significantly higher nutrient concentrations, thicker Ah horizons, and higher organic matter content in dynamic agroforestry systems which were cultivated for more than 20 years. Milz (2010) studied the damage of fruit flies (*Anastrepha* spp. and *Ceratitis captitata)* in citrus trees. He found more than double the amount of aborted fruits due to fruit fly damage in monocultures than in dynamic agroforestry systems. Productivity per orange tree was significantly higher in dynamic agroforestry systems and the sugar content of the fruit was not significantly different beween the two systems (Milz 2010). Gruberg (2011) assessed pests, diseases, and productivity in dynamic agroforestry vs. low-diversity cocoa and citrus systems, and found less incidence of witches' broom and black pot rot, similar cocoa productivity, and a multitude of different products in dynamic agroforestry systems. Schulz (2011) and Vieira et al. (2009)) described how heavily degraded castor bean (*Ricinus communis*) monocultures were successfully restored with dynamic agroforestry systems in Bahia, Brazil. In one study, castor bean production increased by 90 %, and total productivity increased fourfold after the implementation of dynamic agroforestry systems (Schulz 2011).

In Bolivia, Ecotop has facilitated the establishment of 100–150 ha of dynamic agroforestry systems, mainly in the Alto Beni region, and the organization "Shared space of agroforestry systems" (Ecosaf) has established around 50 ha in semi-arid Interandean valleys. With the goal of supporting farmers to increase cocoa productivity, adapt to climatic constraints and pest pressure, and address the challenge of food security, Biopartenaire Ltd., a fully owned subsidiary of the Barry Callebaut Group, and Ecotop introduced the concept of dynamic agroforestry to Côte d'Ivoire

in 2012 with financial support from the Sustainable Trade Initiative (IDH, http://www.idhsustainabletrade.com/) and Barry Callebaut. To date Biopartenaire Ltd. has trained more than 1,000 farmers.

3.2 Selected Case Studies

3.2.1 On-Station Comparison of Agroforestry versus Full-Sun Systems under Organic and Conventional Management in Bolivia

The studies published to date on the long-term agronomic and socio-economic effects of different cocoa production systems have mostly focused on existing cocoa production systems in farmers' fields throughout the tropics (Beer et al. 1998; Belsky and Siebert 2003; Aneani et al. 2011; Clough et al. 2011; Jagoret et al. 2011; Ruf 2011; Jacobi et al. 2013, 2014; Somarriba et al. 2013; Dawoe et al. 2014). Due to the limited data from controlled on-station trials, the Swiss-based Research Institute of Organic Agriculture (FiBL) has set up a long-term on-station experiment in Alto Beni, Bolivia. Alto Beni is a settlement region at the border of the departments of La Paz, Beni, and Cochabamba, in the north-eastern foothills of the Bolivian Andes (Jacobi et al. 2013). The region derives its name from the river Beni, which is part of the Amazon watershed, and lies between 350 and 1,500 m above sea level. Average annual rainfall is 1,440 mm. Temperatures range from 22.4 °C to 26.8 °C with a yearly average of 25.2 °C. The project is part of a larger programme called "Farming Systems Comparison in the Tropics" (www.systems-comparison.fibl.org), and conducts further on-station and on-farm trials in Kenya and India (Forster et al. 2013).

The five different cocoa production systems under comparison include two monocultures and two agroforestry systems, one under conventional and one under certified organic management, as well as a dynamic agroforestry with zero external input under certified organic management. The experiment is set up as a full-factorial, randomized complete block design with four replications, i.e. a pairwise comparison of agroforestry under conventional and under certified organic management, and monoculture under conventional and under certified organic management. The factors tested are: (i) crop diversity in monocultures vs. agroforestry; (ii) management practice, i.e. conventional vs. certified organic; and (iii) cultivar with 12 different cocoa cultivars/hybrids. The combination of the factors "crop diversity" and "management practice" make up the system effect. Figure 3.2 shows example plots of a conventional monoculture and a dynamic agroforestry system 4 years after cocoa tree planting in the long-term on-station field trial in Bolivia.

In this chapter, we present the first 3 years of cocoa harvest (2011–2013), as well as yields of non-cocoa crops, i.e. the by-crops between the start of the experiment 2009 and 2013. We hypothesize that in agroforestry systems, the yields of by-crops

Fig. 3.2 *Left panel*: young cocoa monoculture in Bolivia. *Right panel*: young dynamic cocoa agroforestry system after shade tree pruning in Bolivia. Pictures of both plots were taken 4 years after cocoa tree planting (Source: own research)

lead to higher total system yields as the sum of all marketable goods compared to monocultures. We assessed total system yields by adding up the yields of all the products harvested during the establishment phase from 2009 to 2013, expressed in kg dry matter per ha. These products included cocoa harvested in all systems from 2011 to 2013, plantain harvested in all systems from 2009 to 2011, banana harvested in conventional, certified organic, and dynamic agroforestry systems from 2012 to 2013, maize (*Zea mays*), rice (*Oryza sativa*), pigeon pea (*Cajanus cajan*), achiote (*Bixa orellana*), cassava (*Manihot esculenta*), hibiscus (*Hibiscus sabdariffa*), pineapple (*Ananas comosus*), tannia (*Xanthosoma sagittifolium*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*) harvested in dynamic agroforestry systems from 2009 to 2013 (Schneider et al. under review).

Results showed significantly higher cocoa dry bean yields in the conventional monoculture (+153 %), and significantly lower yields in the dynamic agroforestry system (−70 %) compared to all the other systems in 2013, the third year of harvest. Yields in 2013 ranged between around 600 kg ha^{-1} per year in the conventional monoculture and 100 kg ha^{-1} per year in the dynamic agroforestry system. Furthermore, we recorded significantly higher total system yields in all three agroforestry systems compared to the two monocultures (by +161 % and +81 % in the two agroforestry systems and dynamic agroforestry, respectively). The main explanations of these results are the substantial amounts of bananas harvested in the agroforestry systems in 2012 and 2013, and the considerable amounts of fruits and tubers harvested between 2009 and 2013 in the dynamic agroforestry system. It has to be noted that banana trees were removed from the two monocultures at the end of 2011 in order to achieve the targeted full-sun system. Even though the monocultures had achieved both the highest cocoa dry bean yields and highest plantain yields between 2009 and 2011, total system yields of the monocultures could not reach the level of the three agroforestry systems (Schneider et al. under review). Looking at these results from the farmers' point of view, it is not only about producing more; it also matters how many different products you produce, in which quantity and, perhaps most importantly, if there is a market for the produce. As there is a lack of

evidence about the economic viability of different cocoa production systems, we are currently assessing the systems in our trial in this respect.

3.2.2 On-Farm Comparison of Different Cocoa Production Systems in Bolivia

We assessed dynamic and simple agroforestry systems, as well as full-sun monocultures in Alto Beni by (i) sampling plots of a quarter hectare in size, on a total of 12 farms with four farms per system, (ii) counting the pods and assessing incidences of cocoa mirid, witches' broom, and black pod rot on the ten central cocoa trees of each plot, (iii) categorizing incidences of pests and diseases using the following index: 0 = no visible incidence, 1 = one incidence per tree or pod, 2 = two to ten incidences per tree, and 3 = more than ten incidences per tree, and (iv) comparing the means of each system using Wilcoxon and Kruskal-Wallis rank sum tests with the statistical software R, Version 3.1.2 (R_Core_Team 2014).

We obtained qualitative information on the farmers' rationales behind their respective production system, and identified the major constraints of cocoa farmers in three focus group discussions with about ten farmers each in different parts of the study area. Then, we conducted semi-structured interviews with the owners of the 12 sampling plots, and three additional monoculture cocoa farmers (Jacobi et al. 2013), from which we also obtained information on cocoa yields. Furthermore, we elaborated possible strategies to address the major constraints of cocoa production in a participatory workshop with 30 regional cocoa farmers, and assessed strategies and constraints of sustainable cocoa production through five interviews with local agricultural consultants. As frosty pod rot disease had spread considerably since we gathered our data (2010–2012), we conducted an additional interview on the impacts of this disease with an agricultural consultant of El Ceibo in February 2015 (referred to as: "El Ceibo, personal communication").

3.2.2.1 Cocoa Productivity and Incidences of Pests and Diseases in On-Farm Systems in Bolivia

According to cocoa farmers in Alto Beni, incidences of pests and diseases are more intense and frequent in monocultures than in agroforestry systems (Jacobi et al. 2013). However, with the appearance of frosty pod rot many producers appear to be cutting down shade trees in order to avoid high relative air humidity in their systems, which is believed to increase incidences of the disease (El Ceibo, personal communication). The effect of this adaptation strategy on incidences of frosty pod rot remains to be investigated. Table 3.3 shows the numbers of cocoa pods and the incidences of mirids, witches' broom, and black pod rot on cocoa trees in the different production systems assessed.

Table 3.3 Pod count and incidences of cocoa pests and diseases on 12 cocoa farms in Bolivia

	Pods >5 cm	SEM	Mirids	SEM	Witches' broom	SEM	Black pod rot	SEM
Mean dynamic agroforestry	12.8[a]	1.31	0.58[a]	0.17	0.50[a]	0.10	0.43[a]	0.12
Mean agroforestry	15.5[a]	2.53	0.40[a]	0.13	1.32[b]	0.14	0.30[a]	0.09
Mean monoculture	20.1[a]	3.43	1.00[b]	0.16	2.58[c]	0.10	0.65[a]	0.15

SEM standard error of the mean, agroforestry = "simple" agroforestry system, for a description see Jacobi et al. (2013). No significant difference for numbers sharing the same letter indicates results from Wilcoxon and Kruskal-Wallis rank sum tests

There were more pods >5 cm in monocultures, but the difference was not statistically significant due to a high variability of the data within the groups. The information on cocoa yield from interviews with cocoa farmers showed mean annual yields in dynamic agroforestry systems, simple agroforestry systems, and monocultures of 510, 423, and 350 kg dry beans per ha, respectively (Jacobi et al. 2013). These findings together with the data shown in Table 3.3 indicate that cocoa trees in monocultures developed more pods, but also showed more incidences of pests and diseases which led to a high loss of pods before harvest.

We found more mirids in monocultures than in the agroforestry systems. The difference was greatest for incidences of witches' broom, which increased significantly from dynamic agroforestry to simple agroforestry and from simple agroforestry to monocultures. The fact that witches' broom is among the main cocoa diseases in Alto Beni (Milz 2006) highlights the considerable implications of dynamic agroforestry from our results. As frosty pod rot has only recently begun to spread in Bolivia, no empirical data on its incidence in full-sun and agroforestry systems are available to our knowledge. However, two research studies from Costa Rica found less pressure from frosty pod rot under higher and more complex shade in terms of diversity and spatial distribution of trees in cocoa plantations (Bieng et al. 2013; Gidoin et al. 2014).

Pruning is a crucial management intervention influencing the regulation of pests and diseases in cocoa production systems (Franzen and Mulder 2007). Unfortunately, many cocoa farmers, lacking the equipment and workforce, face difficulties in pruning their cocoa trees. This is why, to regulate humidity in their systems, they often prefer to eliminate shade trees (El Ceibo, personal communication) rather than adequately space or prune them (Schroth et al. 2000). Another reported strategy is the use of different planting materials such as the CCN-51 variety from Ecuador, which is more tolerant to both full-sun conditions and frosty pod rot than other varieties in Alto Beni. Planting CCN-51 is an example of an adaptation strategy which could further favour the shift from agroforestry to monocultures in Alto Beni and other cocoa production areas.

3.2.2.2 Factors Influencing Adoption of Dynamic Agroforestry in Bolivia

The disadvantages of cocoa agroforestry systems in Bolivia are the lack of necessary labour, equipment, technical support, and capacity building to maintain the systems. Elderly farmers face additional difficulties in pruning their shade trees, which is why many cocoa farmers only prune their systems when cocoa prices are high or expected to increase (El Ceibo, personal communication). This is a common strategy for coping with cocoa price volatility (Tscharntke et al. 2011), but works best in agroforestry systems, as monocultures without management face greater ecological pressure (Vaast and Somarriba 2014).

The advantages and disadvantages of dynamic agroforestry systems mentioned by cocoa farmers during our workshops are listed in Table 3.5. All of them said shade trees are crucial to reducing both ecological and economic risks, and to adapting to climate change (Jacobi et al. 2013). Adaptation strategies mentioned were (i) increasing soil organic matter, (ii) incorporating more trees into the land use systems, and (iii) increasing plant diversity for both diversified production and enhanced regulation of pests and diseases. Despite this, only four of the participants managed a dynamic agroforestry system, while about half of them had simple agroforestry systems with few shade tree species.

Those cocoa producers who managed a dynamic agroforestry system indicated that they strongly relate to the plants in their systems. They expressed a high interest in the ecological context and showed a high knowledge of plant species, both domesticated and wild, and their uses. They had all been in contact with an organization or project working on dynamic agroforestry systems, and observed ecological processes and interactions on their farms. In addition, all dynamic agroforestry farmers said that neighbours were taking up at least some dynamic agroforestry management practices such as increased planting densities or diversification. However, managing a dynamic agroforestry system requires a high level of specialized knowledge, as well as the ability and equipment to prune trees. Adoption of dynamic agroforestry involves more than knowledge transfer: social learning and transdisciplinary approaches are important pathways for successful adoption. Capacity building, knowledge exchange networks, and continuous technical support may therefore be important means of enhancing the adoption of dynamic agroforestry systems in Bolivia.

Incentives for dynamic agroforestry systems could be created through projects and extension services, i.e. by a more constant presence of local organizations and projects. Following the examples of Ecotop and Ecosaf in Bolivia, such technical support could help to uphold year-round production of a variety of products and thus lead to a lower dependency on cocoa. Dynamic agroforestry is also a promising way of restoring degraded soils (Milz 2010; Todt 2010) and is suitable for production systems on steep slopes. Dynamic agroforestry farms may therefore be eligible for payments for ecosystem services (PES). However, incentives from PES remain difficult to access and/or unviable for cocoa producers in Alto Beni (Jacobi et al. 2014).

3.2.3   On-Farm Comparison of Different Cocoa Production Systems in Côte d'Ivoire

Our study was located between the departments of Yamoussoukro and Bouaflé, in the forest-savannah transition zone of the Central and Central-West regions of Côte d'Ivoire. This region was part of the main area of cocoa production until about three decades ago, when it became unsuitable for cocoa due to outbreaks of pests and diseases, as well as a prolonged period of drought in the 1980s. The area is a mosaic of mesophilic, semi-deciduous forest and Guinean savannah environments (MEDD 2011). The average annual rainfall is 1000–1200 mm, and the temperature ranges from 14 °C to 39 °C. Farmers are mainly engaged in subsistence agriculture cultivating mainly yams, cassava, plantain, rice, and maize, and production of cash crops such as cocoa and coffee. Both Yamoussoukro and Bouaflé are characterized by low-yielding full-sun systems and high pressure of CSSVD, a disease that requires eradication of affected plants and subsequent replanting.

Biopartenaire Ltd. and Ecotop installed their first dynamic agroforestry trial plots in farmers' mature, low-yielding monocultures (Type 1) by pruning cocoa trees and diversifying plantations at the start of 2013. We studied yields of cocoa and by-crops in these dynamic agroforestry vs. monoculture plots, and, through interviews, assessed the reasons motivating the involved farmers to adopt dynamic agroforestry (Franzel et al. 2001). In addition, we looked at not yet productive plantations which were replanted on sites infected with CSSVD (Type 2). In these, there was no cocoa to harvest yet, so we used interviews to assess cocoa yield levels prior to replanting as well as current by-crop yields (Beerli 2014).

For cocoa yield, we recorded fresh and dry bean weights. Type 1 plantations were split into two study categories, in-depth and trend: (i) for the in-depth study, we did precise yield measurements on 40 plantations; (ii) for the trend study, we obtained less precise information on a further 550 plantations with the help of agroforestry experts who assisted farmers in measuring the yields themselves. Besides cocoa yield, we investigated short-term profitability with net return = marketed yield * current market price, and biophysical performance by measuring the yield of by-crops. We evaluated the profitability of Type 2 plantations by comparing annual yield losses of CSSV-infected monocultures with the installation costs of dynamic agroforestry systems per area. In addition, we documented incidences of pests and diseases, and obtained additional socio-economic information e.g. on the educational level of farmers, installation, maintenance, labour, costs for external inputs through interviews in the in-depth study (n = 43).

Factors influencing adoption of either dynamic agroforestry or monocultures were investigated through farmer interviews. These included (i) initial motivation to try dynamic agroforestry, (ii) comprehension of the dynamic agroforestry approach, (iii) observations made when comparing dynamic agroforestry with monoculture plots, and (iv) objectives regarding the implementation of dynamic agroforestry systems. We also consulted the dynamic agroforestry experts on their experiences with

Table 3.4 On-farm study on adoption potential of dynamic agroforestry in Côte d'Ivoire (Beerli 2014)

	In-depth study (n=40)	Trend study (n=550)
Measurement period	October 2013 to January 2014 (fortnightly)	October 2013 to January 2014 (fortnightly)
Systems	Dynamic agroforestry vs. monoculture (adjacent plots)	Dynamic agroforestry vs. monoculture (adjacent plots)
Plot size	144 m^2 (10 central cocoa trees harvested per plot)	approximately 100 m^2 (all cocoa trees of the plot harvested)
Yield parameters recorded	Pods: number, weight, incidences of pests and diseases Fresh beans: weight Dried beans: weight	Pods: number
Further parameters recorded for plot characterization	Tree density: productive and unproductive cocoa trees Farmer information/estimates: plantation age, varieties, management practices, preceding crop, yield level in 2012, estimation of cocoa quality, soil quality, pests and diseases	Tree density: productive cocoa trees Farmer information/estimates: plantation age, varieties, management practices (frequency of weeding, application of pesticides, fertilizer, pruning)

adoption in the area, and we assessed the willingness to replace a plantation infected with CSSVD by a dynamic agroforestry system using the opportunity cost model. Table 3.4 gives an overview of the study.

3.2.3.1 Cocoa Productivity and Incidences of Pests and Diseases in On-Farm Systems in Côte d'Ivoire

When interpreting our results, we have to consider that our study took place within the first year after pruning and diversification measures to rehabilitate old cocoa stands. We therefore focus in the following on some elements explaining successful implementation of dynamic agroforestry at plot scale, and outline only a preliminary assessment of factors influencing dynamic agroforestry adoption.

Mature, formerly low-yielding monocultures (Type 1 plantations) in the trend study showed significantly higher pod counts and cocoa dry bean yields on dynamic agroforestry trial plots compared to the adjacent control monocultures: 12,747 compared to 11,965 pods ha^{-1} (+7 %), and 478 compared to 426 kg ha^{-1} per year (+12 %), respectively. The in-depth study indicated higher dry bean weight per pod and lower incidences of pests and diseases in dynamic agroforestry trial plots (Beerli 2014).

These positive effects can be mainly attributed to rehabilitation pruning, the principal management practice of dynamic agroforestry systems. It led to higher light inception which induced flowering and reduced losses of young pods, results that

confirm the findings of Petithuguenin (1998). With a reduced cocoa canopy and planting density, the relative air humidity in the systems decreased, in turn creating less favourable conditions for pests and diseases as described by Smith Dumont et al. (2014).

As there is little evidence on the effects of pruning, we compared our results with on-farm surveys which looked at the effects of planting density and shade on cocoa yield; Deheuvels et al. (2012) found highest yields for agroforestry systems with low planting densities which were pruned twice a year, and reported a similar range of tree yields of 0.1–1.0 kg per tree and year as compared to 0.3 kg per tree and year in our study.

3.2.3.2   Preliminary Assessment of Factors Influencing Adoption of Dynamic Agroforestry Systems in Côte D'Ivoire

Short-term profitability of dynamic agroforestry systems in the in-depth study was lower than of monocultures in Type 1 plantations, mainly because of the initial investment to install the systems. Consequently, the net return from cocoa in dynamic agroforestry systems was lower compared to monocultures (−17.2 %), due to higher labour costs (+29.8 %). However, we could not include the by-crops as the farmers did not achieve considerable yields. It is important to mention that the installation of a dynamic agroforestry system is a mid- to long-term investment, and thus needs to be analysed accordingly. The dynamic agroforestry plots investigated in our study had only little time to show their effect, as our study took place only about 8 months after pruning and diversification of low-yielding cocoa monocultures. Production costs are expected to decrease in the near future for two reasons: better labour efficiency as farmers gain experience with an increasing area under dynamic agroforestry, and lower costs associated with pesticides (Clay 2004). In the trend study, net returns from cocoa were higher in dynamic agroforestry systems compared to the monocultures (+10.6 %).

The advantages and disadvantages of dynamic agroforestry mentioned by participants during our workshops are listed in Table 3.5. The results from the interviews were comparable for both plantation types: the higher the degree of perceived problems within their own plantation, the more willing the participants were to install a dynamic agroforestry trial plot, which confirms the findings of existing studies (D'Souza et al. 1993; Sood and Mitchell 2006). We found that farmers who estimated the benefit of dynamic agroforestry systems to be higher than the declining revenue might opt for replacement, since opportunity costs for replacement are lower. The main constraints mentioned for Type 1 plantations were drought and low productivity, while CSSVD was additionally mentioned for Type 2 plantations which confirms reports of farmers being more interested in agroforestry in areas where cocoa is devastated by diseases such as CSSVD (Gyau et al. 2014).

While the experts were able to explain the dynamic agroforestry system approach clearly, most dynamic agroforestry participants found it difficult to explain the principles of dynamic agroforestry in their own words. The advantages and

Table 3.5 Perceptions of and motivations for dynamic agroforestry of (a) dynamic agroforestry farmers/participants and (b) local experts in Bolivia and Côte d'Ivoire

Country		Bolivia[a]	Côte d'Ivoire[b]
Dynamic agroforestry farmers	Pro	Personal relationship to plants To conserve biodiversity and resources, and water quality and availability To host wildlife To produce healthy food Access to know-how: natural succession, plant sociology, year-round production Contact and exchange with research projects and organizations, as well as other dynamic agroforestry farmers	Effect of pruning is perceived to be positive Alternative to CSSVD plantations Access to know-how: natural succession, plant sociology
	Contra	Difficulties in pruning the trees High humidity can favour fungal diseases Market access with a diversity of produce is difficult	Too much work to manage by-crops Time consuming/labour intensive
Local experts	Pro	Soil restoration capacity Enhancement and revival of traditional ecological knowledge Healthier diet Biodiversity conservation, corridor function for fauna Resilient agroecological landscapes Connection to local, regional, and international markets with high quality products	Self-sufficiency Access to know-how Promotes discussion about traditional production methods Long-term positive effects on entire plantation (soil fertility, climate change adaptation, food resilience, etc.)
	Contra	Knowledge intensive Plants and seeds difficult to obtain	Lack of seeds Failure of by-crop Difficulties in sharing dynamic agroforestry knowledge with participants Unfamiliar by-crops are not popular and thus neglected by participants

[a]Dynamic agroforestry systems between 5 and 20 years old
[b]After 8 months of diversification of monocultures using a dynamic agroforestry design, cocoa trees were between 4 and 33 years old

management practices they mentioned were the same their dynamic agroforestry expert had told them, as well as those which were most visible in the short term, such as pruning. However, the majority of the participants were not able to link the applied methods to their long-term effects, indicating that there is a knowledge gap between dynamic agroforestry experts and farmers; this leads to an insufficient implementation of the principles of dynamic agroforestry, and indicates the need for more participatory, transdisciplinary research and social learning processes. Participants expressed their overall satisfaction with their observations, as they felt that cocoa productivity was increasing. Most farmers expressed their motivation to increase their area under dynamic agroforestry, but they felt they could not do it by themselves due to constraints such as labour costs and lack of knowledge, mentioned above. This statement corresponded to the feedback given by the dynamic agroforestry experts, who said that participants did not sufficiently manage the dynamic agroforestry trial plots. Pruning seemed to be the only popular management practice for the dynamic agroforestry systems, with most participants neglecting the by-crops, maybe because these are food crops traditionally cultivated by women. Participants attributed the failure of by-crops to drought and excessive shade. Overall, the acceptance of the implemented dynamic agroforestry extension programme was promising. These initiatives should be promoted, especially in regions affected by CSSVD.

3.3   Discussion

3.3.1   General Trends of Productivity, Soil Fertility and Pests and Diseases in Different Cocoa Production Systems

Tscharntke et al. (2011)) asked: which strategy is more viable for small-scale farmers, risk-averse long-term strategies such as agroforestry systems or short-term yield gains? Vaast and Somarriba (2014) found that full-sun monocultures are not a suitable strategy for small-scale farmers' risk management, and said that to foster agroforestry systems, innovative practices have to be developed, particularly with respect to shade regulation. This includes initiating selection programmes for cocoa genotypes in the context of agroforestry management, as well as appropriate practices of spacing and pruning trees at critical times in the production cycle. Furthermore, adequate combinations of different trees, e.g. with complementary leaf phenology, and local species have to be worked out with the objective of enhancing functional biodiversity.

Our results from Bolivia and Cote d'Ivoire confirm previous findings that farmers prefer to maintain shade trees in their cocoa systems in order to limit their vulnerability against outbreaks of pests and diseases as well as climate change impacts such as drought and heat stress (Johns 1999; Smith Dumont et al. 2014; Vaast and Somarriba 2014). However, sound recommendations for good agricultural practices

in cocoa production systems which work in the farmers' context are scarce, especially when it comes to organic production (Schneider et al. under review). For example, little research has been done on mulching in cocoa. Mulching could contribute to the control of fungal diseases near the soil surface through leachates, as suggested for *Gliricidia sepium* biomass (Inostrosa and Fournier 1982).

However, besides good agronomic practice the most crucial factor in implementing agroforestry systems on a larger scale is arguably the improvement of market conditions. Bolivia is a model case study of how a socio-economic context has facilitated the successful implementation of cocoa agroforestry systems on about half the cocoa growing area. In order to improve the sustainability of worldwide cocoa production, the lessons we learnt from Bolivia may serve as an example for other cocoa growing areas which produce more substantial volumes such as West Africa or Southeast Asia.

Vaast and Somarriba (2014) reported on two recent studies (Gockowski et al. 2013; Asare et al. 2014) which assumed 20 % higher cocoa productivity in full-sun monocultures compared to well-managed agroforestry systems. But the underlying evidence for this assumption is not very strong, as only few studies, all of which were conducted 20–30 years ago, actually document the beneficial effect of removing shade to achieve higher yield (Vaast and Somarriba 2014). In addition, the influence of both cocoa varieties and the composition of shade tree species were not thoroughly addressed in these studies. Full-sun systems can achieve high yields in the short term (Vaast and Somarriba 2014). However, we have seen the prediction that cocoa production will decline within less than 20 years (Beer 1987) become true (Ruf and Zadi 1998). Monocultures thus have to be completely renewed much sooner than shaded systems. They also require the continuous input of agrochemicals and constant management to attain their maximum yield potential. By contrast, higher agro- and wild biodiversity in agroforestry systems is not necessarily negatively correlated with cocoa yield (Clough et al. 2011; Tscharntke et al. 2011; Steffan-Dewenter et al. 2007; Schroth et al. 2014), and shade trees were also associated with a longer lifespan of cocoa production systems (Obiri et al. 2007; Ruf and Schroth 2004; Ruf and Zadi 1998).

Looking beyond the yield of a single commodity such as cocoa, several studies have shown that diversified cropping systems are more productive per area than monocultures (Jaggi et al. 2004; Bellow et al. 2008; Rosset 1999; Tscharntke et al. 2012; Pokorny et al. 2013). These results indicate the significant contributions of agroforestry systems to local food security and risk distribution in smallholder contexts. However, studies on the optimization of total system yields, i.e. total land productivity, and tree-crop interactions in diversified systems are scarce (Bellow et al. 2008). Farmers often have detailed knowledge on their cultivation systems and related processes (Altieri 2004), which is crucial to take into account when trying to understand complex tree-crop interactions and designing projects to support agroforestry systems.

If no external inputs are added, soil fertility declines rapidly in full-sun systems, although it may decline even with the constant addition of mineral fertilizers. This is one of the major reasons for decreasing cocoa productivity worldwide (Vaast and

Somarriba 2014). Leguminous shade trees, for instance, can counteract this decline by continuous inputs of nutrients and organic matter to the soil through litter fall. The nutrients they fix in their vegetative materials can replace around 150 kg urea per ha and year (Tscharntke et al. 2011). This would also help prevent a lack of metabolic energy in the soil caused by the continuous energy and nutrient flux in the form of firewood and charcoal from rural to urban areas (Milz 2012). In addition, the decomposition of litter happens faster under shaded conditions, resulting in higher natural nitrogen and phosphorous levels in the soil and indicating that shaded systems are more sustainable than full-sun systems (Ofori-Frimpong et al. 2007)

Contrary to soil fertility, the findings of different studies on the effects of shade trees on incidences of pests and diseases in cocoa production systems are complex and ambiguous (Beer et al. 1998; Staver et al. 2001; Bedimo et al. 2012). Several authors mention regulatory effects of shade trees on pests (Tscharntke et al. 2011; Rice and Greenberg 2000; Schroth et al. 2000; Clough et al. 2009a, 2010; Daniels 2006; Campbell 1984). For example, they may decrease pest populations directly (Beer et al. 1998; Lin 2007; Jaramillo et al. 2009; Thorlakson and Neufeldt 2012), or indirectly by favouring natural pest antagonists (Opoku et al. 2002). However, some researchers suggested that the cooler microclimate in shaded systems coupled with high humidity and insufficient aeration may increase the incidences of fungal diseases (Schroth et al. 2000; Dakwa 1976), while others found that frosty pod rot was negatively correlated with shade and diversification (Bieng et al. 2013; Gidoin et al. 2014). Furthermore, some shade tree species may act as hosts of pests and diseases such as CSSVD (Ploetz 2007). Physiological stress of cocoa trees is reduced under agroforestry (Beer et al. 1998), which may enhance plant health and its defence against stressors. Ecological conditions such as altitude and slope exposure can also either favour or suppress pests and diseases depending on their effect on microclimatic conditions, i.e. relative air humidity and temperature. In summary, it is often difficult to identify adequate shade levels and tree species compositions that minimize damage from pests and diseases while ensuring favourable growing conditions for cocoa trees. This especially applies because the needed shade levels of the cocoa trees and the periods with the highest likelihood of pests and diseases vary over time. Therefore, research is still needed to assess the suitability of different tree species and optimal planting densities (Koko et al. 2013), and their effects on pests and diseases, as these can vary for different species (Franzen and Mulder 2007).

3.3.2   Resilient Adaptation of Different Cocoa Production Systems to Factors of Global Change

Vaast and Somarriba (2014) have pointed out the threats to ecosystem services of intensification of cocoa systems worldwide. They concluded that removing shade trees reduces the ability of cocoa farmers to adapt to factors of global change such

as demographic pressure, food insecurity, cocoa price volatility, and climate change impacts. While the role of agroforestry systems as a mitigation and adaptation strategy for climate change impacts has been widely discussed, our research from Bolivia adds the component of socio-economic implications of shade trees in cocoa production systems (Jacobi et al. 2013, 2015). For example, farmers like to work in the shade rather than in the scorching sun, and they value the diversification of their production and the knowledge they have about their production system.

Agricultural intensification and climate change are predicted to create synergies which increase the vulnerability of agricultural production (Lin et al. 2008). Cocoa trees are particularly susceptible to climate change impacts (Anim-Kwapong and Frimpong 2006; Laederach et al. 2013), especially to drought (Tscharntke et al. 2011). Diversification is necessary for the adaptability of agroecological systems to climate change impacts (Henry et al. 2009; Steffan-Dewenter et al. 2007; Lin et al. 2008; Tscharntke et al. 2011; Altieri and Nicholls 2013), besides other environmental benefits (Soto-Pinto et al. 2010). In sum, at a time when cocoa production systems need to be more resilient than ever, intensification in terms of the removal of shade has reduced their ecological resilience.

There are more climate change related studies on coffee (*Coffea* spp.) than on cocoa, but as coffee is also a typical perennial cash crop for smallholder families in the humid tropics which is grown under similar agroecological conditions, the results from these studies may also have implications for cocoa. Philpott et al. (2008) found that more diversified coffee sytems suffered less damage from hurricane Stan in Chiapas, Mexico. Shade trees protected the coffee plants from drought, as they reduced evapotranspiration and increased the infiltration capacity of the soil (Lin 2007). Nicholls et al. (2013) described how diversified farms in Cuba lost about 50 % of their production after hurricane Ike in 2008, compared to the 90–100 % lost by monoculture farms.

3.3.3 Next Steps: The Need for Transdisciplinarity in Future Cocoa Research

Designing agroecosystems similar to natural ecosystems may be the only way to sustainably cultivate cocoa (Milz 2012). Ideally, the objective should be to optimize systems for productivity, biodiversity, and food security in the long term, rather than short-term maximization of yield. However, the main constraints for large-scale adoption of approaches such as dynamic agroforestry systems are that they are knowledge and labour intensive. Not only do interested farmers have to understand the underlying principles of these approaches, they also need technical support, as well as help in establishing farmer-to-farmer knowledge and exchange networks, and fair prices for their produce. Organizations such as Ecotop, Ecosaf, El Ceibo, and Biopartenaire Ltd. are thus pivotal for initiating these processes on the ground in order to stimulate bottom-up learning approaches. While this might resolve the

knowledge constraints, labour intensity remains a challenge. Smallholders optimize opportunity costs and invest labour into different activities accordingly. Hence the problem is again on the market side, i.e. producers are paid low prices for their cocoa, which underpins yet again the need to improve market conditions for produce from agroforestry systems.

One of the key factors future cocoa research needs to address is the optimal design of sustainable production systems. The above-mentioned social learning process must aim at the transdisciplinary development of decision support tools for determining optimal shade levels and adequate compositions of tree species under various scenarios, with the aim of minimizing damage from pests and diseases while ensuring favourable growing conditions. This is a complicated task, however, as both ecological conditions such as effects of shade trees on incidences of pests and diseases, and socio-economic factors such as age of farmers, or share of on-farm/off-farm income, impact the success of farmers on the ground and thus the adoption of agroforestry.

As agroecosystems are complex, the above challenges need to be tackled with complexity, i.e. diversity. This refers not only to diversity in production, but also to the ways knowledge is produced and shared, both between people and institutions. We as researchers need to reflect this diversity, complexity, and the processes involved in research by (i) integrating social and natural sciences in the design of our projects, and (ii) taking into account different forms of knowledge while regarding phenomena from a perspective that goes beyond specific disciplines and is based on broad participation (Hirsch-Hadorn et al. 2006). The majority of researchers have come to know that their knowledge is complementary rather than superior to that of farmers and other stakeholders. Researchers need to actively pursue the path of transdisciplinary and participatory action research which allows for the conservation and application of local knowledge, while enabling knowledge co-production and mutual learning among farmers, researchers, and other stakeholders such as consumers and policymakers. As Vaast and Somarriba (2014, p. 953) pointed out: "*The selection of tree species and combinations is likely to be most effective where farmers participate, so that their goals and aspirations are taken into account and their local agroforestry knowledge is incorporated into the design and management of the system.*" Such an integrative approach is also more likely to help identify and implement strategies to adapt to multiple stressors; adaptation to climate change impacts, for example, means much more than identifying and planting resistant crops (Pohl et al. 2010).

Bolivia, with its traditional as well as introduced forms of cocoa production, provides an example for transdisciplinary research and co-production of knowledge. Moreover, the cocoa cooperatives have established market chains for both collected and cultivated cocoa under the guiding vision of sustainable agriculture. The unique socio-economic setting of long-standing, well established cocoa cooperatives that engage in organic cocoa production in Alto Beni makes this region particularly suitable to study current and future economic, ecological, and social problems related to cocoa production.

Our research will be complemented by a new project in Ghana entitled "Transdisciplinary systems research to develop a holistic approach to reduce the spread and impact of cocoa swollen shoot virus disease in Ghana (TransdisCSSVD)". In this project, we aims at (i) quantitatively consolidating 75 years of research about the most promising CSSVD control options (meta-analysis), (ii) identifying the main constraints for adoption of available CSSVD control options (farmers' perspective) and (iii) filling an important knowledge gap about the contribution of cocoa production systems' diversification to reducing the spread of CSSVD (e.g. agroforestry; landscapes fragmentation with hedgerows, etc.). Planned dissemination activities include transdisciplinary workshops with policy makers to determine feasible ways of adapting the existing CSSVD prevention and control program. Furthermore, farmer field days and exchange workshops may stimulate implementation of results on the ground. IN addition, the CGIAR Research Program on Integrated Systems for the Humid Tropics launched a new innovation platform in Ghana earlier this year with the aim of facilitating sustainable intensification of cocoa production. These examples underline that researchers are taking steps towards transdisciplinary and participatory action research, making research more solution-oriented and relevant for the livelihoods of cocoa producers throughout the tropics.

Certification standards hold a certain potential to influence the future design of cocoa production systems. While there is the need to better assess the long-term effects of implementing good practices, including agroforestry, developed by eco-certification schemes across a wide range of ecological and socio-economical contexts (ICCO 2014), certification bodies also need to be open to continuous adaptation of their standards according to research results derived from projects with farmer involvement. Our experiences from Bolivia indicate that organic certification alone may not lead to a diversification of cocoa production systems or the implementation of dynamic agroforestry systems (Jacobi et al. 2013). We think that organic and other certification schemes should emphasize the need to diversify in order to foster the resilience of cocoa production systems to factors of global change. In addition, policymakers should address the costs associated with certification schemes, as these may present a major constraint for farmers wishing to obtain certification, and build incentives for organic and agroforestry produce also on the consumer side.

Sood and Mitchell (2006) found the attitude of farmers towards agroforestry systems to be the most important factor of adoption, which highlights the importance of extension programmes for knowledge sharing between agroforestry experts and farmers. Our experiences suggest that especially in areas where farmers face big challenges in their own plantations, the willingness to adopt agroforestry or dynamic agroforestry is high. However, as the perceptions of farmers about different production systems change according to their underlying motivation of engaging in them, such as expected income or knowledge gain on management practices in agroforestry systems, the way experts interact with farmers and their organizations, e.g. on the principles of system approaches such as agroforestry or dynamic agroforestry, needs to be improved. This was the case especially in Côte d'Ivoire, where we observed that this basic principle is not adequately applied. We advocate for

interactive knowledge sharing methods such as farmer field schools, which stimulate farmers' protagonism and give scientists the role of mediators who integrate different forms of knowledge and make them visible to different stakeholders (Pohl et al. 2010). Much can be learnt from the Latin American agroecological movement, such as the "farmer-to-farmer" (campesino a campesino) movement and its learning approaches in which researchers and external consultants are facilitators rather than instructors (Holt-Giménez 2006).

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Chapter 4
Cacao Nutrition and Fertilization

Didier Snoeck, Louis Koko, Joël Joffre, Philippe Bastide, and Patrick Jagoret

Abstract Cocoa is globally the third agricultural commodity traded in terms of value. The cocoa world production is relatively stable since 2010, amounting to around 4.5 million tonnes. Eight countries account for 90 % of the cocoa production, of which four West African countries. Under traditional cultivation practices, cocoa yields are poor with an average of ten fruits per cacao (*Theobroma cacao* L.), even though it has a potential to yield more than 100 fruits. As for most tree crops, the yields are depending on many factors, of which the more important are planting material, climate, cultural practices, and soil. Cacao is cultivated on many types of soil, and in various conditions, from agroforestry systems to full sun. Soil degradation and low soil fertility are among the main causes of low cocoa productivity. However, despite this inherent low fertility, most of the cocoa farmers do not use fertilizer because they are not well informed of the agricultural and fertilizers issues.

Here we first review why fertilizers are used and how to optimize their effects, particularly farming practices and soil fertility management in full sun or shaded plantations. Secondly, we describe soil diagnosis and the foliar diagnosis, the two complementary approaches that were developed to assess the nutritional needs of cacao. The soil diagnosis provides a means to improve soil nutrient availabilities, while foliar diagnosis provides information on the cacao health. Third, we review the methods used to design fertilizer formulae and doses, and how they are calculated. Fertilizer inputs and mode of application are determined from the local conditions and farming practices. Finally, we review the effects of nutrients on the characteristics of the cocoa tree and cacao product. Finally, some current issues are discussed, such as the use of advising a single formula for a whole region or country and how to develop adoption of fertilizer by cocoa farmers.

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4.1   Introduction

Cacao (*Theobroma cacao* L.) is a tree native to the Amazonian forest. It is grown for the pods that contain the beans from which chocolate is made. The economic importance of the cocoa sector has been amply demonstrated. For example, World cocoa production today is about 4 million tons yearly, of which 75 % are produced in Africa (ICCO 2015). Cacao is grown in 70 countries, all located in the tropical belt and provides livelihoods for more than 5 million small farmers (Rafflegeau et al. 2015). So this is basically a smallholder agriculture which, since the 1990s and the liberalization of the sector in many countries, suffered the brunt of the fluctuations of the world market. In terms of agriculture, this crop is characterized by relatively low productivity and a displacement of the production areas. Indeed, after 20–30 years of cultivation or even less, cocoa plantations installed after a forest clearing must be regenerated because of the depletion of soil fertility and biological decline of cacao trees in the absence of adequate pest and disease control and mineral fertilizers (Ruf 2009). At the same time, a consequence of the improvement of living standards in emerging countries like India, China, Brazil and Russia, is the increasing demand for cocoa. According to the International Cocoa Organization, experts estimate that around 2020–2025, one extra million tons of cocoa will be needed to meet consumers' demand (ICCO 2015). To achieve this goal, to secure the supplies and increase the sustainability of current cacao growing areas in order to limit the impact of this crop on the environment, recent initiatives have been launched to provide support to farmers, especially in terms of fertilizing cocoa plantations (Adjehi 2014; IDH 2014).

Under traditional cultivation practices, cocoa yields are poor with an average of ten fruits per cacao, which is very low for a crop that has a potential to yield more than 100 fruits per tree (Aneani and Ofori-Frimpong 2013). The yields depend of many factors, of which the more important are environment, climate, cultural practices, and the soil. Among these, management practices have to take into account that the cacao is cultivated on many types of soils, either in agroforestry systems or in full sun. Moreover, after many decades of cacao cultivation and changing generations of cocoa farmers, soil fertility and available cultivation areas per family are shrinking. Finally, soils are getting depleted because of continuous cropping with little or no added inputs.

Both cacao growers and development experts know how much the cacao plots are getting degraded and need fertilisation to replenish the soil nutrients and recover their production levels and income. Conversely, the mechanisms for adopting fertilizers and their impact are widely ignored. Now, after many years with very little use of fertilizer linked to low prices and low income from cocoa, the cacao growers are willing to increase their production, especially in countries where cacao is mostly grown under full sun or very low shade, as it is the case in Côte d'Ivoire and Ghana.

Fertilisation is therefore a major issue for the future of cacao cultivation and cocoa farmers (Ruf 2012).

Being a shade plant, the ecological requirements of cacao are quite adapted to many regions in the humid tropics. Altogether, cacao turns out to be a plant that can adapt easily to various growing conditions and to many soil types (Smyth 1980), as long as the nutrients can be provided to correct the soil nutrient levels and balances (Jadin 1975). Initially, cacao was grown under shade, but scientists quickly realized that productivity was much better when the trees were grown in full sun (Alvim 1965; Beer 1987). Thus, research has favoured intensive models based on the use of selected varieties, vigorous hybrids, conducted on total forest clearings or light shade and high use of inputs. The example of the Cocoa High Technology Program developed in Ghana in the early 2000 has underlined that such systems allow to obtain high yields. With the help of the project, yields of exceeding one ton of cocoa per hectare could be achieved during a period of more than 10 years thanks to good management, improvement of the soil fertility and reduction of parasitic pressure, following a clearing of the number of shade trees.

The objective of this paper is to review research findings from Africa, Asia, Latin America and the Pacific regions with the aim of coming up with suitable answers to the questions raised by cocoa farmers about fertilization, particularly on how to supply the soil nutrients in correct and balanced amounts to improve and maintain optimal yields on the long term.

4.2 Cacao Nutrition and Nutrient Requirements

To provide adequate and balanced nutrient amounts to support the cacao growth and yields, researchers and development agents need to consider the cacao needs, the current available soil contents and how these soil nutrients can be made available to the cacao tree.

Each plant species has its own nutritional needs and ways of taking up nutrients from the soil. Plant tissues are made up of a number of elements, 16 of which are essential for their physiological development. Three of these, carbon (C), oxygen (O), and hydrogen (H), can be supplied by the atmosphere and water or taken up from the soil. The other 13 can only be absorbed as mineral nutrients from the soil through plant roots. These 13 nutrients are divided into macronutrients that are required in large quantities: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) and into micronutrients that are required in micro quantities: manganese (Mn), boron (B), iron (Fe), zinc (Zn), copper (Cu), aluminium (Al) and molybdenum (Mo).

Each soil has different amounts and balances of nutrients. Their availability depends on many factors, but primarily the origin of the parental material and its geological and pedological development. Soil origins are wide ranging, from basaltic soils in Brazil to granitic gneiss and schist soils in Africa or sedimentary or metamorphic soils in Trinidad (Verlière 1981). Consequently, the soil pedology parameter is essential to determine the types of available nutrients and to calculate their amounts to meet cacao requirements. The initial soil fertility may have to be

adjusted. More specifically, the soil nutrient availability varies with the type of soils, but also with regard to its ease of being taken up by the cacao in relation with the soil cation exchange capacity (Jadin 1975; Loué 1961). Light, soil moisture and associated crops also influence the soil fertility (Afrifa et al. 2009; Ahenkorah et al. 1987; Somarriba et al. 2001; Wessel 2001). The structure, texture, fertility and nutrient availability of a soil depend also on its history. Some important factors influencing the evolution of soil are described below.

The purpose of fertilization is to feed the crop and refill the missing and exported nutrients and achieve the optimum nutrients balance for maximum, sustainable cocoa production. Once the right balance is reached, fertilization has to be continued to maintain the optimum quantities and combinations of ingredients in the soil to replace the nutrient removed by the cacao. For fertilization to be effective, it is essential that all required nutrients are present in the soil (naturally or introduced) and in an available form that the plant can take up. This requires an adequate choice of fertilizer formula, an healthy root system, and conditions favourable to the uptake, especially climate (Hartemink 2005).

The balance between available nutrients supplied by the soil and the demand of the plant will determine the nutritional quality and thus the extent to which the cacao will benefit in each cultivation system. Indeed, even in case of severe deficiency, the only correction of this deficiency is not sufficient to improve yield and the effects of interactions between nutrients are often superior to the effects of nutrient taken alone (Verlière 1981).

4.2.1 First Research Results

The need to fertilize cacao has been known since the time when high yielding cacao trees were first grown in commercial plantations. The first fertilizer trials are found in Trinidad in the 1930s (Mac Donald 1934), then in Cameroon and other countries (Loué 1961). These first results highlighted the strong interaction between fertilization and shade showing that cacao without shading, but with fertilizer inputs, can give huge returns. In the beginning, the trials focused on the effect of each nutrient supplied separately. Trials in Ghana have shown that, under shade, soluble P can increase yields by 20 %, but no response were found for N, K, Ca, Mg and micronutrients (Cunningham and Arnold 1962). Other trials in Côte d'Ivoire have also found that N has no effect when cacao is grown under shade and that the fertilizer should mainly contain P and K, with K being effective only if P levels are correct. Positive effects of Ca and Mg on yields were also observed, but to a lesser extent (Verlière 1965). Later, Murray (1965) showed that, if K fertilization was not efficient on cacao grown under shade, it was because high K contents under shade are common in Trinidad. However, in full sun, it is the combination of N–K inputs that predominates in the choice of nutrients to be used in the fertilizer formulae.

To determine the nutrients requirements, researchers have started investigating the foliar diagnosis as a mean to assess yield requirements. In 1935, the first article

on foliar diagnosis was published by researchers having worked at the Trinidad Imperial College of Tropical Agriculture on a shaded NPK trial (Hardy et al. 1935). The authors observed that best yields were obtained with relatively high levels of leaf K compared to N or P. Later in Costa-Rica, leaf content differences were found between treatments in a fertilizer trial, but only at particular periods of the year (Machicado and Alvim 1957). The correlations between leaf nutrients levels and the cocoa yields were described by Verlière (1965) who could also determine the best period to sample the leaves. His results confirmed previous works done in Trinidad showing that the growth of pods is the most demanding period in nutrients, which are partly derived from the leaves, but no direct correlation is possible. Similar observations were also found in Ghana, Côte d'Ivoire, Nigeria, Malaysia, Colombia, Ecuador, West Irian, DR Congo, São Tome and Brazil (Eernstman 1968; Murray 1965; Wessel 1971).

Verlière (1981) also assessed how ratios between leaf nutrients can be correlated two by two with the plant health or soil nutrients. The studies were done first in a green house on sandy soil receiving various amounts of nutrients, then in fertilizer trials planted in areas of various geological origins. In these trials, the nutrients were added independently one by one. He found that K fertilizer increased significantly leaf K, Ca and Mg, but significantly decreased Cu; Ca fertilizer increased K, Mg but decreased N, P, Cu; Mg fertilizer increased nothing, but decreased K, Ca, Mg; high K/Ca ratio was significantly associated with higher P, Ca, Mg, Cu but low K; high K/Mg ratio was significantly associated with higher Ca, Mg, but low K; high Ca/Mg ratio was significantly associated with higher Mg, but low K, N, Cu.

However, despite the interest of the foliar diagnosis method, Verlière (1981) felt that the method suffers from major limitations that hamper its use. Particularly, he reported that:

– Cacao is very tolerant with respect to the soil. The wide range of soils on which cacao trees are grown obviously implies variations in nutrient absorption, so a broad range of mineral compositions may be observed in cacao trees without major variations in cocoa yields.
– Vegetative and generative activities of cacao trees have very different rates compared to those normally found in many other crops. Equally, the leaf nutrient content is influenced by fertilization, shade, rain, light, temperature, sunlight, evaporation and pod maturation.
– The period when the leaf is sampled is the main factor of variation in leaf mineral composition, even if a leaf of the same age growing in the same position relative to the branch is sampled. It is thus difficult to predict if the tree has enough reserves to produce a large harvest.
– As cacao is cauliflorous, the maximum of pods is located between the roots and the leaves and intercept part of the nutrients before they reach the leaf. Thus, pods may receive sufficient mineral nutrients for their growth while the leaves may not and analysing the leaves may suggest deficiencies that do not influence yields. There may also be serious competition between the cambium of the trunk and branches, pods and leaves for food, growth substances and water. This is

why some researchers have tested using bark as an indicator of pod yields (Humphries 1950; Moss 1964). However the technique had not much success and was abandoned after Verlière (1981) demonstrated good relationships between foliar diagnosis and all other parts of the tree (leaves, branches, trunks, roots) for K, Ca and Mg.

All of this shows that standardization of leaf sampling and its relation with cocoa yield is quite difficult. Particularly, the mineral content of cacao leaves can adapt to light conditions (Cunningham and Burridge 1960; Lachenaud and Oliver 1998). Shaded leaves are able to use very low light while sunlit leaves seem to have an internal resistance to the penetration of light rays. By acting on leaf size, shading causes a dilution effect in the nutrients levels without the total quantity of these necessarily being reduced. Depending on its position on the cacao foliage, a leaf can have diverse foliar density and nutrient levels (Bastide and Jimmy 2003). Cacao leaves also vary greatly with the environment. The leaf chemical content follows the rainfalls distribution and seasons in relation with leaf flushes.

Finally, most researchers found that foliar diagnosis is insufficient to be used as a means to determine the cacao fertilization requirements and that the use of soil testing is preferable For example, Murray (1967) wrote that: "Despite the work that has been done, we must accept the fact that foliar diagnosis is of limited value to plan a cacao fertilizer program". Similarly, Wessel (1971) wrote that the main advantages of the cacao leaf analysis are the detection and identification of nutritional deficiencies and its assistance in interpreting the results of fertilizer trials.

Researchers then focused on soil diagnosis with the objectives to correct the soil nutrient deficiencies and imbalances, firstly to enhance cacao yields and secondly to adapt the soil before planting cacao in new land envisaged for growing cacao. There is need to add nutrients in order to tailor the soil composition to meet cacao requirements. This generally means increasing the nutrient content and correcting the balance between the main cations. It also entails eliminating the toxic effects of nutrients, such as aluminium, manganese or iron, in acidic soils. Fertilizer trials have been carried out in all cocoa producing countries. They have shown that good correlations exist between cocoa yields and soil nutrients levels, thus confirming that the method is well adapted for cacao growing and production improvement (Appiah et al. 2000; Wessel 1971; Ahenkorah et al. 1987; Bénac and Dejardin 1970; Morais 1998; Ojeniyi et al. 1982; Paviot 1977; Santana et al. 1971; Souza Júnior et al. 1999b; Tossah et al. 2006; Wyrley-Birch 1987).

However, despite the many limitations described above, foliar analysis remains a practical tool for detecting nutrient deficiencies, and particularly in the field through visual diagnosis (Machicado and Alvim 1957). The authors concluded that it is a good option to combine both approaches: (1) the soil diagnosis for soil correction, to assess the need for fertilization and to calculate the most suitable fertilizer formula and dose and (2) foliar diagnosis for information on the cacao status and direct overview of imbalances and fine-tune the fertilizer recommendations.

4.2.2 Soil Diagnosis

The principle is based on the fact that the crop feeds from soil nutrients, which have to be available in the soil in diversity and amounts required by the crop. The required thresholds were previously determined from fertilizer trials setup in different regions of the country.

Three methods are currently used for fertilizer calculations: (1) based on thresholds (most of the cacao growing countries. (2) Based on soil nutrient thresholds and the ratios of some specific nutrients; this method is developed in Brazil, mainly and described below (Sect. 4.2.2.1). (3) Based on soil nutrient thresholds and the ratios of all nutrients; this method is developed in Sect. 4.2.2.2).

A fourth method using a combination of both some soil and leaf nutrients also exist; it has been developed in Malaysia only. It is described in Sect. 4.2.4.

4.2.2.1 Method Based on Soil Nutrient Thresholds and Ratios

The method consists in calculating more or less precise fertilizer formulae that are designed to fill in the missing nutrients due to exportation revealed by soil analysis to compensate for nutrient deficiencies. The required thresholds obtained from fertilizer trials were used to draw up tables giving lower or upper thresholds. The recommendation is then to correct the soil assuming that cacao growth and productivity will be optimum if each of the nutrients (macro and micro) is in between these limits. Standards were developed in Côte d'Ivoire, Ghana, Nigeria, Brazil and Malaysia (Mac Donald 1934; Cunningham and Arnold 1962; Machicado and Alvim 1957; Lockard and Asomaning 1965b; Liabeuf and Lotode 1969; Bartley 1970).

Table 4.1 shows a synthesis of the information given in the literature.

Locally, the thresholds might be slightly different because of the influence of local conditions and cacao genotypes (Ribeiro et al. 2008; Rosand and Mariano 1998; Schroth et al. 2001; Snoeck and Jadin 1992; Obiri et al. 2007).

In a second step, researchers added the imbalances between some of the nutrients to improve the formulae recommended. For example, in Brazil, the decision to fertilize is monitored through soil analysis (Malavolta 1997). As recently recalled (Chepote et al. 2013), the fertilizer formulae are calculated from the critical levels of two soil nutrients, P and K and two sets of formulae are given, one for each of the main cacao growing regions (Table 4.2).

In Brazil, specific recommendations for N and soil acidity correction are given separately in addition to the recommendations given in Table 4.2:

– N applications: they are based on light intensity and field observation regarding possible deficiency symptoms.
– Liming is recommended if the concentration sum of Ca plus Mg is lower than 2.0 $cmol \cdot kg^{-1}$ of soil in Amazonia or below 3.0 $cmol \cdot kg^{-1}$ of soil in Bahia at the dose calculated by the formula: CaO (t/ha) = $1.5 \times Al$. Otherwise, Ca and Mg are not recommended.

Table 4.1 Average soil macronutrients and micronutrients thresholds for cacao

	Parameter	Unit	Medium
Macronutrients	pH (H_2O)		5.1–7.0
	C org	%	1.7–3.2
	N total	%	0.2–0.4
	C/N		9.5–15.5
	P avail. (Mehlich)	ppm	6.0–15.0
	P avail. (Olsen)	ppm	12.0–25.0
	K (Ac. Am. pH 7)	me/100 g	0.2–1.2
	Ca (Ac. Am. pH 7)	me/100 g	4.0–18.0
	Mg (Ac. Am. pH 7)	me/100 g	0.9–4.0
	Al (Ac. Am. pH 7)	me/100 g	0.1–1.5
	CEC (Ac. Am. pH 7)	me/100 g	12–30
Micronutrients	Fe (Mehlich)	ppm	19–45
	Mn (Mehlich)	ppm	3–12
	Cu (Mehlich)	ppm	0.4–1.8
	Zn (Mehlich)	ppm	0.5–2.2
	B (Hot water)	ppm	0.16–0.90

Soil data below the lower limit are deficient in the corresponding parameter or nutrient
Note: 1 me/100 g = 1 cmol$^+$·kg^{-1} of soil

Table 4.2 Calculation of soil nutrient requirements for cacao in the two main cacao growing region of Brazil based on soil P and K levels

Fertilization criteria		Nutrients (kg/ha)					
P (ppm)	K (ppm)	Amazonia			Bahia		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
<6	<47	30	90	60	60	90	90
<6	47–117	30	90	30	60	90	45
<6	>117	–	–	–	60	90	–
7–15	<47	30	60	60	60	45	90
7–15	47–117	30	60	30	60	45	45
7–15	>117	15	15	10	60	45	–
>15	>117	–	–	–			

The amounts of N, P, K fertilizers are calculated from the combination of soil P and K limits. The limits are different in Amazonia or Bahia

Realising that it is important to consider both the nutrient levels and their ratios in the soil pool, a more sophisticated model was developed in Côte d'Ivoire (Jadin and Snoeck 1985).

4.2.2.2 Soil Diagnosis Model

Nutrient balances and their levels and ratios measured in many different soils were compared to the vigour and yields of cacao crops in a large number of fertilizer trials. Assumptions were made once the balances were known and classified. They were then implemented and tested in greenhouse and field trials. The results were used to develop a software programme which calculates nutrient levels and the relationship between them with the aim of achieving optimum vigour and yields in the soil concerned (Jadin 1975; Jadin and Snoeck 1985; Snoeck et al. 2006). This method was developed parallel to the other approaches in the four main African cocoa growing countries (Jadin 1975; Loué 1961). The results were satisfactory and the tool has been validated in Côte d'Ivoire (Jadin 1975; Koko et al. 2009, 2011), Togo (Jadin and Vaast 1990) and Ghana (Snoeck et al. 2006).

Required Parameters

Physical The percentage of clay plus fine silt helps agronomists understand the soil type as it is related to the pH and cation exchange capacity (CEC) and can consequently help them choose the right type of fertilizer.

Chemical Analyses The following are necessary (Jadin and Snoeck 1985). The methods are standard laboratory analyses (Blakemore et al. 1987):

- N: total N by the Kjeldahl method.
- C: organic C by the method of Walkley and Black.
- pH: pH in water (pH_{H2O}) is measured at a soil to water ratio of 1:2.5.
- Total phosphorus and available phosphorus: The method used must be appropriate (e.g. the Olsen method, the modified Dabin or Truog method, or the modified Bray2 method).
- Exchangeable cations (K, Ca, Mg, Na) and CEC: Extraction with ammonium acetate at pH 7 is preferred because it gives the real potential of cation levels and CEC in the soil. Therefore, it is best suited for fertilizer determination than methods that give the current levels, but will be modified by the fertilizer applied (e.g. cobaltihexamine).

Calculation of the Nutrients Required for Soil Correction

Acidity: Cacao trees can grow in soil with a pH ranging from 4.6 to 7.5 in the top 20 cm. However, severe production limitations are observed when the pH is below 5.0. Particularly, soils with a pH of less than 5.0 usually lack calcium and should be limed. In acid soils, the amount of lime is computed either to reduce the Al saturation or to increase the base saturation. The two formulae below are possible (Jadin and Snoeck 1985; Kamprath 1970):

- Cao $(kg \cdot ha^{-1}) = Al \times 1.5 \times 28.1 \times 1.4 \times 20$, where Al is in $cmol \cdot kg^{-1}$, 1.5 is to obtain the quantity of CaO to neutralize 85–90 % of exchangeable Al (in a soil with 2–7 % SOM).
- CaO $(kg \cdot ha^{-1}) = CEC \times (BS_{corr} - BS_{soil}) \times 28.1 \times 1.4 \times 20$, where BS_{corr} is the corrected Base Saturation, BS_{soil} is the actual soil BS.

With 28.1 is the ratio to convert 1 $cmol \cdot kg^{-1}$ of Ca into mg of CaO, 1.4 = soil density in $g \cdot cm^{-3}$, and 20 = soil depth in cm.

Moreover, phosphorus availability starts reducing at pH below 5.5 (Jadin and Truong 1987).

Nitrogen: The N demand is calculated from the ratio of exchangeable bases (EB) to nitrogen (N). The EB:N ratio is pH dependent. For each pH, there is an optimal zone where cacao produces the highest yields. These privileged zones can be joined by a line according to the formula: $EB = 89 N + 6.15$; where EB is the sum of the exchangeable bases in $cmol \cdot kg^{-1}$ of soil or mili-equivalent per 100 g of soil (me/100 g) and N is the total nitrogen in % (Fig. 4.1).

C:N Ratio: This provides an indication of the type of OM present in the soil and, in particular, the degree of humification. In tropical soils, the C:N ratio is quite low due to high temperatures and intense microbial activity. The incorporation of partially decomposed organic residues can greatly modify the C:N ratio. Non-decomposed straw residues tend to increase the ratio whereas leguminous residues, with high N content, tend to reduce it. This factor should be taken into consideration when applying organic fertilizers because it could lead to N deficiency. The C:N ratios of several materials are equal to 40 for straw, 20 for acid humus, 12 for good soil (including average cocoa plantations), and below ten for soil poor in organic matter. Verlière (1981) reported a positive correlation between the organic matter content and the C:N ratio of the same horizon on the one hand, and the cacao yield on the other. This demonstrates that the C:N ratio of soil can be a useful indicator of soil fertility in cocoa production.

Available Phosphorus: Critical level of 10 ppm (Olsen-Dabin method) or 5 ppm (Truog or Mehlich extraction). The optimal P value is calculated from the N:P ratio. The value of the regression coefficient will depend on the extraction method used. The optimum ratio is equal to 100 mg/kg with Olsen-Dabin analytical method and 50 mg/kg with the Truog 2 method (Jadin and Snoeck 1985).

By default, the software program proposes an immobilization factor of 50 % if the soil pH is <5. The agronomist can accept or adjust this value.

Similarly, if the soil already has sufficient N and extra N-based fertilizer is added, this may have a negative effect (Ofori-Frimpong et al. 2003; Wyrley-Birch 1987). In such a case, further addition of P will likely improve yields.

Total Phosphorus: The N:P ratio is more important than the threshold level of P. The best yields are obtained with an N total (%):P_2O_5 total (%) ratio of 2.0 (Fig. 4.2). P becomes a limiting factor if this ratio is higher than 2.0. For example,
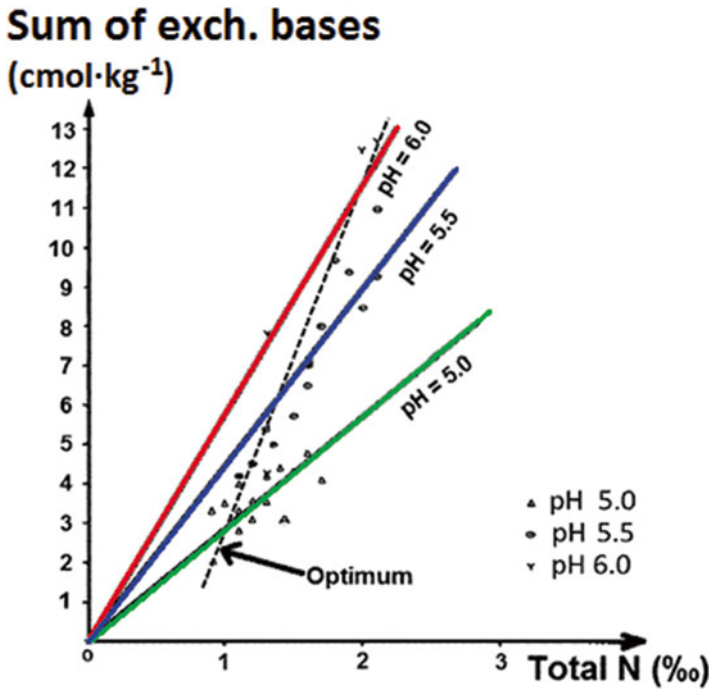
Fig. 4.1 Ratio of total exchangeable bases to N and comparison with the optimum for cocoa yield showing that the exchangeable bases are dependant of both the soil acidity and N. Soils on the right of the optimum require N fertilization (Adapted from Jadin and Snoeck 1985)

in Fig. 4.2, the dots represent the $N:P_2O_5$ ratio in different cocoa plots monitored during a fertilizer trial in the Central Region of Ghana (Snoeck et al. 2006).

The graph suggests the following recommendations for N and P fertilizers to be as close as possible to the optimum. If the soil is below the optimum line, P should be added to increase cocoa yields, thus moving the dot closer to the optimum. However, if the soil is above the line, adding P will move the dot away from the optimum line which is likely to have a negative rather than positive effect.

Potassium, Calcium and Magnesium The K, Ca, and Mg levels must be above threshold values which vary with the soil type. However, the %K, %Ca and %Mg are the most important ratios. These ratios correspond to the % of the three main cations relative to their sum (K+Ca+Mg = 100 %). Optimum cation levels are determined in three steps: (1) Consider the %K ratio, because best yields are obtained with a %K of 8 %; (2) Then, adjust the ratio between Mg and K, which should be Mg:K = 3; (3) The %Ca is calculated from the difference: %Ca = 100 − (8 + 24). Finally, the optimal balance between cations is 8 % K; 68 % Ca; 24 % Mg.

The ratios were confirmed through multi-local trials done in other countries (Jadin 1988; Jadin and Vaast 1990; Snoeck et al. 2006).
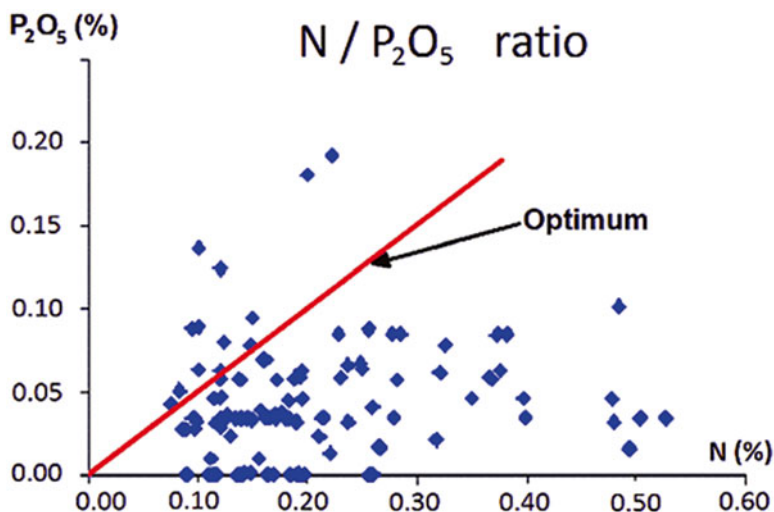
Fig. 4.2 N:P$_2$O$_5$ ratio in cocoa plantations in Ghana and comparison with the optimum for cocoa yield. *Dots* are indicating the N:P$_2$O$_5$ ratios of soil samples taken in cacao plots. *Dots* below the *red optimum line* indicate P-deficient soils that will respond to P fertilization; *dots* above the *optimum line* will respond to N fertilization (Snoeck et al. 2006)

Base Saturation: It is the sum of the basic cations (K+Ca+Mg) divided by the cation exchange capacity (CEC). The minimum threshold is 40 %; but satisfactory yields can only be obtained when the base saturation is higher than 60 %.

4.2.3 Leaf Diagnosis

The principle is based on the idea that a healthier plant can produce higher yields, and on the opinion that the number of pods produced could be linked to the level of nutrients or nutrient balances in leaf (Murray 1956).

4.2.3.1 Chemical Leaf Diagnosis

Leaf analysis is particularly recommended to detect nutrient depletion in the cacao and to follow nutrients variations and imbalances in the cacao over time (years). This means that samplings have to be repeated on the same trees at successive intervals. The diagnosis is based on variations in nutrient levels and their ratios. For example, in soils with low phosphorus contents which are poorly buffered but have a fairly high fixing power, leaf diagnosis can supplement the soil diagnosis to determine whether phosphate fertilization is required. It can also help understand if the

plant will benefit from a given nutrient without causing a deficiency in another one. It gives information on the status of the cacao tree.

The use of models integrating both the levels and ratios between leaf nutrients was recently studied in Brazil (Marrocos et al. 2012). Particularly, the authors tested the Diagnosis and Recommendation Integrated System (DRIS) developed in USA (Walworth and Sumner 1987) which has already been tested on other tropical crops such as coffee. The method is based on the calculation of norms to compute an index for each nutrient. The norms consist of averages and coefficients of variation of relationships among the leaf nutrients. The data linked to lowest productivity cacao trees are used to determine the lower thresholds.

Unlike the works of Verlière (1981) who determined the thresholds and ratios in controlled medium (both green house and long term fertilizer field trials), the DRIS method is based on the thresholds and ratios that are previously determined by comparison with the contents recorded in a reference population.

4.2.3.2 Visual Leaf Diagnosis

This method provides the advantage that it can be used in the field to rapidly detect nutrient deficiency symptoms and take quick action. The deficiency symptoms normally occur when the levels are lower than the minimum thresholds as given in Table 4.1 (above).

The description of deficiency symptoms were described by Loué (1961) and Alvim (1961) and recently recalled by De Souza (2012).

For survey purpose, both macro (N, P, K, Ca, Mg) and micro (Fe, Mn, Zn, Cu, B) nutrients are analysed. For routine assessment, only macronutrients are required.

The interpretation must take into account the numerous factors able to modify the chemical composition of the leaves. Among the factors the influence of shade is fundamental (Burridge et al. 1964; Murray 1956).

The standards were determined from fertilizer trials observations. The ones most used were established in Côte d'Ivoire (Loué 1961), Brazil (Alvim 1961), and Trinidad (Murray 1967; Spector 1964). They are not very different, and served to develop other methods. The data obtained from a combination of data given by various researchers cited above were used to build the thresholds described in Table 4.3.

Particularly, De Souza (2012) showed that the visual leaf analysis interpretation is based on the comparison of actual nutrient levels with the thresholds divided into three zones as described in Fig. 4.3: a zone of deficiency where visual deficiencies are likely visible, a zone of adequate nutrition and a zone of toxicity.

The leaf nutrient contents refer to samples taken from the third leaf of the last maturing flush at the height of 1.5 m above the soil. They were 2–3 months old and were fully active.

The lower thresholds of chemical leaf analysis correspond to the apparition of leaf deficiency. Thus, both leaf diagnosis methods lead to the same interpretation (Malavolta 1997).

Table 4.3 Cacao leaf optimum nutrient thresholds

Macronutrients (%)					
N	P	K	Ca	Mg	S
1.8–2.5	0.17–0.25	1.2–2.4	0.3–1.5	0.2–0.8	0.10–0.25
Micronutrients (ppm)					
B	Cu	Fe	Mn	Mo	Zn
25–70	8–20	50–250	150–750	0.5–1.5	30–150

Actual leaf sample data below the lower limit indicate a nutrient deficiency in the cacao tree; Data above the upper limit indicate a risk of toxicity (Compilation after Loué 1961; Murray 1967; Malavolta 1997; Egbe et al. 1989)
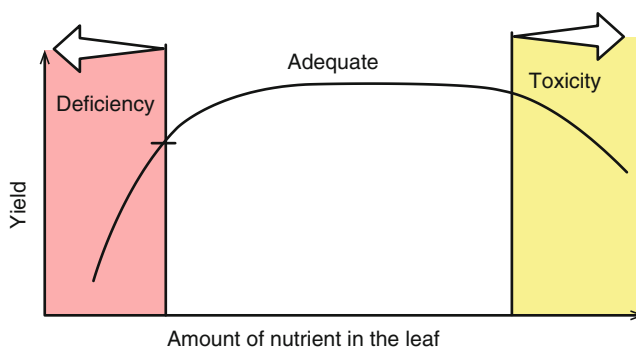
Fig. 4.3 Relation between leaf nutrient and cacao growth or yields. The three zones are used to interpret foliar analysis. Leaf nutrient data in the deficient zone indicates inadequate growth and leaf showing visual deficiency symptom (adapted from De Souza 2012)

4.2.4   Method Based on a Combination of Soil and Leaf NPK Thresholds

In Malaysia, an integrated approach is promoted (Ling 1984). It takes into consideration factors such as leaf nutrient content, trees age, cropping level, soil type, amount of shade, leaching losses and other agronomic factors, such as tree vigour and harvesting system (Table 4.4).

In Sabah (Malaysia), trials revealed that P and K were equally important, but not N (Wyrley-Birch 1987).

This last method is a first step of combination of both approaches: the soil and leaf analyses.

Table 4.4 Calculation of nutrient requirements for mature cacao in Malaysia (after Ling 1984)

Mature cacao – (kg/ha/year)			
Nutrient	Soil	Leaf (%)	Fertilizer rate
N		<2.0	100–150
		2.0–2.6	60–80
		>2.6	–
P_2O_5	<15 ppm[a]	<0.2	90–150
	<15 ppm	>0.2	30–60
	>15 ppm	>0.2	–
K_2O	<0.3 me/100 g	<2.0	120–180
	<0.3 me/100 g	>2.0	80–100
	>0.3 me/100 g	>2.0	–

The amounts of fertilizer to apply are calculated from the combination of soil N, P, K and leaf N, P, K limits

[a]Available P by Bray and Kurtz no. 2; exchangeable K in me/100 g

4.2.5 Practical Recommendations for Fertilizer Use

4.2.5.1 Prerequisite to the Use of Fertilizers

Cropping practices should be properly implemented to maximize the efficiency of nutrients to the benefit of pod production. Good cultural practices begin with the selection of an appropriate growing area, followed by an effective land preparation. Once all is done, the cocoa grower has to decide whether the cacao will be planted in agroforestry (complex or simple association with other tree crops) or in direct sunlight. This should normally influence the choice of planting material, tree planting density and cultural practices that will follow; proper pruning techniques, weed control, integrated pests and diseases control, soil management and tillage to ensure suitable water and nutrients supply. Proper installation of the root system is essential to enhance cacao tree growth and its productivity.

For example, the decision to plant in agroforestry system will influence the soil fertility and amounts of nutrients required (Jagoret et al. 2012; Snoeck et al. 2010).

The selection of appropriate planting material is very important. Indeed, in a plot planted with traditional or hybrid cacao trees, only half of them will produce pods, while the other half produces virtually very few pods or nothing (Bénac 1970). Fertilizers are applied at the foot of each cacao tree in the plot. Ideally, 100 % of the cacao trees on a plot should be productive (Bartley 1970).

Similarly, the importance of good pruning to maximize the fruit:vegetative ratio and optimize the effects of nutrients inputs can be deduced from the study of Thong and Ng (1978). The authors have shown that a 5–6 years-old cacao contains 45.5 kg (dry matter) of the vegetative parts (leaves, stem, branches and roots) but only 1.5 kg of pods (i.e. equivalent to 195 kg cocoa beans ha^{-1}). This clearly indicates that, when fertilizers are applied, nutrient consumption is used to a greater extent for growth than for fruiting. This should prompt farmers to improve their pruning

practices in order to maintain shorter cacao trees, but bearing a maximum of pods. Pruning also improves air circulation and reduces humidity, which limits black pod diseases for healthier pods to thrive.

The potential fertility of a soil depends on how nutrient fluxes are managed in terms of inputs (mainly chemical fertilizers) and factors that could influence the nutrient availability (e.g. environment, type of associated trees, shade trees, farming practices, soil nutrient availability, biomass recycling, etc.) and outputs (leaching, evaporation, erosion, and harvested crop). In this context, the decision to plant in agroforestry system and how to manage them is an important aspect (Schroth et al. 2001).

In addition, fertilization of a tree crop requires long term management because mature trees have a buffer capacity that may differ responses to fertilization over several years (Viroux and Jadin 1993). The levels of nutrients in the soil are changing continuously, and once the soil is corrected regarding the nutrient levels and ratios, the fertilization programme should continue and focus on the nutrients exported through pods harvested and used for tree growth.

Selection of Suitable Area

Prior to plant a cacao field, the cocoa grower has to select suitable land. Particularly, he should consider the quality of the soil by carrying out physico-chemical analyses, supplemented by soil profiles studies.

The physical and chemical characteristics of a soil, at a given time in its history, are the result of the development of a combination of factors: environment, bedrock, rainfall, temperature, farming practices, including the level of shade and, to a certain extent, the variety (Afrifa et al. 2009; Ekanade 1987; Lotodé and Jadin 1981; Malavolta 1997; Snoeck et al. 2006).

Substratum

The potential fertility of a soil depends on the bed rock from which it was formed. For example, Verlière (1981) showed that soils in Côte d'Ivoire derived from:

- Tertiary sands that are often deficient in N and quite always very deficient in K;
- Granitic rocks poor in P;
- Schist rocks deficient in both P and K.

The influence of the soil origin on the soil fertility under cacao was also demonstrated in Brazil (Cabala-Rosand et al. 1971; Santana and Igue 1972). The authors showed that Nitosols are much richer than Latosols, particularly in N, P, and micronutrient reserves. The suitable soils for cacao growing were described by Smyth (1980).
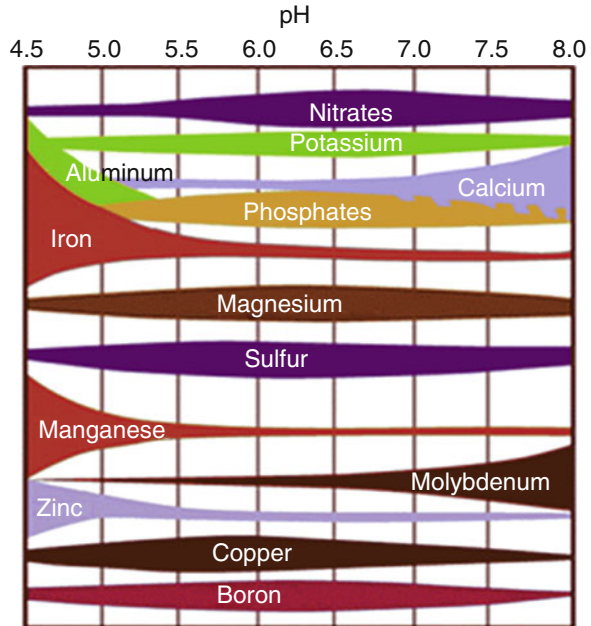
Soil Texture and Depth

The ideal soil texture contains 30 % clay, 50 % sand, and 20 % silt. However, the most important factor is the soil depth, to enable good development of cacao roots, which must be free of physical limitations (Smyth 1966).

Soil should allow root growth to at least 1 m depth (Smyth 1966). However, the presence of pebbles or stones in the soil profile, as well as the colour of the horizons are not significantly correlated to the tree yield (Souza Júnior et al. 1999a, b).

Water availability, oxygen and growth are impaired under high density planting, compaction, continuous rocks, concretions and/or deficient drainage (Silva and Carvalho Filho 1969).

Soil Acidity and Aluminium Toxicity

Soil acidity, either native or due to use of acidifying fertilizer, has detrimental effects on nutrient availability (Fig. 4.4) and leads to aluminium toxicity. Aluminium toxicity is partly responsible of poor phosphorus availability because the Al^{+++} ion tends to accumulate in the roots and inhibits uptake and translocation of both P and Ca to the aerial portion of the plant (Sanchez 1976).

Fe ions also have a detrimental effect on P fixation in the soil (de Geus 1973; Kamprath 1970).

It is worth mentioning that cacao appears to be highly sensitive to Al toxicity in acid soil. This makes cacao an important exception among native Amazonian species, which are normally highly tolerant to acid soils. Soil pH is the parameter

having the most significant effect on fertilizer efficiency; thus, it can be used as a good predictor of cacao yields (Fearnside and Filho 2001; Hardy 1961).

Green house trials have confirm the reduction of root biomass and root length in acid soils. There is also a reduction of Ca, Mg, K, Cu, Fe, Mn and Zn uptake (Baligar and Fageria 2005).

Soil Water Availability

Cacao being a typical plant of the humid tropics, it requires a high quantity of water. It is sensitive to a lack of soil moisture and a water deficiency causes dire problems (Jadin and Snoeck 1981). The cocoa yields in quantity and distribution are determined more by the rain than by any other ecological factors (Alvim and Alvim 1980). Trees grown on soils with a low buffer capacity and low organic matter content are the most affected by water stress in drier years.

Soil salinity should not be higher than 0.6 dS/m; yield reduction of 10 % was noticed in soils having 1 dS/m (Smyth 1966).

Where rainfall is below 1,200 mm and poorly distributed in the year with a dry period than 30 days, cacao can only develop successfully under irrigation. This is the case in Venezuela, where the precipitation is 700–800 mm/year (Alvim 1965) and in the North of Espírito Santo, where rainfall occurs during a few months of the year, in spite of an annual precipitation of 1,200 mm/year (Malavolta 1997).

In Côte d'Ivoire, Verlière (1970) studied the influence of soil humidity regimes on the development of three different cacao varieties groups (Amelonado, Upper Amazon and Trinitario). He found very different reaction of the varieties to the ecological factors. In particular, different water regimes produced different effects regarding the trunk circumference, number of foliar shoots, and water use by the cacao. One reason could be that cacao cultivars with an efficient stomatal regulation mechanism lose less water by transpiration under water stress, which indicates an important adaptation strategy (Balasimha et al. 1988).

Fertilization trials in Côte d'Ivoire showed that with fertilization and irrigation, the number of harvested pods was 63 % higher than in non-irrigated control cacao plots (Jadin and Paulin 1988).

The scarcity of studies on how soil moisture influences production in cacao plantations might be due to the fact that cacao is mainly grown in regions where, characteristically, the total annual precipitation outstrips water losses by evapotranspiration (Moser et al. 2010).

Soil Organic Matter

Soil organic matter (SOM) plays an important role in the cacao nutrition because of its influence on the physiological, chemical and biological characteristics of the soil. It also makes the soil more porous and favours water infiltration, while

Effective CEC (cmol ·dm⁻³)
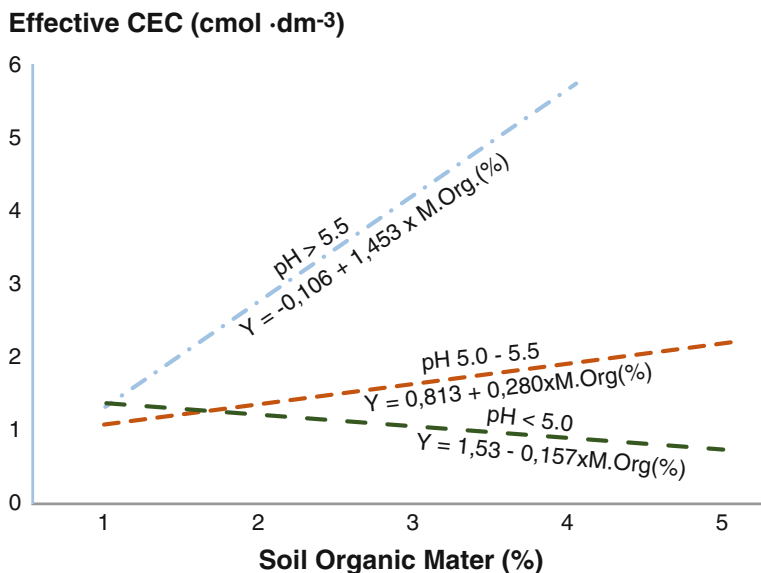
Fig. 4.5 Variation of e-CEC in relation with soil organic matter and pH showing that the CEC is dependant of both the soil acidity and the soil organic matter. *CEC* cation exchange capacity (Adapted from Malavolta 1997)

reducing erosion and activating animal life. SOM considerably improves the cation exchange capacity (CEC) of tropical soils and helps limit soil acidity (Fig. 4.5). This is important because a high level of soil acidity reduces microbial activity, as well as toxicity caused by the presence of available aluminium or manganese. SOM in the soil also encourages the activity of various microorganisms like mycorrhiza, rhizobia and other organisms. Microbiological activity plays a role in protecting crops but also in soil fertility and nutrient availability (Rousseau et al. 2012; Silva Moço et al. 2009; Snoeck et al. 2009). Consequently, it is very important to preserve the native humus level in the soil. This must be taken into consideration right from the start, particularly when preparing the land prior to planting cacao (Smyth 1966).

SOM should be preserved and, where possible, improved by good cultivation practices. At least 3 % of organic matter is required for minimum cacao growth (Smyth 1980; Somarriba et al. 2013).

The use of mulch significantly reduces the need for chemical fertilization and ensures a considerable input of SOM, which enriches the soil in nutrients, mainly potassium. However, mulching requires regular checks of the cations ratio to avoid imbalance between magnesium and potassium. Excess potassium shortens leaf life and accelerates leaf fall. It also reduces the efficiency of N fertilizers. In all cases, mulching enhances the efficiency of mineral fertilizers and the water retention capacity of the soil.

Type of Planting Material

The choice of planting material should be done considering whether the varieties or hybrids will be adapted to the cocoa farmer's decision. Particularly: local soil and climatic conditions, decision to plant in full sun, crop associations or agroforestry, and ability to practice good cultural practices and cacao pruning.

For example, in Brazil, trials have shown that clones or hybrids do not respond similarly to phosphate fertilizer, mainly because clones were planted without tap root (Pacheco et al. 2005; Rosand and Mariano 1998). Similarly, different responses to N and P by different varieties were also observed in Ghana (Afrifa et al. 2003) and Brazil (Ribeiro et al. 2008).

4.2.5.2   Role of Nutrients in Fertilizer and Recommendation for Their Application

Macronutrients

Nitrogen: N is not systematically required. For example, in a legume-cacao association, Kurppa et al. (2010) found limited net N transfer from associated legume tree species to cacao in spite of active N_2 fixation. No response to N application has generally been found in fertilizer trials, particularly in Côte d'Ivoire (Lotodé and Jadin 1981), Ghana (Ofori-Frimpong et al. 2003), or Sri-Lanka (Heenkende and Gunarantne 2000). In Cameroon, the application of N produced a depressive effect on the cocoa yields (Liabeuf and Lotode 1969). Some reasons are that N is already provided from other natural sources, such as Rainfall or litter decomposition, in amounts that are sufficient for shaded cacao trees (Ojeniyi et al. 1982). It is therefore important to apply N fertilizer only when it is a limiting factor, which is computed from the N:P and N:exchangeable bases ratios (Jadin and Snoeck 1985).

N requirements are also dependant of the cultural practices and amount of other nutrients applied. For example, in Ghana, the soil diagnosis has shown a need for N in plots that received repeated applications of P and K and in highly anthropized plots, where N was not recommended 20 years ago (Snoeck et al. 2010).

If N is needed, the agronomist selects a suitable formula (N alone or NPK) depending on the recommendation and the soil conditions. N can be brought in multiple forms as N uptake by the cacao occurs in both nitric and ammoniac forms (Santana 1982). Urea requires to be buried to avoid the lost by ammonia (NH_3) volatilization of a part of N. The rest will convert in the form of ammonium ($NH4^+$). Finally, both forms have an acidifying effect: on the one hand, the ammonium form is an active energy consumer releasing H^+ in the rhizosphere developing a very localized but strong acidifying effect; on the other hand, if the nitrification is not impaired by too acidic soil conditions, the conversion from ammonium ($NH4^+$) to nitrate ($NO3^-$) also releases H^+, thus contributing to soil acidification.

Urea is a cheap source of N, but as it acidifies the soil it might not be the best choice for acid soils.

N added as ammonium (e.g. ammonium sulphate) has a worse acidifying effect because the conversion from ammonium to nitrate releases two ions H$^+$ (instead of one in the conversion of urea to nitrate). The N uptake through ammonium fertilizers or urea, competes with the uptake of other cations, K, Mg, and Ca.

N added as nitrate (e.g. Calcium nitrate) is directly available but can be easily leached; thus, any application has to be avoided during heavy rains. It is the best form of N for its synergy with K, Mg, and Ca (passive uptake, concomitant uptake of cations and anions, "+" and "−", without antagonism). As a result, nitrate does not release H$^+$ and so, it has no acidifying effect. Or the contrary: the nitrate form (NO3$^-$) can have a favourable basifying effect in the rhizosphere, releasing OH$^-$ ion if no concomitant cation is taken by the roots.

Phosphorus: The type of phosphate fertilizer depends on the soil pH. In acid soil, the use of soft rock phosphate (RP) or triple super-phosphate (TSP) are recommended because they have a high CaO content (20 % for TSP and up to 45 % for RP) helping the release of phosphorus in acid soil. It also helps to increase the soil pH. However, the associated CaO must be controlled because improper use could unbalance the cations ratios beyond a point where the correction becomes impossible. At pH >5.5, either triple-super, or single-super, or di-calcium phosphate can be used. The choice will depend on the amount of CaO required. The amount of P also depends on the immobilization factor of the soil towards the (P$_2$O$_5$) oxide (Jadin and Truong 1987).

Phosphorus is not very mobile in the soil, but its behaviour will depend on its solubility in the soil. The part of the quick P that is not rapidly taken by the roots will be fixed by aluminium and iron in acid soils (and by calcium in calcareous soils). Alternately, the P form that is not water soluble requires a soil reaction.

When using water soluble phosphorus (e.g. TSP), localized application is preferable. It will provoke localized saturation that will minimize the P immobilization by less reaction with Al and Fe.

When using non water soluble phosphorus, broadcasted application are required to maximize the contact with the soil to facilitate its solubility. Di-calcium Phosphate and Fuse Magnesium Phosphate are close to 100 % citric acid soluble (2 % citric acid solution method). Rock Phosphate can be hard (or very hard if it has an igneous origin) and requires strong (or very strong) acid attack to become soluble (like with phosphoric, sulphuric or nitric acids in fertilizer factories). Alternately, it can be soft if it has a sedimentary origin and can be directly used on acid soils. Reactive Rock Phosphate have a "crystal" shape that is "more like a coral than a sphere" enabling more contact surface with the soil. The best reactive phosphate rocks have a surface area of more than 20 m^2 per gram of product and about 70 % of P$_2$O$_5$ soluble in formic acid (2 % formic acid method) as demonstrated in the BET theory (Brunauer et al. 1938). To maximize the contact with the soil, the particle size of a rock phosphate must be less than 100 μ.

As phosphorus increases flowering, it must be applied at the beginning of the first heavy rainfalls.

Potassium: K is often supplied as KCl. The chloride accompanying ion can be toxic, leading to tip leave scorching, particularly in dry season. KCL releases 60 % of K_2O, but also 47 % of Cl. Cl is more rapidly leached than sulphate and will contribute to soil acidification by loss of cations in the top soil. If N is also needed and applied as NPK fertilizer, then, part of the K_2O is already added through NPK fertilizer.

The best period to apply K_2O fertilizer is the pod set and development, as they are very rich in K. As potash fertilizer is highly soluble and therefore easily leached, it is recommended that the applications be split: half the dose during flowering and the other half 2–3 months later.

Calcium: Ca is normally supplied in the form of lime; or as dolomite if CaO:MgO requirements are close to dolomite values. CaO and MgO can be applied together or separately, along with the first potassium application. If N was required and calcium nitrate was used, or if P was required and Ca-based fertilizer (e.g. TSP) was used, the amount of applied Ca along with the other fertilizers must be taken into account when calculating the amount of CaO fertilizer.

Magnesium: Mg is normally supplied in the form of dolomite (Magnesium carbonate requiring long time to be dissolved, an option for very acidic soils adequate quantity according to Soil Diagnosis) or kieserite (Epsom salt – a soluble Magnesium Sulphate to prefer in NPKMg fertilizers for quicker supply for the tree uptake, avoiding short term antagonism with Potassium coming from soluble fertilizers). If magnesium is given alone, the best time is at the end of the rainy season, because magnesium supports the leaf retention and delays their senescence.

Aluminium: Al concentration over 2 mg/kg hinders the absorption of calcium, magnesium, ammonium N, iron, boron, zinc, and manganese. Toxicity will most likely occur at low soil pH along with low cation contents. The aluminium content in the soil should be measured to calculate the required calcium fertilizer to correct soil acidity. Agricultural gypsum can contribute to CaO supply without impact on the soil pH and, thanks to the relative water solubility of this form as Calcium Sulphate, penetrate deeper in the sub-soil (sub-soil liming with aluminium detoxification as main benefit thank to the combination of the gypsum based sulphate in non-root toxic aluminium sulphate (vs. the high toxicity of Al^{+++}).

Micronutrients

Boron: B is absorbed through the roots as non-dissociated boric acid. Correction is done by applying 20–30 kg/ha borax (11.3 % B) or other B-containing substances to the soil in a ring around the tree, or by foliar spray (200–300 g/l Solubor, Polybor or boric acid) repeated 3–4 times per year. There may be a danger of toxicity after several years (Malavolta 1997).

On an industrial plantation in Côte d'Ivoire, where yields were low, Loué and Drouineau (1993) could increase the yields by 180 % after an application of borax at the rate of 4.4 g B per tree. More recently, in a trial conducted in Côte d'Ivoire, a

significant positive correlation was noted between cocoa yields and soil boron levels, either already present in the soil or added through fertilization (Stemler 2012). Upper Amazon are more sensible to boron deficiency than Amelonado trees (Lachenaud 1995). This author also found that boron deficient cacao has incomplete pod filling. Thus, the application of borax to cacao grown in full sun and deficient soil, can increase the number of normal-sized beans per pod of three (deficient SCA6: 33 beans, with borax: 36 beans) and reduce the number of flat beans (from 3.5 to 1.9 %).

The positive effect of boron on cacao productivity was also observed in Nigeria (Ojeniyi et al. 1981).

Deficiencies are more common on sandy soils, with low organic matter and low pH.

Zinc: Zn is absorbed through the roots as a bivalent ion Zn^{2+} or as a chelate. Its deficiency is common in acid or exhausted soils. Correction is done by applying 10–20 kg/ha $ZnSO_4$ to the soil, or by foliar spray of 1 % zinc sulphate or zinc oxide repeated 2–3 times per year.

Main causes of zinc deficiency: highly weathered acid soils, but also in calcareous soils with high pH and poor soil aeration. Typical symptoms of zinc deficiency were in particular noticed on field borders and "light holes" in Ivory Coast (Loué and Drouineau 1993).

Copper: Cu is more common on soil with pH >7.5, or if excess N is applied.

Manganese: Mn is more common on soil with pH >7.5. Deficiencies occur with increasing soil pH and aeration, when insoluble Mn oxides are formed.

Iron: Fe deficiency is more common on alkaline soil.

4.2.5.3 Soil and Leaf Sampling

Soil Sampling

Soil sampling is an important step in soil fertility evaluation. Precise recommendations of fertilizers doses and formulae are directly proportional to the extent of good sampling. If the sample is not representative of the area, this could lead to wrong recommendations, whatever the quality of the laboratory.

Soil samples should be taken at the beginning of the rainy season when the soil organic matter is undergoing active mineralization and nitrates are being generated. This also corresponds to the period of vegetative growth and pod development.

Soil is collected in the area which normally receives fertilizer, i.e. a ring under the canopy. After removing any decayed debris on the soil surface, samples should be taken with an auger to a depth of 20 cm, i.e. where more than 80 % of the feeder roots are concentrated (de Geus 1973; Leite and Valle 1990; Wessel 2001; Moser et al. 2010). For larger farms, soil diagnosis can be performed to draw up specific recommendations at the plot level.

Soil samples are taken in the whole plantations. To reduce the amount of samples without reducing the area representativeness, it advised to mix some samples of the same area into one composite sample, which will be analysed. A composite sample consists of a mix of 30 borings collected under different trees in each homogeneous type of soil within a plot. At least 30 borings are required to reduce the variation under 20 % (Jadin 1988).

Soil analysis should be performed regularly every 4–5 years to check for possible variations in the soil fertility of the plantation and adjust the fertilizer formulae, if required.

Leaf Sampling

Sampling Number: In trials, four leaves per cacao tree are harvested on 25 cacao trees at random in each plot. In surveys, a visual evaluation is done to delimit the blocks, then 50 pairs of leaves are taken on 25 cacaos.

From 50 cacaos sampled in a same field, Acquaye (1964) noted that leaf nutrient variations between trees were very large and differences could range from one- to twofold or even threefold for phosphorus. A minimum of 40 trees must be sampled to obtain an acceptable standard deviation (below 10 %).

Sampling Position: Sampling position: Depending on its position on the tree, cacao leaf can contain very different nutrient amounts. It is therefore very important to well determine which leaves to harvest and do not modify the position from 1 year to the other (Orchard et al. 1981). The authors suggest harvesting the leaf at the fourth development stage (stage I.2: Newly produced leaves dark green with a thick cuticle and dormant apical bud). Loué (1961) recommends harvesting the second leaves of the first flush that has become mature. But he noticed that other leaves can be green and that mature leaves can be of different colours. Verlière (1970) suggested sampling leaves with the petiole still green, but becoming brownish.

Sampling Period: The hour of the day is also important as it can be a source of irregularities; the early morning is the most regular period (Acquaye 1964). The leaf age is another important parameter that can modify the dry matter content. Foliar diagnosis is sensitive to the sampling period because the nutrient levels vary with the seasons; particularly, N, P and K are higher in the dry season than in the rainy season. Calcium varies inversely of potassium and magnesium has low amplitude fluctuations following those of calcium (Alvim 1961).

Recommendation for Leaf Sampling: The most commonly used recommendation is to harvest the third or fourth leaf at breast height that is lighted (i.e. the Southern leaves in the North hemisphere and vice-versa) and after the start of rainy season when foliar activity is highest (Bastide et al. 2003).
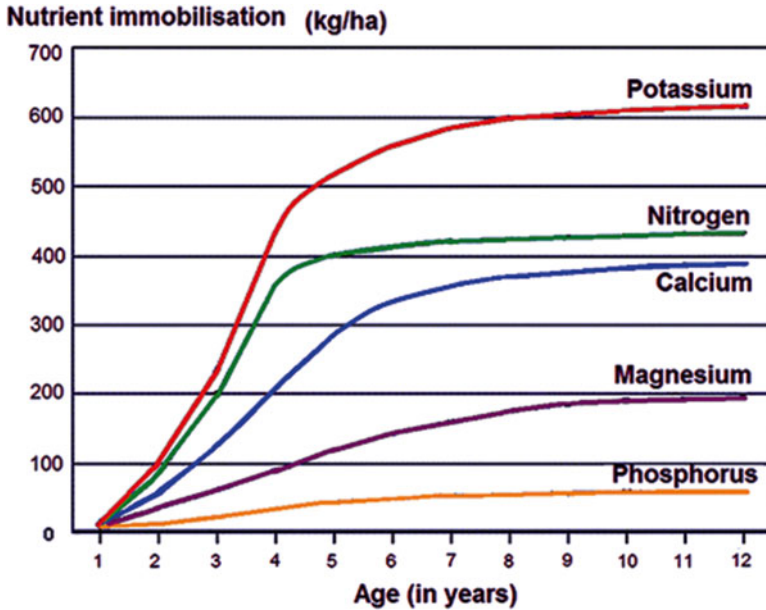
Fig. 4.6 Nutrient uptakes increase with the age of cacao from 0 to 12 years (Thong and Ng 1980)

4.2.5.4 Mode of Application of Fertilizer

Fertilizer Requirements by Age

Nutrient uptake by cacao increases with the tree age but at different speeds depending on the nutrient. For example, N uptake increases during the young stage and levels off after year 5. However, K and Mg uptake increase from year 10, i.e. when the cacao had already started producing (Fig. 4.6).

It is important to know that, regardless of environmental conditions and any effects of the soil, the nutritional requirements are growing from seedlings to mature cacao trees, with a maximum yield is attained after about 10 years (Thong and Ng 1980). Then fertilizer amounts can be reduced because the cocoa yields will drop while age increases as demonstrated by the age-yield curves (Ryan et al. 2009), either in full sun or associated (Obiri et al. 2007; Snoeck et al. 2013). This suggests that a fertilization programme should be based on actual nutrient requirements in plots containing mature cacao; therefore, for cacao above 20–25 years old the fertilizer impact will be reduced. However, it is important to be aware that nutrients applied have a residual effect that can last up to 3-year after having stopped the fertilizer application (Cabala-Rosand et al. 1971).

Practically, this shows that:

– For young cacao, the requirements can be reduced in proportion to the corresponding age.

– For mature cacao, the fertilization programme involves two steps: (i) correcting the soil nutrient levels and ratios to optimize nutrient availability; (ii) supplying nutrients to compensate for those used for physiological maintenance, lost through leaching, pruning, or those exported from the field when the pods are harvested.

Fertilizer Application Periods

Normally, fertilization is applied in four stages: in the nursery, in the planting hole, on young plants and on adult trees in production.

In the nursery, fertilizer can generally be applied every 2 months once the seedlings have two or three pairs of leaves. Fertilizer in solution or in a leaf spray is recommended.

In the planting hole, application of well-rotten cattle manure or compost (about 20 L per tree) is usually recommended. Lime should be applied prior to planting and incorporated in the soil to obtain a pH >5.5 at the minimum.

On young cacao growing in the field, three applications of fertilizer can be carried out during the rainy season. The formulae should be based on the results of the soil analysis. Fertilizer applications have to be more frequent (i.e. 3–4 times per year) to minimize losses.

Mature cacao producing fruits can be fertilized at the same time as growing trees, but the formulae and fertilizer rates should be tailored to the soil analysis results and expected yields. Fertilizer applications should be split into at least two applications per year, with the basal dressing applied at the onset of the rainy season (Chepote et al. 2013; Jadin and Snoeck 1985; Malavolta 1997).

As a general rule, 3 or 4 applications per year of N in the rainy season are advisable. The first two applications can be combined with other nutrients like potassium, phosphorus, magnesium and, where necessary, boron.

As phosphorus is less mobile, it can be supplied in one application at the beginning of the rainy season. Phosphate dislocates nitrate and the accompanying cation can dislocate potassium in the adsorption complex. As a result, large quantities of phosphorus fertilizers should not be applied at once.

As P increases flowering, it is best applied at the start of rainy season (April in the Northern Hemisphere).

As K is required for pod set and pod growth, and since it is very soluble, it is advised to split the applications in two; one after flowering and 3 months later (in the North: June and September).

Calcareous and N fertilizer doses should be separated by a period of at least 2 months to limit denitrification, or the diameter of the rings should be increased to apply both compounds in a larger ring so that the lime concentration is smaller.

Ca and Mg should be applied at the same time as the first K applications.

Fertilizer Application Localisations

To reduce the fertilization cost, it is recommended not to fertilize the entire plot, but rather to apply the fertilizers either in a ring or a crown around the base of the trunk as roots are mainly growing under the cacao tree canopy (Jadin 1975) or in strips (alleys) along the rows. On big farms, mechanization of fertilizer application can be done on alleys in a 0.8 m band in the middle of cacao rows planted at 3 m spacing. The area corrected represents about 27 % of the total surface. Application in a ring around the base of the trunk is preferred for young trees. The diameter of the ring should be similar to that of the tree canopy. Fertilizer applied in strips along rows is a method generally used for high density adult plantations. To avoid a toxic salt concentration, chemical fertilizers should not be applied to young cacao until at least 3–4 weeks after field planting.

Nitrogen fertilizers can be applied on the soil surface, but their application should be spaced out to avoid losses through rainfall or flooding and to ensure regular feeding of the cacao plants. When using urea N, which is rather volatile, it is advised to place the fertilizer under the litter.

Lime should be incorporated before planting, before rainy season. In mature plantation, applications should be split to prevent the formation of a hard crust on the soil surface.

The quantity of fertilizer to apply must also take the fertilization efficiency into account, which depends on soil pH. For example, at pH 4.5, only 30 % of N supplied in fertilizer will be available to the plants, but in the same soil at pH 6.0, 80 % of the N will be available (Jadin and Snoeck 1985).

Besides, fertilizers do not need to be applied to all cacao trees. Indeed, only a portion of the cacao trees are producing (Bartley 1970; Bénac and Dejardin 1970). The authors found that, in a cacao plot, 7.9 % of the trees are unproductive, 34.5 % produce little yields (<12 pods/tree), 27.2 % produce average yields (12–20 pods/tree), 21.7 % produce good yields (20–50 pods/tree), and the remaining 8.7 % produce high yields (>50 pods/tree).

Use of Organic Fertilizers

The recommended amounts of organic matter are normally calculated from the amounts of equivalent nutrients provided by the organic compounds.

Studies carried out in an Oxisol showed that the use of $8 \, t \cdot ha^{-1} \cdot year^{-1}$ of cocoa husk compost promoted an increment of 133 % in cocoa dry seed production, as compared to the treatment without fertilizer (Chepote 2003). It was also found that the application of $4 \, kg \cdot plant^{-1} \cdot year^{-1}$ of cocoa husk and cattle manure compost +50 % of mineral fertilizer (13 % N, 35 % P_2O_5 and 10 % K_2O) promoted a 188 % increase as compared to the plot without fertilizer.

Similarly, in Côte d'Ivoire, the use of compost enhances the effect of P fertilizer by increasing the soil P status (Koko et al. 2013). The authors showed that the

application of 8 t·ha⁻¹·year⁻¹ of compost of cocoa +184 kg·ha⁻¹·year⁻¹ (as TSP) increased the cocoa yield of 204 %.

Similar recommendations are done in other countries, such as Brazil (Lima et al. 2012) or Nigeria (Ogunlade et al. 2012).

4.2.5.5 Fertilizer Recommendations Once the Soil Is Corrected

When fertilizing cacao, two separate but necessary aspects should be considered: (1) compensating for real deficiencies revealed by soil analyses and (2) replacing nutrients removed by harvested beans to ensure growth.

All recommendations found in the literature and described above apply to deficient or imbalanced soils with the objective to correct the nutrient levels while also correcting imbalances. Once the soil is corrected, new recommendations have to be applied. For example, in the tables given for Brazil or Malaysia, no fertilizers are necessary when available P is >15 ppm and available K is >0.3 cmol·kg⁻¹ soil. Similarly, calculations using the soil diagnosis model will conclude on no fertilization for soil correction and only the amounts computed to compensate for nutrients exported by the harvests will be applied.

However, once the soil is corrected, fertilization must not be stopped but continued to compensate for the nutrients exported through pod harvest but also through leaching or immobilised by the cacao for its growth. Indeed, the nutrient cycling balance is negative in the cocoa production system and mineral or organic fertilizer compensation is essential (Afrifa et al. 2009; Appiah et al. 1997; Hartemink 2005). The authors highlighted the negative balance of the nutrients cycles in a cacao plantation, showing that outputs are greater than inputs. These parameters therefore have to be taken into account in the calculation of nutrients to include in the routine fertilizer formula.

– Outputs are due to immobilisation by the crops (uptake for growth and maintenance) plus harvests and soil leaching. In particular:

  • Exports through harvests of 1000 kg of cocoa dry beans=21.1 kg N+8.6 kg P+11.1 kg K+1.1 kg Ca+4.0 kg Mg.
  • For each 1000 kg of dry beans, there is 1400 kg of pod husk produced. If not returned to the plantation field (after composting), then the following amounts of nutrients are also exported: 14.0 kg N+4.2 kg P+68.0 kg K+6.6 kg Ca+6.5 kg Mg.
  • Immobilisation: 4 kg.ha⁻¹.year⁻¹ N+2 kg.ha⁻¹.year⁻¹ P+6 kg.ha⁻¹.year⁻¹ K.
  • Leaching: 5.2 kg.ha⁻¹.year⁻¹ N+0.5 kg.ha⁻¹.year⁻¹ P+1.5 kg.ha⁻¹.year⁻¹ K.

– Inputs come from rainfall deposition (5–12 kg·ha⁻¹·year⁻¹ N+0.2–3.0 kg·ha⁻¹·year⁻¹ P+2.5–12 kg·ha⁻¹·year⁻¹ K), litterfalls (130 kg·ha⁻¹·year⁻¹ N+12 kg·ha⁻¹·year⁻¹ P+65 kg·ha⁻¹·year⁻¹ K), and fertilizers.

A big part of the output is the nutrient export with the husks. Altogether, it is quite an important exportation of K. In field, husk recycling should be promoted to decrease the net nutrients removal. In addition, the husk left in the cocoa plantation will contribute to better maintain soil carbon and organic matter.

Foliar diagnosis can be a good tool to monitor variations in a cacao field under controlled farming management over years because leaf analysis can provide a good idea of the nutritional status of a plant at a given time. Particularly, once the soil has been amended, leaf diagnosis can play a useful role in monitoring changes in the behaviour of cacao influenced by different cultivation practices. In particular, it is useful to determine continued fertilization requirements as this depends partially on the ability of the varieties to assimilate different nutrients (Chepote et al. 2013).

Both soil and foliar analyses have to be repeated at successive intervals because the nutrients levels and ratios in both soil and plant are in constant evolution. Particularly because in good soil conditions, the cocoa yields will increase and these surplus will induce new imbalances in the soil and will require new fertilizer doses (Viroux and Jadin 1993).

4.3 Effect of Nutrients on Some Cacao Physiological Characteristics

Fertilizers are required to increase yields and replace for nutrients exported by harvest or leached. However, if not properly used they can have negative effects. There is need to better know the impact of fertilizers on other characteristics of the tree and other production parameters.

4.3.1 On Cacao Growth and Yields

4.3.1.1 Macronutrients

Nitrogen

N is essential for the vegetative growth of the trees. It boosts the development of branches and leaves. It also greatly influences yields by increasing the number of flowers and pods, and by extending leaf life. It helps to fight dieback (Santana and Cabala 1982). In case of deficiency, the leaves fall and the branches gradually wither from tip to base, provoking the dieback of the tree. Tree growth slows down (Verlière 1981). Higher levels of nitrate N in the 14–110 ppm range in the nutrient medium usually produce marked increases in the cacao growth (Lockard and Burridge 1965; Loué 1961), while a level of 220 ppm tends to delay growth and produce

characteristic symptoms of leaf scorch reminiscent of Ca deficiency suggesting a possible interaction between N and Ca in the cacao tree (Chepote et al. 2013). Similarly, this author noticed that leaf K was found to decrease with increasing N levels in the nutrient medium.

Phosphorus

P is necessary for the development of roots, wood and young buds, and for flowering. It is absorbed in the form of $H_2PO_4^-$ and HPO_4^{2-} ions to produce organic components (Morais 1998). P deficiency is responsible of older leaves fall. P deficiency also reduces cacao root development (Malavolta 1997).

Potassium

K is of major importance for cacao physiological development, particularly for the pod development and maturation. High K contents are found in soils that are regularly mulched. K is an antagonist of magnesium and calcium, which means that soils with a high K content commonly show Mg and Ca deficiencies and vice versa (Loué 1961). K deficient leaves fall off and are responsible of dieback at the final stage (Malavolta 1997).

Calcium

Ca is important for the development of terminal buds and flowers. Ca deficiency affects leaves but also root development (Malavolta 1997).

Higher levels of Ca in the soil increased the leaf concentrations of Ca, Fe, Zn and Mb but decreased the leaf N, Mn and Na.

Magnesium

Mg is one of the constituents of chlorophyll and is therefore important for photosynthesis. A prolonged period of Mg deficiency will cause older leaves to abscise whereas young leaves remain unaffected. Trees soon become defoliated (Chepote et al. 2013).

Sulphur

S facilitates the conversion of nitrate to ammonium in the amino-acid production process, ensuring high N use efficiency. Leaf veins are often paler than the lamina (Loué 1961).

4.3.1.2   Micronutrients

Boron

Boron is a micronutrient essential for all higher plants as a component of cell walls in shoots and roots, and for bean growth. Main causes of B deficiency are high pH and dry weather, or heavy rains in sandy soils. Boron is not a mobile element and is poorly mobilised within organic substances (Loué 1961). According to (Tollenaar 1967), foliar analysis data is less unreliable than soil data, and the author found the soil threshold to be 0.2 mg/kg (water-soluble B method). In boron deficient soils, cacao shows yield reductions of up to 40 %, deformed fruits, reduced bean size and are more prone to black pod disease (Lachenaud 1995).

Zinc

Zinc acts either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes. Zn deficiency is the most common micronutrient deficiency. Zinc is a component of enzymes found in the cocoa beans fermentation process (Loué 1961). Deficiencies occur first on the youngest parts of plants (Santana and Igue 1972). Zn deficiencies are more frequent where soils have high pH (overliming), high levels of P, or sandy soils with very low CEC.

Manganese

Mn is involved in chlorophyll production and photosynthesis. It helps in the proteins metabolism and synthesis. Deficiencies are more frequent where soils have high pH (overliming), organic soils, excess of Ca, Mg and K, or high levels of Fe, Cu and Zn.

Copper

Cu deficiencies occur in organic soils, or when pH is outside the range of 5.0–6.5, or where there are high levels of other metal ions such as Fe, Al and Mn. High doses of N can also be responsible of Cu deficiency.

Iron

The majority of iron is found in chloroplasts where it is essential for photosynthesis. In cases of Fe deficiency, chlorophyll synthesis is inhibited and leaf chlorosis develops. The leaves turn yellow, then white, but the veins remain green. Older leaves often remain green.

4.3.2   On Pods and Beans

The amount of nutrients provided by fertilization can increase or decrease some physiological characteristics of the pods and beans.

4.3.2.1   Number of Pods per Tree

(Lachenaud 1991; Verlière 1981)

- N has a positive effect on the number of pods per tree only when soil P and K are both present.
- P has a significant negative effect in the absence of K.
- K has a positive effect. The effect is increased (2.7-fold) when N is supplied at the same time.
- Ca and Mg deficient pods are susceptible to black pod, thus reducing the pod number per tree. This can also be observed with boron deficiency.
- Acid soils are often linked with higher black pod disease. However this might be due to the lower Ca level also observed in acid soil.
- The pod index (number of pods for 1 kg of dry beans or beans per pod) is not soil dependant, but a genetic trait.

4.3.2.2   Bean Size

(Lachenaud 1995)

- P and K have a significant effect on the bean size. The effect is negative when both P and K fertilizers are applied separately, but it is positive when they are supplied together.
- The individual effects of P and K are roughly the same (1.6 % of the control value), which explains why the main effects of either P or K are not significant.

4.3.2.3   Weight of Beans per Pod

- Fertilizers have no significant influence on the weight of fresh beans per pod or on the average weight of a bean. The number of beans per pods and their weight are influenced by water availability and the location of the pod on the cacao with the biggest pods being at the base of the trunk (Lachenaud 1995).

4.3.2.4   Mineral Composition of Beans

- In Côte d'Ivoire, no significant effect of fertilizer on the mineral composition of cocoa beans was found (Verlière 1981).

- The average mineral composition of the dry matter of a cocoa bean in Brazil was found to be: 2.34 % N; 0.41 % P; 0.97 % K; 0.08 % Ca; 0.15 % Mg (Malavolta 1997).
- Mg improves the chocolate taste by increasing the polyphenol and sugar contents in the beans.
- Zn can contribute to an improvement of the fermentation through producing enzymes that can help cocoa bean fermentation.
- The taste of chocolate was found correlated to the terroir; this includes many factors of which are the environment and varieties, and the type of soil (Araujo et al. 2014).

4.3.3   On Other Characteristics

4.3.3.1   Pest and Disease Resistance

- A positive effect of N fertilizer applied as urea was found on the population density of mealybugs *Planococcus citri* (Campbell 1984; Adomako 1972). The fact that N applications increase the swollen shoot because N makes cacao more attractive to mealybug, the main vector of the disease, was confirmed recently (Manu 2006). It could be worth to carry on further studies comparing the sap or bark soluble sugar profile and the amino acids profiles with nitrate N fed cacao versus ammonium N fed cacao.
- Boron in the soil (and applied as fertilizer) has a positive effect on pod rot reduction (Stemler 2012).
- Potassium and boron have an important role in increasing the resistance of cacao to vascular streak dieback (VSD), as demonstrated in Indonesia (Abdoellah and Nur'Aini 2012).
- Mn is the nutrient with the greatest influence on witches' broom tolerance (Nakayama 1995) because manganese is essential in the phenol compound formation process, which are important for tolerance to the disease.

4.3.3.2   Leaf Toxicity

- Application of N in the form of NO_3-N when not necessary can induce severe leaf-edge scorch. Cacao receiving NO_3-N showed leaf-scorch and deficiency symptoms for K, Ca and Mg, while those receiving urea-N showed only the P deficiency (Lockard and Asomaning 1965b; Lockard and Burridge 1965).
- High N levels (as nitrate) increase the amount of leaf-edge scorch (Lockard and Asomaning 1965a).
- Excess N increases the risk of black pod disease.

4.3.3.3 Tree Growth and Management

- Application of N promotes high vegetation growth. This will favour branch lodging, as observed in Côte d'Ivoire (Stemler 2012). Consequently, more intensive pruning will be required.

4.4 Current Challenges

Fertilization is a key practice for the sustainability of intensive cocoa production. However, there is still much to be done to better understand how to use them and the conditions to improve the recommendations. We will discuss below some particular points:

1. There are still many countries where extension services still recommend the use of a unique formula that does not take into account variations in soil and environmental conditions; this is particularly the case of the two largest world cocoa producers even though the use of several formulae has been proven (Koko et al. 2009; Snoeck et al. 2010). Fertilization must be adapted to the local soil and climate conditions; therefore, the use of more than one formula is encouraged. The model was developed and validated in West and Central Africa. It can be used for development and recommendations at different farm, regional or country level.
2. There is a need to better understand the functioning of cacao in various environments, particularly the effect of shade (agroforestry systems) or slightly shaded (often for tree-crop associations) as compared to full sun. There is a need to investigate the fluxes of the soil nutrients and how they are taken by the cacao trees.

4.4.1 Limitation of Using a Single Fertilizer Formula

Based on the observation that P and K are important and usually the only nutrients required, many countries recommend a single fertilizer formula containing P-K alone or plus a small amount of Ca-Mg; some micronutrients are also sometimes added, particularly when visual deficiencies have been noted. For example, in Ghana, the COCOBOD recommends the use of 'Asaase Wura', a concentrated fertilizer with 21 % P2O5, 18 % K2O, 9 % CaO, 6 % MgO, 7.5 % S, and 0.7 % Zn. In Côte d'Ivoire, 'Engrais cacao' is recommended, with 23 % P_2O_5, 19 % K_2O, 10 % CaO, 6 % MgO, and a small amount of S and Zn. This is the only fertilizer formula recommended throughout the country, regardless of the type of soil and environment.

Nevertheless, on the long term, the use of a single standard formula cannot fulfil the real needs of the cacao in the broad range of different situations because of the many possible interactions between the environment and farming practices, as explained above. This is why, as early as 1961, Loué had proposed using three different fertilizer formulae adapted to the three different geological origins of Côte d'Ivoire (Loué 1961). In other countries (e.g. Malaysia, Brazil, and Columbia), even more specific recommendations are given to cacao growers: fertilizer formulae are computed on the basis of critical soil N, P and K levels (Ling 1984).

The variability of responses to a single fertilizer formula was again demonstrated by Appiah et al. (2000) in 20 farmers' fields in the Eastern Region of Ghana comparing unfertilized plots with cocoa plots receiving P and K (such as triple super phosphate and potassium chloride) during. In 65 % of the farms, cocoa yields were increased thanks to fertilization; but in 33 %, the increase in yield was not significant and in 2 % there was no increase, even though the same farming practices and pest and disease controls were used. The main explanation for these differences is that the single formula with only two nutrients (P and K) cannot be suited to all farms being in so many environments and with so many cultural practices. A globally positive trend was found between cocoa yields and the use of 'Asaase Wura' fertilizer. However, statistical analysis revealed a low coefficient of correlation due to significant differences at the farm level in response to the single fertilizer used. In fact, 61 % of the cocoa plots benefited from the fertilizer application (with 6 % more than doubling the yield compared to the unfertilized control plot), while the remaining 39 % of cacao plots showed yield losses after the same fertilizer application.

In addition, regardless of the crop, soil nutrients interact with each other and when one nutrient is supplied in large quantities whereas the amounts of the others remain low, the latter may counteract the effect of the added nutrient. The balances between nutrients thus need to be taken into account to ensure optimum use of each nutrient by the crop and avoid limiting factor incident. These relationships were identified after long-term fertilizer trials on various types of soils by Jadin (1975) who concluded the need to develop localized and adapted fertilizer formulae and built up a first thematic map of cacao fertilizer requirement in Côte d'Ivoire. The formulae were given per administrative region, based on an extrapolation of the results of fertilizer trials conducted in the different regions (Fig. 4.8).

4.4.1.1 Validation of the Soil Diagnosis Tool

In Côte d'Ivoire, fertilizer trials were conducted at different locations under different types of soil and climatic conditions. At each location, unfertilized control cacao plots were compared with cacao that had been fertilized according to the soil diagnosis results, including soil correction and amounts to compensate for nutrients exported per yield of 1 t.ha^{-1} of cocoa beans.

In Cameroon, Paviot (1977) used soil diagnosis to fertilize cocoa in a nursery and in young and mature plantations. He confirmed that the method is also suitable for nursery use.
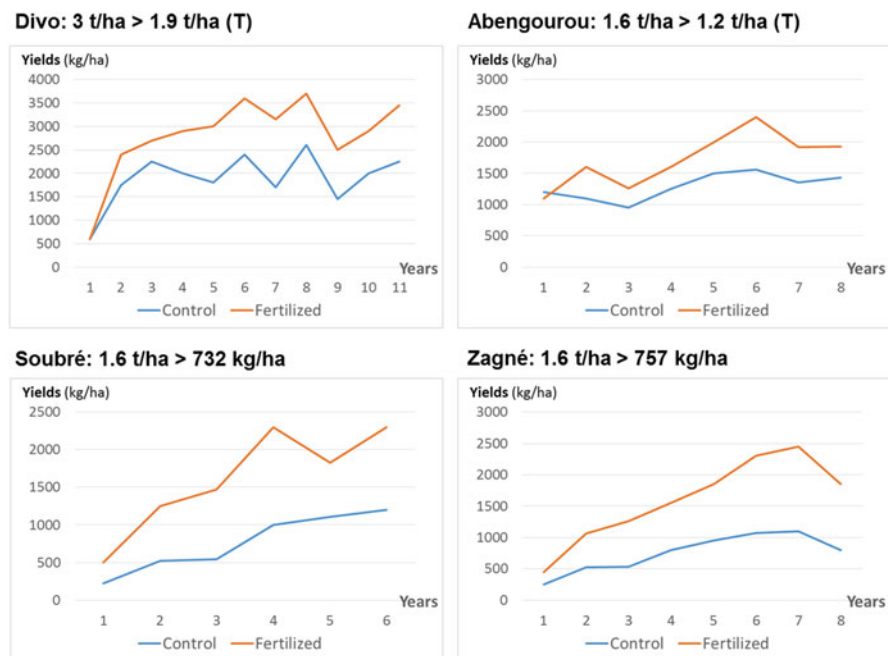
Fig. 4.7 Effect of fertilizer use per department in Côte d'Ivoire showing the significant efficiency of fertilizers in the various zones of Côte d'Ivoire. Fertilizer formulae were determined using the soil diagnosis tool (Koko et al. 2011)

In Ghana, validation of the soil diagnosis model began in 2009 on 120 young cocoa farms distributed throughout the cocoa belt. On each farm, unfertilized control cacao trees are currently being compared with trees that receive fertilizer in quantities that were computed according to the soil diagnosis method and trees receiving the traditional single fertilizer.

The advantage of using formulae calculated by the soil diagnosis model was demonstrated in a recent trial conducted in four regions of Côte d'Ivoire (Koko et al. 2011) over an 11-year period. Figure 4.7 shows that the average yields of fertilized plots were increased by at least 40 % compared to those in unfertilized plots (i.e. an increase of 580–1120 kg · ha^{-1}). Better results were obtained in the Central (Divo) and Western (Zagné) regions, where the rainfall was higher than in the Eastern Region (Abengourou). In Divo, the average yields of fertilized plots were increased by at least 130 % compared to unfertilized plots (i.e. an increase of 1500 kg · ha^{-1}) for 76 % of the plots.

In Togo, the soil diagnosis model was used to compare fertilized and unfertilized cacao plots. The results showed that most soils were poor in N, but also deficient in P and K (Tossah et al. 2006).

Formulae are expressed in N – P2O5 – CaO – K2O– MgO (doses × 10)
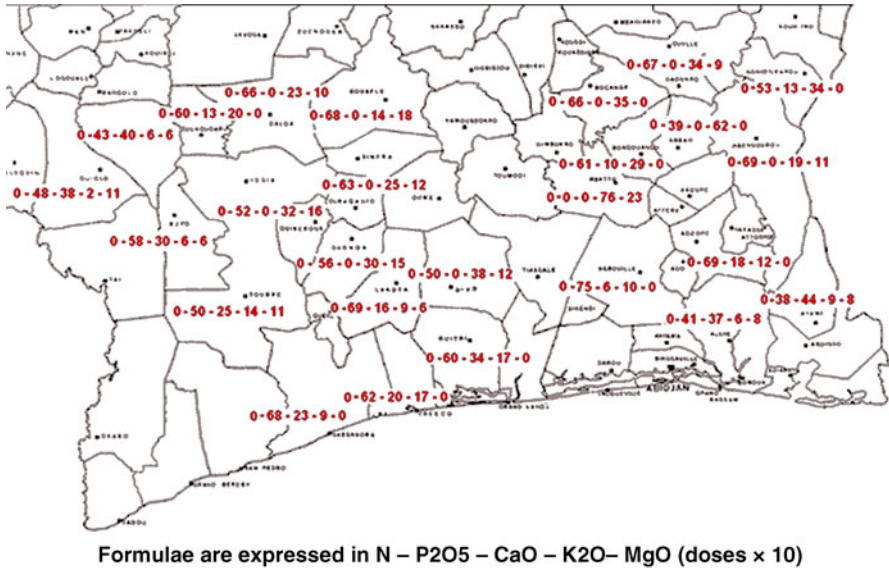
Fig. 4.8 Map of fertilizer needs per department in Côte d'Ivoire. Formulae were computed using the soil diagnosis tool (Jadin 1975)

4.4.1.2 Some Applications Using Soil Diagnosis

In Côte d'Ivoire, Jadin (1975) used the soil diagnosis model to build a map of the fertilizer formulae per department (Fig. 4.8). Twenty-six formulae were calculated from the results obtained in the regional research centres and were proposed to farmers.

In Ghana, the soil diagnosis was combined with a geographic information system (GIS) to begin a process of precision but sustainable agriculture by optimizing fertilizer application (Snoeck et al. 2010). The study was based on the analysis of soil samples collected in land units defined by combining climate data with soils from different soil associations that were defined using digital pedological (i.e. on different bed rocks) and climate maps of Ghana (Fig. 4.9).

At least, 30 different fertilizer formulae are required to fulfil the demand of the vast majority of cocoa farms. Extension services have a direct online access (www.wajae.org) to see what type of recommendation should be applied in their area.

In Togo, the soil diagnosis was used to determine the formulae required for cocoa production. It was demonstrated that all soils in the region were exhausted by over-exploitation and all required N in addition to other nutrients, mainly P and K (Tossah et al. 2006). These results could be compared with those obtained in the Eastern Region of Ghana where the same types of soils are found, thus confirming the results obtained in both countries.

In Central Cameroon, the soil diagnosis model was used to monitor changes in nutritional status under young and old cacao plantations, compared with secondary
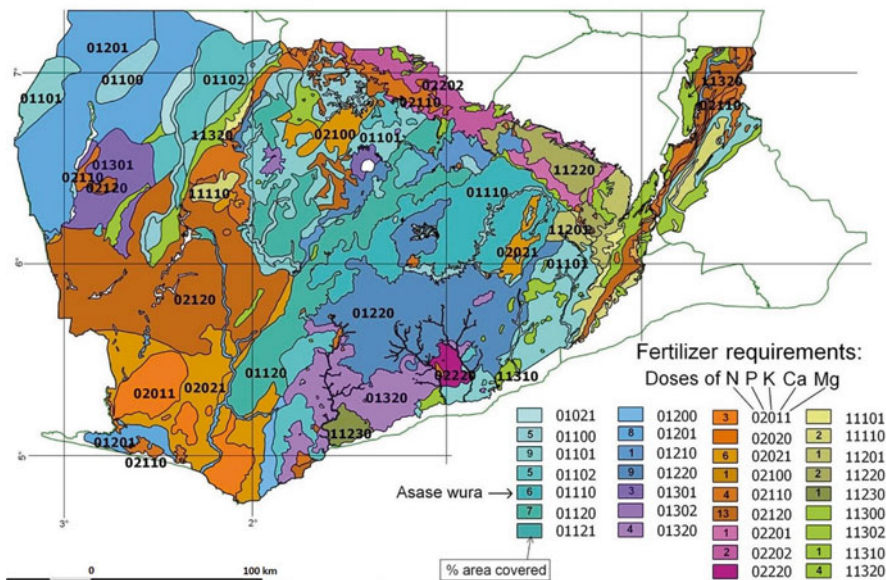
Fig. 4.9 Thematic map of cacao fertilizer requirements according to different climates and soil pedology in Ghana. Formulae were computed using the soil diagnosis tool (Snoeck et al. 2010)

forest soils (control). It was thus possible to assess the sustainability of cacao-based agroforestry in terms of soil development (Snoeck et al. 2009).

4.4.1.3   Limits to the Adoption of Fertilization by Farmers

After a century of cocoa production, after massive deforestation, after production of hundreds of thousands of tonnes of cocoa, much of the land of cocoa-producing countries is depleted (Appiah et al. 1997). Fertilization is required to sustain cocoa yields in the long-term, except in traditional cocoa agroforests where cocoa yields can be maintained for more than 70 years at level of 350 kg per hectare without noticing any yield depletion (Jagoret et al. 2011). In a cacao trial under permanent shade of Gliricidia, the cocoa productivity could even be maintained at around 700 kg cocoa beans per hectare without fertilization (Bastide et al. 2007).

However, despite its importance in maintaining cocoa yields, smallholder farmers do not use enough fertilizers. Three reasons are often given to explain this phenomenon (Ruf 2009): (1) farmers are not well informed about the correct use of fertilizers; (2) access to chemical fertilizers is difficult; and (3) chemical fertilizers are costly.

It is true that the fertilization process is somewhat complicated as productive cacao tree has specific requirements and the possibilities are endless if we consider that, for each plot, nutrients must be supplied in a balanced way to be effective. By

reducing the number of formulae by grouping plots with similar needs, extension services can make fewer recommendations which hopefully will then be more easily accepted by farmers. In Ghana, for example, we were able to reduce the number of formulae to 33 (Fig. 4.9), which we felt to be an acceptable number to cover as many different situations as possible without jeopardising the quality of the recommendations. In Côte d'Ivoire, the final number of formulae proposed by Jadin (1975) was of 26 (Fig. 4.8).

However, cacao growers are aware of the importance of using fertilizers and are buying them as soon as they have access to them. The correlation between fertilizer adoption and cocoa price was clearly demonstrated by Ruf (2012). That is the reason why it is important that access to fertilizer for smallholders should be made as easy as possible. Ghana, which subsidizes 70 % of the price of fertilizer, is an example of a State that recognizes the importance of fertilizers in improving cocoa yields.

To reduce fertilizer uses, other sources of nutrients are currently being studied, for example the reuse of cocoa pod husks as a source of K, one of the most important cations (Ahenkorah et al. 1987).

4.4.2 Associated Trees

4.4.2.1 Shade Trees

Cacao being a plant native to the forest, it is particularly adapted to agroforestry systems. Particularly, such systems are known for their capability to improve soil fertility, particularly the soil organic matter (Jagoret et al. 2012; Snoeck et al. 2010). Moreover, shaded cacao plots have the advantage to provide environmental services and improved C sequestration (Gama-Rodrigues et al. 2010). However, under shade, cacao trees are not very productive and fertilization will consequently only result in a very slight increase in yield (Asomaning et al. 1971). On the opposite, because cacao can reach maximum photosynthesis with 400 μm photons.m^{-2}.s^{-1} (Balasimha et al. 1991; Bastide et al. 2003), no shade or light shade is leading to a higher overall nutrient requirement and thus a higher requirement of specific nutrients according to their physiological importance.

The impact of shading was demonstrated by Ahenkorah et al. (1987) at the Cocoa Research Station in Ghana in a trial comparing cacao crops with or without shade trees and with or without fertilization. The results showed that, over a 30 years period, cacao grown in full sun could produce twice as many cocoa beans as those under shade (Fig. 4.10).

However, the works also demonstrated the limits of full sun cultivation in the absence of fertilization (dotted lines). Indeed, in the no-shade and no-fertilizer treatment, the yields dropped after about 18 years, and thereafter, they were not better than those in the shade and no-fertilizer treatment. The decrease in yield followed the same trends as that of soil P, which was originally 24 mg.kg^{-1} in all treatments at the beginning of the trial and then dropped to less than 5 mg.kg^{-1} after 20 years in

Yield (kg cocoa / ha)
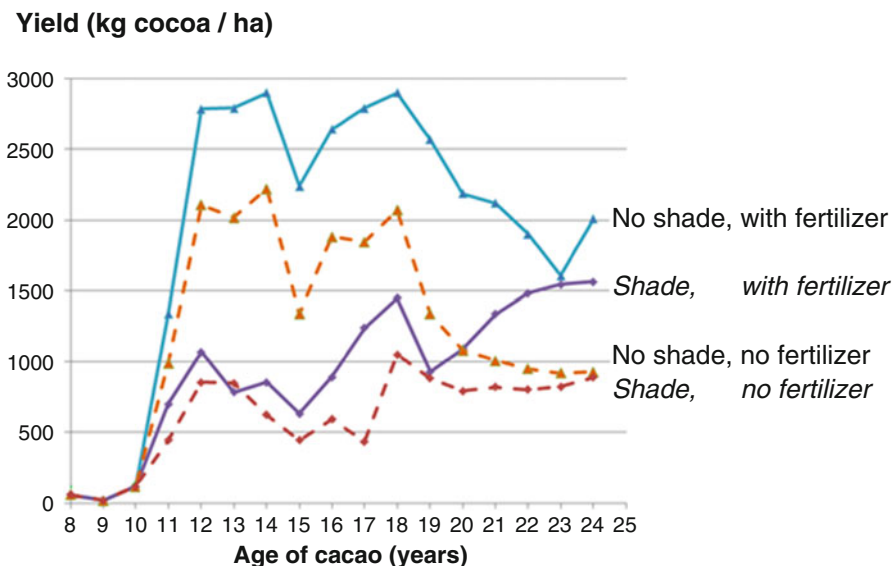
Fig. 4.10 Impact of shade and fertilizer on cocoa yield over 24 years (Adapted from Ahenkorah et al. 1974, Ghana)

both unfertilized treatments, while it remained at around 20 mg.kg^{-1} in both fertilized treatments.

The trial also demonstrated the limits of using a single formula based on only two nutrients (P and K) over a very long period. Indeed, in the no-shade and with-fertilizer treatment, the yields dropped and were only slightly better than those in the shade and with-fertilizer treatment, with the former showing an upward trend and the latter a downward trend. This suggests that the addition of only two nutrients (P and K) over a 23-year period led to imbalances in relation to other nutrients (particularly Ca, Mg, N) in the no-shade and fertilized treatment; while the ratio between cations remained more balanced in the shade and fertilized treatment thanks to associated shade trees.

Under shade, N has no effect and, depending on the type of soil, phosphorus or potassium needs will predominate, whereas potassium will have a positive effect only if phosphorus nutrition is adequate. Considering the results described above, we can hypothesize that the poor response to N fertilizers is the result of the combined effects of litter and rainfall deposits which are sufficient to feed the system with N.

Without shade, N is essential. The role of potassium is related to that of N whereas high doses of phosphorus appear to be of secondary importance. With fertilization, cocoa yields increase significantly, whereas without fertilizer, yields quickly drop once the soil nutrients have been consumed.

In Ghana, Acquaye (1964) found that shade increased foliar levels of K by 14.3 % and of P by 12.9 %, but N only by 3.4 %. Shade reduced Ca by 19.5 %.

Cacao growing under shade was more balanced, but, in both cases, it is advisable to carry out soil analysis at least once every 5 years to adjust the fertilizer formulae and doses.

4.4.2.2 Tree Crop Associations

Intercropped trees can affect potential soil fertility, either by providing external nutrients, or on the contrary, by competing for nutrients.

For example, legume crops can provide N to associated cacao via their ability to fix atmospheric N thus making it available for the cacao. A 16 % increase in the litter N level was observed in cacao associated with *Erythrina* sp. compared to cacao associated with *Cordia* sp., a non-leguminous shade tree (Alpizar et al. 1986). However, legume intercropping does not systematically benefit the cacao as it depends on the levels of associated nutrients that may have limiting effects if their levels are low compared to that of N (Nygren and Leblanc 2009).

Conversely, the availability of certain nutrients for cacao may be reduced due to competition with associated trees. For example, competition for phosphorus between cacao and shade trees such as iroko (*Milicia* sp.) was observed in Ghana (Cunningham and Arnold 1962; Isaac et al. 2007).

4.5 Conclusion

This review highlights that fertilization is an important parameter for cacao cultivation sustainability. The soil should be able to provide the necessary nutrients to compensate for those lost in cocoa production. Although rainfall and the transfer of nutrients through litter can compensate for the nutrients removed by cocoa harvesting (up to 700 kg dry beans per hectare), more intense cacao cultivation induces an ecosystem imbalance, responsible of continuous nutrients depletion.

The first fertilizer trials revealed that significant yields can be achieved when cacao is fertilized and grown without shade. Mineral fertilization thus quickly emerged as a key way to increase cocoa yields. However, in light of the diversity of cropping situations, fertilizer doses and formulae should be adjusted according to the prevailing conditions, particularly regarding shade and soil.

In nature, cacao feeds from nutrients taken from the soil. Soil correction is thus the first step required to optimize cacao growth and productivity. Works carried out in various cocoa producing countries have given rise to guidelines for soil nutrient applications. The recommendations currently used can be divided into three levels: (1) a generic all-purpose formula; (2) more accurate formulae calculated on the basis of the soil fertility status. They are based on the comparison of current nutrients levels with predefined thresholds which were first defined in local fertilizer trials; (3) even more accurate formulae that take both the thresholds levels and balances between nutrients into account. The latter approach is more complex and has

required the development of a decision support tool called "soil diagnosis". This tool has been validated for different soils and environments.

It is generally accepted that leaf diagnosis is not adequate to predict cocoa productivity and that it is insufficient for determining the nutritional needs (in the form of fertilizers) of existing cocoa farms whose cropping history and techniques are not known. Soil diagnosis is preferable in these circumstances. However, leaf diagnosis is useful to detect nutrient imbalances in the plant to detect incorrect use of fertilizer. Its advantages will be that it enables fine-tuning the fertilizer formula by highlighting any nutrient deficiency. Therefore, it is a good option to combine both approaches (soil and leaf) when the history of the cacao plot is known and after having corrected the soil to fit the cacao requirements and looking for intensive cacao cultivation.

Cacao responds well to fertilizer applications only if the management, cropping practices, soil and climatic conditions are favourable for optimum growth and yield and if the soils can supply the nutrients required on time. How to apply the fertilizer and the choice of ingredients used in the formula can determine the nutrient absorption efficiency, especially in marginal soil conditions. Also the fertilizers should be applied only on top of active roots to ensure optimum uptake. Poor application techniques are detrimental in view of the scorching and damage caused to the superficial root system especially in immature cocoa.

Cocoa farmers are aware of the importance of using fertilizers but fertilizers are costly and need to be used with a minimum of knowledge because nutrient excess or deficiency can affect yields and soil health as well as production costs.

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Chapter 5
Agroecological Principles from a Bibliographic Analysis of the Term Agroecology

Zachary T. Brym and Jennifer R. Reeve

Abstract Agricultural developments over the previous half-century have highly increased food, feed and fiber production. Yet, global food output and distribution still falls short of feeding the world with unintended harm to the environment and society. Agriculture requires new approaches that meet the challenges of sustainable and equitable food production. One prevailing alternative, agroecology, is an approach that promotes environmental conservation, ecosystem health and social equality in the global food system. However, the field of agroecology remains disjointed by a number of working definitions and conflicting agendas. Lack of a clear definition of the term can lead to misuse or overgeneralization that hinders effective dialog, collaboration, and development of the discipline.

We conducted a literature review to determine trends in current usage of the term 'agroecology' and to offer an approach to developing a unified agroecological framework. Our findings suggests that diverse agendas in agroecology can be unified through the fundamental principles of systems thinking, resilience, biodiversity, and production. We found that the agroecological literature continues to grow at a rapid rate. Agroecological practices are discussed more often than principles, though almost half of publications already use the term systems approach. Biodiversity and resilience are not as well represented in the literature, though resilience is increasingly used in recent papers. The diverse perspectives and agendas encompassed by agroecology are a strength of the discipline when communicated within a clear and open dialog. Improving cohesion among agroecologists through a focus on defining foundational principles will broaden the credibility of agroecology in science and public opinion.

Keywords Agroecology • Agroecosystem • Systems approach • Resilience • Biodiversity • Science communication

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5.1   Introduction

Agriculture faces many challenges globally. The food supply must support a rising population and an increasing demand for high-calorie foods (Snapp and Pound 2008; Tilman et al. 2011). Agriculture currently uses an abundance of non-renewable resources to maximize production that can cause negative impacts to the environment and society (Cassman 1999; Altieri 2002; Rosegrant and Cline 2003). Intensive cropping systems often rely on high rates of pesticides and fertilizers. These practices persist despite the risk of pest resistance and nutrient loss that requires farmers to continually increase inputs to maintain crop productivity (Matson et al. 1997; Perfecto et al. 2009; Davis et al. 2012). Once harvested, as much as 70 % of the food calories produced in our agricultural system are used for animal feed or biofuel, with even more lost as waste products (Cassidy et al. 2013). Urban centers around the world are increasingly isolated from agricultural lands leading to a populous less connected to where and how their food is grown (Loomis and Connor 1992). The result has been a general erosion of a cultural relationship to healthy fresh food, reduced participation in food production and preparation, and an increase in diet related disease (Popkin 2011; Popkin et al. 2012).

Over the years, there have been increasing calls for new approaches to agriculture to help solve these challenges. Agroecology emerged as one prevailing alternate approach that considers ecology, evolution, and social equality as the foundation for evaluating farming practice and food distribution success (Altieri 1987; Snapp and Pound 2008; Wezel et al. 2009; Weiner et al. 2010; Francis and Porter 2011). The concept of agroecology has developed over the course of nearly a century across many related disciplines and increasingly has entered common usage (Wezel et al. 2009). Since the term's first use, many divergent definitions and philosophies have evolved (Altieri 1987; Wezel et al. 2009; Tomich et al. 2011). Contradictory understandings of agroecology among researchers, practitioners, political activists, and policy makers result in unnecessary suspicion and conflict that raise barriers to the effective development and implementation of the discipline (Rosset and Altieri 1997; Dalgaard et al 2003; Oenema et al 2003; Phelan 2009; Altieri 2012).

A unified agroecological framework will improve the dialog among the disparate groups interested in the intersection of food production, sustainability, and social justice and cohesively work to face the modern challenges of agriculture (Wezel et al. 2009; National Research Council 2010; Tomich et al. 2011). In this paper we present a literature review that surveys the unique aspects and uses of the term agroecology and the fundamental unifying principles of the discipline. Our review suggests the field can build cohesion by focusing on principle over practice to facilitate meaningful dialog among scientists and practitioners and broaden the credibility of agroecology in science and public opinion.

5.1.1 History and Background

Agroecology and its principles first appeared in the late 1930s as a scientific discipline that combined agronomic and ecological methods in research on soil health and crop production (Wezel et al. 2009). Early agroecology was influenced by a debate on how to view and interact with an agricultural system and its parts (Steiner 1924; Howard 1940; Northbourne 1940). Sir Albert Howard is credited with an approach to farming that recognizes the soil as a complex evolving biological system, a perspective influenced by the philosophy of Charles Darwin (Conford 2001). Howard's views were counter to the prevailing 'Law of the Minimum' approach formalized by Justus von Leibig. The 'Law of the Minimum' defines soil fertility as limited by a suite of essential elements required for plant growth (von Liebig 1840). The result was a general shift in focus away from recycling organic materials that maintain soil fertility and health to a focus on replacing individual essential elements in readily available form. Deficiencies in soil fertility were now easily and cheaply corrected with the specific limiting element while problems with pests were controlled with new biocides.

Despite the phenomenal success of the modern agriculture methods in increasing farm productivity while reducing farm labor, there was growing concern among scientists, farmers and the general public that unforeseen consequences were negatively affecting both the wider environment and society at large. Widespread soil loss, pollution of water bodies, loss of biodiversity, the erosion of rural communities and a general decline in public health were increasingly seen as the direct result of an increasingly industrialized food system (Altieri 1987; Perfecto et al 2009; National Research Council 2010). The search for alternatives arose among scientists, farmers and the general public. Many of these new agendas allied themselves with the ideas of agroecology, using and defining the term in subtly different ways.

While discrete boundaries in usage do not always exist, we find it useful to break agroecology into four main categories. Simultaneously, agroecology is (1) an ecologically based systems research approach, (2) an agricultural design that mimics nature, (3) an agricultural practice implemented to achieve sustainability, and (4) a socio-political movement that promotes social and environmental integrity in the food system. These categories converge to facilitate a system of resilient agriculture that minimizes external resource requirements while producing an adequate supply of food and fiber and preserving social and environmental integrity.

5.1.2 Defining Terms and Concepts

5.1.2.1 The Current Framework

The current agricultural framework strives to maximize yields with the efficient use of resource inputs (Jackson 1997). Agricultural systems are improved through use of a reductionist approach. Reductionist research is extremely effective in

identifying mechanisms that improve upon expert knowledge, dubbed the industry standard or best management practice. The components of agriculture split into an open system with discrete inputs and outputs as a result of the focus on underlying mechanisms and simple systematic levers (Altieri 1987). The open system is maintained to function at the greatest efficiency and advanced to use fewer inputs per unit output. The current framework misses potential system-wide impacts of its modular design, such as environmental degradation from waste products and sociological disruptions in health and economy (Matson et al 1997; Cassman 1999; Phelan 2009).

The current agriculture framework considers increased production and economic gains as the primary goal. Decision-making by the farmers is heavily constrained by available markets, large agribusinesses that monopolize agricultural inputs, such as seeds or fertilizer, and by food processing industries that demand unblemished uniformity in large quantities (Howard 2009). Farmers are forced to increase production to meet loan payments or leases on land from local elites or foreign investors (Snapp and Pound 2008; Vandermeer 2011). The remainder of the population is disconnected from their food source and the great challenges facing modern agriculture seemingly content to pay a smaller percentage of their earnings for food than ever before (National Research Council 2010).

5.1.2.2 The Agroecosytem Concept

The term agroecosystem can be thought simply as an agricultural field, farm or region. It describes a coherent agricultural unit, the boundaries of which include aspects normally outside the primary agricultural interests of productivity and profitability including environmental, biological, economic and sociological processes. Diverse biological processes and ecological relationships drive a healthy agroecosystem that expresses long-term maintenance of the biological, physical, and social qualities of the farmland. Model agroecosystems support adequate farm production, regulate and balance the flow and timing of nutrients, actively build healthy soils, maintain and regulate species interactions, conserve biodiversity, and adapt to dynamic conditions (Okey 1996; Altieri 1999; National Research Council 2010; Lemaire et al. 2014).

These healthy qualities of an agroecosystem, called 'ecosystem function', provide additional benefits as a result of an agroecological approach (Swift and Anderson 1994). With strong ecosystem function, external inputs can be applied sparingly and are efficiently recycled (Oenema et al 2003; Gliessman 2007; Schramski et al. 2011). Production driven economic considerations may require increased energy and resource demands to maintain yields, but then ecosystem function can be leveraged to the benefit of other economic returns (Reganold et al. 2001). Ecosystem function and the resulting goods and services can be maximized when considering the agroecosystem as a whole (World Commission on Environment

and Development 1987; Costanza et al. 1997; Klein and Sutherland 2003; Millennium Ecosystem Assessment 2005).

Agroecosystem boundaries can be drawn at a broad range of scales (e.g., soil, plant, plant-pest, field, farm, region, food system) and describe the spatial and temporal context of practical recommendations in agroecology (Levin 1992; Altieri 2012). Some agroecologists seek to understand the nutrient cycles and biotic interactions in the soil defining their agroecosystem boundaries at relatively small scales (Lundquist et al 1999; Arshad and Martin 2002), while others define boundaries at the intermediate farm or regional level (Reganold et al. 1987; Reganold et al. 1993; Drinkwater et al. 1995; Letourneau and Goldstein 2001; Reganold et al. 2001). Still, others work at the level of the food system, including economic and sociological processes (Gliessman 2007; Wezel and David 2012). The findings from these multiple scales must then be linked and synthesized to be sure comprehensive knowledge is available for successful agroecological outcomes and recommendations. Overall sustainability in agriculture can only come from understanding the interactions of all components of the food system.

5.1.2.3   Agroecology I: A Scientific Research Approach

The first definition of 'agroecology' is a rigorous systems approach to compare and evaluate the characteristics of agricultural production systems, such as productivity, profitability, and broader impacts on the environment and society. The agroecological research approach explores linkages among physical, chemical, biological, and social components of an agricultural system across space and time (Jackson 1997; Klein and Sutherland 2003; Doré et al. 2011). Alternative modern farming systems developed through the agroecological research process integrate traditional farming, modern farming and improved management practices and technologies to build and maintain a healthy agroecosystem (Matson et al. 1997; Altieri 2002).

Agroecologists use complex systems analysis tools with detailed observations to evaluate agroecosystems and describe successful management strategies (Lockeretz et al. 1981; Drinkwater 2002; Mäder et al. 2002; Verma et al 2005; Reganold et al. 2010; Doré et al. 2011; Davis et al. 2012). Some ideas and tools are integrated from other related scientific fields, like evolutionary biology (Weiner et al 2010) or physics (Deng et al. 2012). Although many systems studies do not allow for the direct identification of factors responsible for the observed relationships, a strength of the current reductionist agricultural research model, they can effectively compare differences between complex biological systems. Systems can be identified at any scale from soil to food system, though many of the analytical tools perform better over large spatial and temporal scales and benefit from long-term research programs (Bawden 1991; Drinkwater 2002; Robertson et al. 2008; Hufnagl-Eichner et al. 2011). Such research programs build understanding of agroecosystem processes to improve the performance of the farm as a whole.

5.1.2.4 Agroecology II: An Agricultural Design

Agroecology is also defined as a method of agricultural design that is informed by observations of traditional farming systems, natural ecosystems and agroecological theory. The extreme examples of agroecosystems under this definition look more like natural ecosystems than farms, but the term is most often used in this context to describe agroecological systems that are moving in the direction of greater complexity and resilience.

As the design of agroecology developed to mimic naturally occurring ecological systems and traditionally sustainable farming systems, a number of additional terms arose to describe an agroecological farming system, including biological agriculture, sustainable agriculture (Hahlbrock 2007; Pretty 2008), organic agriculture (Zehnder et al 2007; Vogt 2007), biodynamic agriculture (Reeve et al. 2011), natural systems agriculture (Glover et al. 2010; Franzluebbers et al. 2014), agroforestry (Huxley 1983; Anderson and Sinclair 1993), restoration agriculture (Shepard 2013), permaculture (Ferguson and Lovell 2014), and traditional agriculture (Altieri 2002; Perfecto et al. 2009). All of the types of farming listed can be considered 'agroecology' under the definition of an agricultural design and share the goal to intensify ecological processes (Altieri 1999; Pretty 2008; Doré et al. 2011). Components of current industrialized agriculture can also be viewed as an agroecological design, especially when incorporating natural processes occurring in ecological systems (Cassman 1999; Cassman et al 2002; Fuhrer 2003; Zehnder et al 2007; Wezel et al. 2014).

Often designs are then communicated broadly through the description of an agroecological practice, a slight permutation of this second term that we discuss in the following section.

5.1.2.5 Agroecology III: An Agricultural Practice that Meets the Highest Standard in Sustainability

Agroecology describes an agricultural system or set of practices that is deemed sustainable. Farmers expect scientists to recommend practices suitable to their area, and they tend to be less concerned with abstract, nuanced principles behind the practical decision. This need for concrete advice encourages researchers to focus on practices, especially as some may have broad applicability and are easily replicable. Practices generated from the agroecological framework motivate further research and can provide well-supported recommendations to the public if used within appropriate contexts (Uphoff 2002; Wezel et al 2014).

Agroecological practices are the building blocks and spokesmen of the agricultural approach but they can lead to misunderstood recommendations and inappropriate adoption if overgeneralized. For example, the benefits of organic fertilizer for soil health are likely universal, but the question of how much to apply is very site specific. As with any fertilizer, improper application can negatively affect the environment, cause nutrient imbalances, or reduce yield (Mäder et al 2002). Cover crops

can be extremely beneficial in many situations but may utilize scarce soil water reserves or confer negligible ecosystem benefit such as weed suppression (Smith et al 2014). Increasing biodiversity more generally can promote pest and disease control but the effectiveness is often very site-specific (Ratnadass et al 2012). The over generalization of agroecological recommendations is likely to remain an ongoing challenge, but greater awareness of the issue will help avoid the impression that certain practices represent a universal solution.

The appropriate use of agroecology as a 'practice' must include the allowance for a developmental process towards sustainability; otherwise, it erroneously assumes that the recommended practice will achieve sustainability regardless of context. This extreme usage implies that a specific type of farming system or set of practices is the most sustainable option regardless of potential system-specific, socio-economic, or environmental conditions. This assumed result easily leads to misuse or overgeneralization. A preferred perspective would refer more loosely to a developmental process that is designed to move in the direction of the highest standard but has yet to reach the goal.

It is exceedingly important to be precise when using 'agroecology' as a descriptor of the highest standard of practice and resulting agroecosystems. Very few, if any, truly sustainable and equitable agroecosystems exist where this would be appropriate. Indeed, agroecological systems are tremendously diverse in outward appearance and management practice, but share a common set of ecological and socioeconomic principles. Unless we are careful to define our terms, this permutation of the term 'agroecology' is very difficult to separate from the other meanings and opens us to the criticism that we are over-extrapolating and failing to appreciate the complex and context-dependent nature of agriculture.

5.1.2.6   Agroecology IV: A Socio-political Movement

Another definition of agroecology is a socio-political research and policy movement at the food-system level. This agroecology focuses on the practical application of the science of agroecology with the people as central to the system (Altieri 1987; Reijntjes et al. 1992; Chazdon et al 2009). Food production and distribution processes are linked in a complex coupled system of people and their environment, with diverse climates, cultures, and decision-making principles involved in the success of the food system.

Agroecology provides a scientific basis for a sustainable development strategy emphasizing conservation of natural resources and biodiversity through the empowerment of rural social movements (Rosegrant and Cline 2003; Perfecto and Vandermeer 2008; Snapp and Pound 2008; Wezel et al. 2009). One important goal, food security, promotes the availability, stability, and access to food (Altieri et al. 1999; Schmidhuber and Tubiello 2007). The failings of thinking purely in terms of food security have been challenged by the concept of food sovereignty, which requires social equity and the ability for consumers to have a supply of food from an ethically acceptable source (Perfecto et al. 2009; Rosset et al. 2011). The socio-

political movement of agroecology advocates for the equitable and participatory approach to food production and distribution at the intersection of food security and food sovereignty.

5.2 Word Use in Agroecological Publications

We conducted a quantitative literature search in order to determine how agroecological publications are using key terms that are associated with the field. From this analysis, we address trends in the terms we describe as fundamental to the field and evaluate the overall usage of additional terms related and synonymous to 'agroecology'.

5.2.1 Methods

We searched the large academic publication database Scopus for a number of key terms. We separated the terms into four groups; primary, focal, additional, and synonyms. Our primary search returned all publications with 'agroecology' or 'agroecological' in the title, abstract, or keywords (Table S1). The searches for focal and additional terms were a subset of the primary search (Table 5.1). Focal terms correspond to the main descriptions of the term 'agroecology' in Sect. 5.1.3 and the terms we suggest for use as unifying principles. The additional terms are words strongly related to the field, but did not fit the previous groups. Synonyms are words or phrases that may be used interchangeably with 'agroecology', many listed in Sect 5.1.2.4, and were analyzed independent of the primary search (Table 5.2). We recorded the count for all publications returned in the search and the publication count per year for 1994–2014 for the primary search and 2004–2014 for the focal terms (Figs. 5.1 and 5.2). For the focal terms, we recorded the ten publications with the highest citation record (Tables S1–S9).

5.2.2 Results

The primary search ('agroecology' OR 'agroecological') returned 2722 results. This is a relatively small number of publications given the size of the database, suggesting a low representation of the field among scientific disciplines. For reference, a search for 'agriculture' returns 189,540 publications. Regardless, the publications per year are continuing to rise at a very rapid rate (Fig. 5.1; Wezel and Soldat 2009). Publication count has more than doubled each decade from 1994 to 2014 to more than 250 for three of the last 4 years.

The search for focal terms helps us determine the trajectory of the field as it relates to moving towards a unified framework. Many more publications are written about 'practice' than 'principle' (Table 5.1). Use of the term 'practice' continues to rise every year while 'principle' has minimal fluctuation (Fig. 5.2). Among the focal terms that define our 'agroecology' term, 'research' is much more represented than 'design' and 'movement'. This may largely reflect our use of an academic publication database, though we would expect 'design' to be similarly represented as

Table 5.1 Search term totals for publications including *focal terms* and *additional terms*

Term	Count
– Focal terms	
agroecolog –y/-ical	2722
~ principle	165
~ practice	580
~ research	620
~ design	231
~ movement	97
~ systems	1171
~ biodiversity	293
~ resilience	48
– Additional terms	
~ production	955
~ sustainable	486
~ organic	413
~ conservation	362
~ biological	283
~ farming systems	243
~ traditional	235
~ food security	142
~ agroforestry	115
~ food systems	56
~ restoration	47
~ food sovereignty	40
~ systems approach	21
~ communication	19
~ systems research	13
~ natural systems	13
~ mixed farming	13
~ equity	10
~ systems thinking	5
~ ecological intensification	5
~ biodynamic	4
~ silviculture	3

Table 5.2 Search term totals
for publications including
synonymous terms.

Term	Count
– Synonymous terms	
agroforestry	6016
sustainable agriculture	5385
silviculture	5189
organic agriculture	1869
traditional agriculture	990
mixed farming	772
conservation agriculture	642
biological agriculture	79
biodynamic agriculture	42
restoration agriculture	22
natural systems agriculture	7
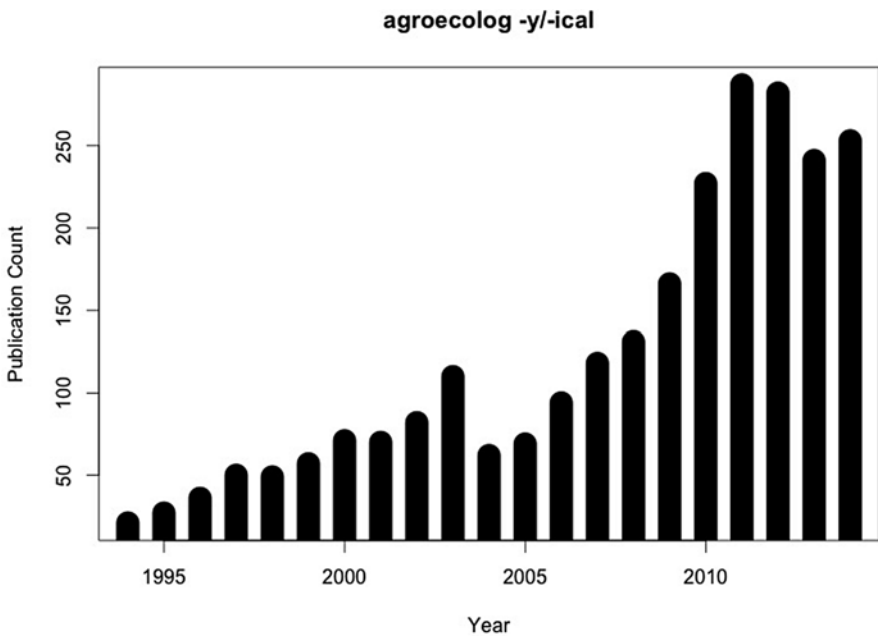
Fig. 5.1 Timeline of agroecological publications from 1994 to 2014

'practice'. Of our focal terms that relate to our proposed unifying principles, 'systems' is most represented, being used in almost half of all the papers in the primary search. 'Biodiversity' was moderately represented and 'resilience' much less. All six focal terms continue to increase in use each year.
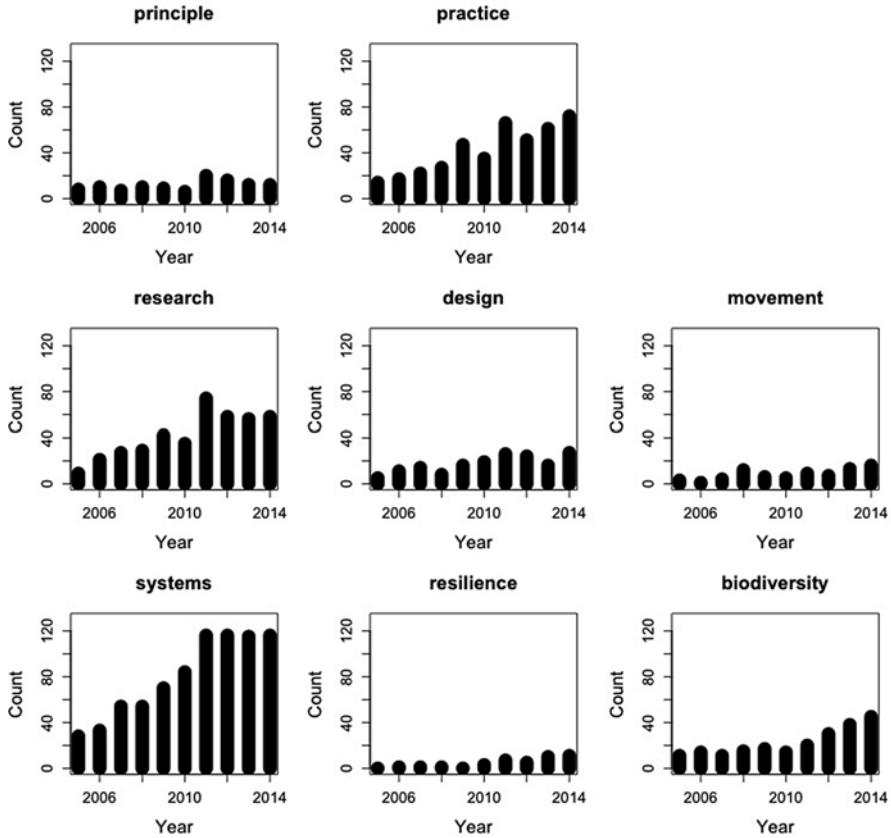
Fig. 5.2 Timeline of focal terms used in agroecological publications from 2004 to 2014

The search for additional terms, such as 'production', 'sustainable', and 'organic' helps us understand the use of related terms that could represent principles important for unification. Perhaps unsurprisingly, 'production' returned the highest number of publications used in about one-in-three publications. It returned more than double the publication results of the next two most used, 'sustainable' and 'organic', which are used in about 1:6 publications. 'Conservation', 'biological', and 'farming systems' are the next most frequent additional terms used in 1:8 to a little less than 1:10 publications.

We determined the rank of the most commonly used synonyms to 'agroecology' through an independent search of the database. 'Agroforestry', 'sustainable agriculture', and 'silviculture' returned more results than 'agroecology', with 6016, 5385, and 5189 publications respectively. 'Organic agriculture' (1869) and 'traditional agriculture' (990) were the next most used synonymous terms.

We compiled and investigated the top ten citations lists of focal terms to add further commentary on the application of terms we describe as the foundation of

agroecology (Table S1–S9). We suggest these citations represent the core usage of the focal terms and identified many interesting debates occurring in the discipline. It is clear many are still working to define ecological intensification as a synonym for agroecology. 'Ecological intensification' has garnered much interest as a term, but appears very sparsely in the agroecology literature (Table 5.1). The debate between those who promote agroecological practices for incremental improvement to agriculture and those who advocate for transformational change is ongoing. In support for our focus on 'resilience', a majority of the top citations for the term were published recently unlike many of the other terms (Table S9). Many of the same publications appear under multiple search terms, suggesting the terms we offer as focal are used together by the most widely read and cited contributions to the agroecology literature. Publications appearing in multiple top ten lists are noted in the tables and received special attention to be cited in our review.

5.3 Developing a Unified Agroecological Framework

5.3.1 Guiding Principles of the Agroecology Framework

With roots in several diverse disciplines, a single definition of agroecology has remained elusive; however, the goals and defining characteristics of an agroecological approach are strikingly consistent. Broadly, the agroecology framework integrates principles of agriculture, ecology, social equity, and sustainability. By combining all four uses of the word 'agroecology', we define it as a field of study motivated to understand ecological, evolutionary, and socioeconomic principles and use them in an improvement process that sustains food production, conserves resources, and maintains social equality.

We conducted a literature search to discover emergent principles that could guide a unified agroecological framework. We propose the principles of systems thinking, production, biodiversity, and resilience as fundamental components of a unified agroecological framework. We find that agroecologists effectively place emphasis on a systems approach to agriculture and strive to integrate environmental, ecological, and socioeconomic integrity in agroecosystems (Pretty 2008). The principle of production is also already a strong component of agroecology; though, the relative importance of production is one of the major rifts among scientists in the field. Indeed, the problems associated with an emphasis on production and bottom line can be mitigated through a systems thinking approach to agriculture and a greater emphasis on resilience and biodiversity, currently lacking in the field.

We encourage greater emphasis in agroecology on the principle of resilience. The term resilience is used in many highly cited recent publications to describe food systems that rely on ecosystem function, system regeneration, biological diversity, and equitable participation (Fig. 5.2 and Table S9). Resilience can be measured in relation to sustained production, overall agroecosystem health, and the system's ability to respond to distress over long time periods (Altieri 1987; Okey 1996; National Research Council 2010). External disturbances that might stress an agro-

ecosystem include drought, disease, pest outbreaks, economic recession, and market fluctuations. Shocks to the system from external disturbances can cause major disruption in the functioning of simplified farming systems that rely heavily on inputs. More complex agroecosytems may recover quickly and require less intervention following distress (Altieri 1987; Franzluebbers et al. 2014).

Agroecologists should also unviersally emphasize the principle of biodiversity. High species diversity may lead to self-regulation of pests and resource regeneration in an agroecosystem (Matson et al. 1997; Swift and Anderson 1994; Altieri 1999; Shea and Chesson 2002; Vandermeer 2011). It has been demonstrated that increased biodiversity in the soil improves water use, nutrient uptake, and disease resistance of crop plants (Brussaard et al. 2007; Franzluebbers et al. 2014). Crop diversity, in both space and time, can improve overall biomass production in the system and reduces required inputs (Tilman et al. 2001; Davis et al. 2012). It may be equally important to consider the composition of a biodiverse agroecosytem in addition to the species count to ensure favorable production and pest suppression (Ratnadass et al 2012). Overall, we find biologically and culturally diverse farming systems promote resilient, sustained, and equitable food production.

5.3.2   Building Cohesion with a Unified Agroecological Framework

In many ways the field is converging on a standard approach to innovation and implementation that can rapidly progress towards the development of new and effective agroecological systems (Fig. 5.3). In other ways, a diverse set of goals and agendas diminish the effective communication and credibility of the discipline (Rosset and Altieri 1997; Dalgaard et al 2003). We encourage further dialog regarding a decision-making strategy that would develop a standard set of principles fundamental to unify agroecology.

The ultimate goal of the agroecological framework is to develop sustainable agricultural systems through an understanding of complex ecological processes and prioritizes resource conservation and social equity. Through decisions supported by an agroecological framework, agriculture would strive to feed the world (Tilman et al. 2011), provide better nutrition (Brandt et al. 2011), restore ecosystem processes (Drinkwater et al. 1998), maintain biodiversity (Perfecto et al. 2009), adapt to climate change (Fuhrer 2003; Schmidhuber and Tubiello 2007) and foster healthy communities (Snapp and Pound 2008). However, very few agricultural systems meet all of these goals, and even more fall short as the food product moves through the processing and distribution chain (Cassidy et al. 2013).

For widespread behavioral and institutional shifts, agroecologists must facilitate dialog that promotes the development of productive agricultural systems that are economically viable, environmentally safe, resource conserving and socially just (Chazdon et al 2009; National Resource Council 2010). Future dialog must address the diverse agendas among agroecologists and misconceptions that have emerged among agroecologists and the public. The goal of the dialog should be to establish
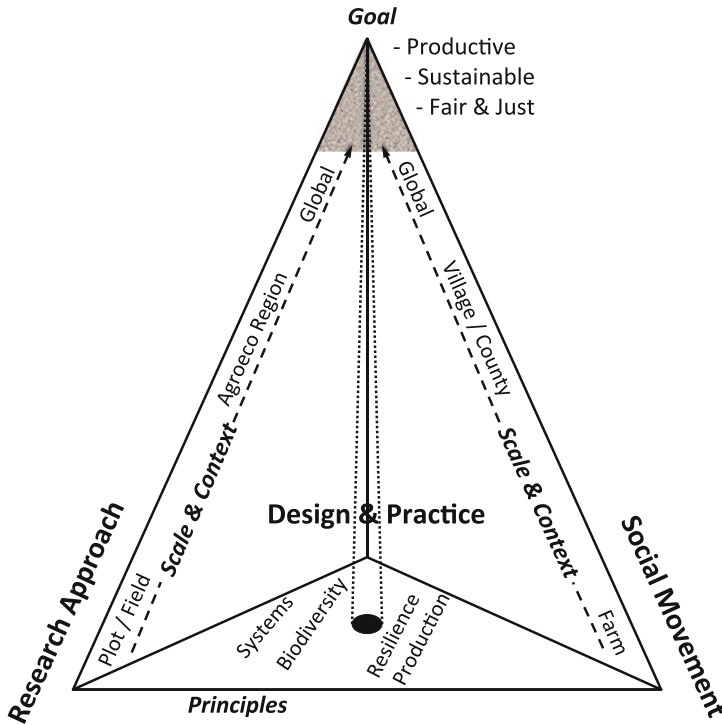
Fig. 5.3 Conceptual diagram of the unified agroecological framework. The framework builds a foundation from the major facets of the discipline (research, design & practice, sociological movement) and the major principles (systems, biodiversity, resilience, production). The agroecological framework develops its systems along various scales and contexts to move towards the goal of the ideal agroecological system

the core values and principles of the discipline and strive to place those concepts at the forefront of the conversation. The dialog has faced a number of barriers from agricultural scientists, farmers, and the public, but many promising shifts are taking place.

One barrier that must be addressed is the idea agroecology has become synonymous with a faction firmly against the trajectory and goals of conventional industrialized agriculture. As a consequence, agroecology can be misrepresented as immediately sustainable agriculture or practice that is inherently superior and mutually exclusive to the current conventional system, as in our third term (Sect. 5.1.2.5). In reality, very few agroecological systems are entirely sustainable given the current global food production and distribution system. They benefit from context-dependent integration of conventional and agroecological practices to work towards achieving sustainability (Cassman et al 2002). It is insufficient to mimic an existing practice or adopt a recommendation without considering the environmental and social context of the new location. Rather than defining agroecology as a prescriptive list of practices that may or may not be well suited to any given setting, it is

important to describe it as a process and set of principles to move towards sustainable agriculture.

Another barrier is the trade-off between system-wide resilience and the stability of a single entity. Biodiverse agroecosystems are resilient as a whole, but their component parts, such as the commodity crop, might fluctuate widely within the overall system stability (Fischer et al. 2006; Tilman et al. 2006; Ratnadass et al 2012; Sabatier et al. 2013; Lemaire et al. 2014). This fluctuation of target crops poses an ecological challenge for the evolution of biodiverse agroecosystems (Weiner et al. 2010). Economically, farmers must have access to diversified markets so that agricultural production is not limited to a single commodity whose production may fluctuate with climatic and biological shifts, or whose price may fluctuate with the markets (Kleijn and Sutherland 2003). When farmers do rely on one commodity crop, they create simpler agroecosystems that compromise diversity in an effort to improve production stability to meet market demands (Tilman et al. 2006; Sabatier et al. 2013). Resilience of the whole agroecological system is important, and tradeoffs are sometimes necessary if we value the economy, the environment, and society equally.

We urge agroecologists to emphasize general principles of resilience and biodiversity aimed at improving productivity, environmental conservation, economic viability, and social equity. From a systems approach based on foundational principles, context-dependent solutions will arise using the breadth and depth of agroecological knowledge and application. Our unified agroecological framework encourages increased farmer and consumer demand for information about growing and distributing food that must be met with a clear and open dialog.

5.4 Summary and Conclusion

Agroecology emerged in the 1930s alongside early agronomic research and first diverged during a debate over whether soil should be treated as a simplified mix of plant growth media or as a complex biological agroecosystem. The current simplified agricultural framework focuses primarily on production and fails to adequately feed the world while also causing unintended harm to the environment and society. The agroecological approach includes environmental, biological, economic and sociological processes within a defined set of agroecosystem boundaries.

Agroecology has developed as a field and as a descriptive term with a myriad of uses that can be grouped into four main descriptions: (1) a rigorous systems research approach that compares and evaluates the impacts and improvements of agriculture on the natural and socio-economic environment, (2) a method of agricultural design that is informed by observations of traditional farming systems, natural ecosystems, and agroecological theory, (3) a moniker for an agricultural practice that has achieved the highest measure of sustainability, often used erroneously out of context, and (4) a socio-political research and policy movement that focuses on the broad effects agricultural choices have on people.

We conducted a literature review of the academic literature that suggests agro-ecologists can build cohesion through emphasis on systems thinking and the integration of environmental, ecological, and socioeconomic principles in agriculture, such as resilience, biodiversity and production. Future research could expand on our analysis to include the agroecological dialog in trade and popular literature also. Publications in the field of agroecology continues to increase at a rapid rate and can improve quality of communication through a deliberate use of principles and clear terms. Many goals and characteristics of an agroecological approach are strikingly consistent that can build cohesion and unification in the discipline. We propose the principles of systems thinking, resilience, biodiversity and production as fundamental components of a unified agroecological framework. Agroecology aims to produce sufficient agricultural products in a system that emphasizes biological processes and ecological relationships, limits external inputs and maximizes nutrient recycling, and gives equal consideration to the economy, the environment, and the society.

Agroecology has a long history influenced by several fields of study and continues to promote a rapid increase of interest and use. Interdisciplinary teams of researchers and visionary farmers use an agroecological framework to promote a systems approach to developing resilient agroecosystems that produce food, conserve resources, and provide a fair livelihood for practitioners of agriculture in the long term. However, subtle permutations of the understanding of agroecology among researchers, farmers and the public have led to communication barriers that impede the field's development. This multitude of meanings requires us to define our terminology precisely. As we move forward, we must emphasize the principles that define an agroecological framework in order to develop a sustainable farming and food system. To promote understanding among disciplines, we urge agroecologists and their colleagues to clearly describe their work within the broader context of agroecological definitions, as well as to highlight the relevant principles and constraints to their particular system.

An agroecological framework uses a systems approach to feed the world that improves resilience and diversity in the economy, environment, and society. While this simplified framework is broad in scope and based on general ecological principles, we realize that developing agroecosystems is very complex and involves site-specific decisions. Indeed, the agroecological framework encourages this diversity in systems and solutions moving toward a sustainable agricultural future.

Supplementary Tables

Table S1 Top ten citations for the search term 'agroecology' OR 'agroecological'

Title	Author	Year	Journal	Citations
[a]Agricultural intensification and ecosystem properties	Matson PA, Parton WJ, Power AG, Swift MJ	1997	Science	943
[a]Soil fertility and biodiversity in organic farming	Mäder P, et al.	2002	Science	842
[a]How effective are European agri-environment schemes in conserving and promoting biodiversity?	Kleijn D, Sutherland WJ	2003	Journal of Applied Ecology	624
[a]The ecological role of biodiversity in agroecosystems	Altieri MA	1999	Agriculture, Ecosystems and Environment	593
[a]Agroecosystems, nitrogen-use efficiency, and nitrogen management	Cassman KG, Dobermann A, Walters DT	2002	Ambio	385
[a]Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture	Cassman KG	1999	PNAS	366
[a]Agroecosystem responses to combinations of elevated CO_2, ozone, and global climate change	Fuhrer J	2003	Agriculture, Ecosystems and Environment	244
[a]Global Food Security: Challenges and Policies	Rosegrant MW, Cline SA	2003	Science	215
[a]Annual carbon dioxide exchange in irrigated and rainfed maize-based agroecosystems	Verma SB, et al.	2005	Agricultural and Forest Meteorology	208
[a]Agricultural sustainability: Concepts, principles and evidence	Pretty J	2008	Philosophical Transactions of the Royal Society B: Biological Sciences	200

[a]Denotes publication included in multiple top ten lists

Table S2 Top ten citations for the search term 'principle'

Title	Author	Year	Journal	Citations
[a]Agricultural sustainability: Concepts, principles and evidence	Pretty J	2008	Philosophical Transactions of the Royal Society B: Biological Sciences	200
[a]Biodiversity conservation in tropical agroecosystems: A new conservation paradigm	Perfecto I, Vandermeer J	2008	Annals of the New York Academy of Sciences	146
[a]Farming for the future: an introduction to low-external-input and sustainable agriculture	Reijntjes C, Haverkort B, Waters-Bayer A	1992	*Farming for the future: an introduction to low-external-input and sustainable agriculture*	132
[a]Agroecology: The ecology of food systems	Francis C, et al.	2003	Journal of Sustainable Agriculture	85
SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different megaenvironments	Dreisigacker S, et al.	2004	Crop Science	69
[a]Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge	Dore T, et al.	2011	European Journal of Agronomy	68
The role of the concept of the natural (naturalness) in organic farming	Verhoog H, Matze M, Van Bueren EL, Baars T	2003	Journal of Agricultural and Environmental Ethics	62
[a]The greening of the "barrios": Urban agriculture for food security in Cuba	Altieri MA, et al.	1999	Agriculture and Human Values	57
Communicating complexity: Integrated assessment of trade-offs concerning soil fertility management within African farming systems to support innovation and development	Gillet KE, et al.	2011	Agricultural Systems	52
Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: A review	Ratnadass A, Fernandes P, Avelino J, Habib R	2012	Agronomy for Sustainable Development	49

[a]Denotes publication included in multiple top ten lists

Table S3 Top ten citations for the search term 'practice'

Title	Author	Year	Journal	Citations
[a]Agroecosystems, nitrogen-use efficiency, and nitrogen management	Cassman KG, Dobermann A, Walters DT	2002	Ambio	385
[a]Annual carbon dioxide exchange in irrigated and rainfed maize-based agroecosystems	Verma SB et al.	2005	Agricultural and Forest Meteorology	208
[a]Agricultural sustainability: Concepts, principles and evidence	Pretty J	2008	Philosophical Transactions of the Royal Society B: Biological Sciences	200
Identifying critical limits for soil quality indicators in agro-ecosystems	Arshad MA, Martin S	2002	Agriculture, Ecosystems and Environment	183
[a]Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies	Oenema O, Kros H, De Vries W	2003	European Journal of Agronomy	182
Evaluation of the environmental impact of agriculture at the farm level: A comparison and analysis of 12 indicator-based methods	Van Der Werf HMG, Petit J	2002	Agriculture, Ecosystems and Environment	163
Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle	Lundquist EJ, Scow KM, Jackson LE, Uesugi SL, Johnson CR	1999	Soil Biology and Biochemistry	160
Soil health and global sustainability: Translating science into practice	Doran JW	2002	Agriculture, Ecosystems and Environment	147
Determinants and effects of income diversification amongst farm households in Burkina Faso	Reardon T, Delgado C, Matlon P	1992	Journal of Development Studies	138
[a]Farming for the future: an introduction to low-external-input and sustainable agriculture	Reijntjes C, Haverkort B, Waters-Bayer A	1992	*Farming for the future: an introduction to low-external-input and sustainable agriculture*	132

[a]Denotes publication included in multiple top ten lists

Table S4 Top ten citations for the search term 'research'

Title	Author	Year	Journal	Citations
[a]How effective are European agri-environment schemes in conserving and promoting biodiversity?	Kleijn D, Sutherland WJ	2003	Journal of Applied Ecology	624
[a]The ecological role of biodiversity in agroecosystems	Altieri MA	1999	Agriculture, Ecosystems and Environment	593
[a]Agroecosystems, nitrogen-use efficiency, and nitrogen management	Cassman KG, Dobermann A, Walters DT	2002	Ambio	385
[a]Global Food Security: Challenges and Policies	Rosegrant MW, Cline SA	2003	Science	215
[a]Agroecology: The science of natural resource management for poor farmers in marginal environments	Altieri MA	2002	Agriculture, Ecosystems and Environment	188
[a]Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies	Oenema O, Kros H, De Vries W	2003	European Journal of Agronomy	182
[a]Arthropod pest management in organic crops	Zehnder G, et al.	2007	Annual Review of Entomology	176
An overview of some tillage impacts on earthworm population abundance and diversity – Implications for functioning in soils	Chan KY	2000	Soil and Tillage Research	147
[a]Beyond reserves: A research agenda for conserving biodiversity in human-modified tropical landscapes	Chazdon RL, et al.	2009	Biotropica	135
International approach to assessing soil quality by ecologically-related biological parameters	Filip Z	2002	Agriculture, Ecosystems and Environment	121

[a]Denotes publication included in multiple top ten lists

Table S5   Top ten citations for the search term 'design'

Title	Author	Year	Journal	Citations
[a]How effective are European agri-environment schemes in conserving and promoting biodiversity?	Kleijn D, Sutherland WJ	2003	Journal of Applied Ecology	624
[a]The ecological role of biodiversity in agroecosystems	Altieri MA	1999	Agriculture, Ecosystems and Environment	593
[a]Beyond reserves: A research agenda for conserving biodiversity in human-modified tropical landscapes	Chazdon RL, et al.	2009	Biotropica	135
Soil fertility management and insect pests: Harmonizing soil and plant health in agroecosystems	Altieri MA, Nicholis CI	2003	Soil and Tillage Research	95
Environmental benefits of conservation buffers in the United States: Evidence, promise, and open questions	Lovell ST, Sullivan, WC	2006	Agriculture, Ecosystems and Environment	86
[a]Agroecology: The ecology of food systems	Francis C, et al.	2003	Journal of Sustainable Agriculture	85
[a]Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge	Dore T, et al.	2011	European Journal of Agronomy	68
Object-based crop identification using multiple vegetation indices, textural features and crop phenology	Peña-Barragán JM, Ngugi MK, Plant RE, Six J	2011	Remote Sensing of Environment	67
Developing incentives and economic mechanisms for in situ biodiversity conservation in agricultural landscapes	Pascual U, Perrings C	2007	Agriculture, Ecosystems and Environment	64
An agent-based simulation model of human-environment interactions in agricultural systems	Schreinemachers P, Berger T	2011	Environmental Modeling and Software	58

[a]Denotes publication included in multiple top ten lists

Table S6 Top ten citations for the search term 'movement'

Title	Author	Year	Journal	Citations
[a]Biodiversity conservation in tropical agroecosystems: A new conservation paradigm	Perfecto I, Vandermeer J	2008	Annals of the New York Academy of Sciences	146
[a]Beyond reserves: A research agenda for conserving biodiversity in human-modified tropical landscapes	Chazdon RL, et al.	2009	Biotropica	135
Agroecology as a science, a movement and a practice. A review	Wezel A, et al.	2009	Agronomy for Sustainable Development	76
Agroecology versus input substitution: A fundamental contradiction of sustainable agriculture	Rosset PM, Altieri MA	1997	Society and Natural Resources	60
[a]The greening of the "barrios": Urban agriculture for food security in Cuba	Altieri MA, et al.	1999	Agriculture and Human Values	57
The agroecological revolution in Latin America: Rescuing nature, ensuring food sovereignty and empowering peasants	Altieri MA, Toledo VM	2011	Journal of Peasant Studies	50
Organic and conventional agriculture: Materializing discourse and agro-ecological managerialism	Goodman D	2000	Agriculture and Human Values	43
Measuring farmers' agroecological resistance after Hurricane Mitch in Nicaragua: A case study in participatory, sustainable land management impact monitoring	Holt-Gimenez E	2002	Agriculture, Ecosystems and Environment	37
Traditional agroecological knowledge, adaptive management and the socio-politics of conservation in Central Sulawesi, Indonesia	Armitage DR	2003	Environmental Conservation	34
[a]The Campesino-to-Campesino agroecology movement of ANAP in Cuba: Social process methodology in the construction of sustainable peasant agriculture and food sovereignty	Rosset PM, Sosa BM, Jaime AMR, Lozano DRA	2011	Journal of Peasant Studies	31

[a]Denotes publication included in multiple top ten lists

Table S7 Top ten citations for the search term 'systems'

Title	Author	Year	Journal	Citations
[a]Soil fertility and biodiversity in organic farming	Mäder P, et al.	2002	Science	842
[a]Agroecosystems, nitrogen-use efficiency, and nitrogen management	Cassman KG, Dobermann A, Walters DT	2002	Ambio	385
[a]Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture	Cassman KG	1999	PNAS	366
[a]Agroecosystem responses to combinations of elevated CO2, ozone, and global climate change	Fuhrer J	2003	Agriculture, Ecosystems and Environment	244
[a]Annual carbon dioxide exchange in irrigated and rainfed maize-based agroecosystems	Verma SB, et al.	2005	Agricultural and Forest Meteorology	208
[a]Agricultural sustainability: Concepts, principles and evidence	Pretty J	2008	Philosophical Transactions of the Royal Society B: Biological Sciences	200
[a]Agroecology: The science of natural resource management for poor farmers in marginal environments	Altieri MA	2002	Agriculture, Ecosystems and Environment	188
[a]Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies	Oenema O, Kros H, De Vries W	2003	European Journal of Agronomy	182
A model for fossil energy use in Danish agriculture used to compare organic and conventional farming	Dalgaard T, Halberg N, Porter JR	2001	Agriculture, Ecosystems and Environment	178
[a]Arthropod pest management in organic crops	Zehnder G, et al.	2007	Annual Review of Entomology	176

[a]Denotes publication included in multiple top ten lists

Table S8 Top ten citations for the search term 'biodiversity'

Title	Author	Year	Journal	Citations
[a]Agricultural intensification and ecosystem properties	Matson PA, Parton WJ, Power AG, Swift MJ	1997	Science	943
[a]Soil fertility and biodiversity in organic farming	Mader P, et al.	2002	Science	842
[a]How effective are European agri-environment schemes in conserving and promoting biodiversity?	Kleijn D, Sutherland WJ	2003	Journal of Applied Ecology	624
[a]The ecological role of biodiversity in agroecosystems	Altieri MA	1999	Agriculture, Ecosystems and Environment	593
Social capital in biodiversity conservation and management	Pretty J, Smith D	2004	Conservation Biology	200
Human geography and the "new ecology': the prospect and promise of integration	Zimmerer KD	1994	Annals – Association of American Geographers	193
[a]Arthropod pest management in organic crops	Zehnder G, et al.	2007	Annual Review of Entomology	176
Global food security, biodiversity conservation and the future of agricultural intensification	Tscharntke T, et al.	2012	Biological Conservation	160
Conservation of biodiversity in coffee agroecosystems: A tri-taxa comparison in southern Mexico	Perfecto I, Mas A, Dietsch T, Vandermeer J	2003	Biodiversity and Conservation	150
[a]Biodiversity conservation in tropical agroecosystems: A new conservation paradigm	Perfecto I, Vandermeer J	2008	Annals of the New York Academy of Sciences	146

[a]Denotes publication included in multiple top ten lists

Table S9   Top ten citations for the search term 'resilience'

Title	Author	Year	Journal	Citations
[a]Agricultural sustainability: Concepts, principles and evidence	Pretty J	2008	Philosophical Transactions of the Royal Society B: Biological Sciences	200
Tropical Soils and Food Security: The Next 50 Years	Stocking MA	2003	Science	88
Assessing a farm's sustainability: Insights from resilience thinking	Darnhofer I, Fairweather J, Moller H	2010	International Journl of Agricultural Sustainability	46
Agroecologically efficient agricultural systems for smallholder farmers: Contributions to food sovereignty	Altieri MA, Funes-Monzote FR, Petersen P	2012	Agronomy for Sustainable Development	42
Ecosystem services in biologically diversified versus conventional farming systems: Benefits, externalities, and trade-offs	Kremen C, Miles A	2012	Ecology and Society	32
[a]The Campesino-to-Campesino agroecology movement of ANAP in Cuba: Social process methodology in the construction of sustainable peasant agriculture and food sovereignty	Rosset PM, Sosa BM, Jaime AMR, Lozano DRA	2011	Journal of Peasant Studies	31
Agroecology: A review from a global-change perspective	Tomich TP, et al	2011	Annual Review of Environment and Resources	28
Prospects from agroecology and industrial ecology for animal production in the 21st century	Dumont B, Fortun-Lamothe L, Jouven M, Thomas M, Tichit M	2013	Animal	25
Modelling loss of resilience in agroecosystems: Rangelands in Botswana	Perrings C, Stern DI	2000	Environmental and Resource Eonomics	23
Systems approaches and properties, and agroecosystem health	Okey BW	1996	Journal of Environmental Management	23

[a]Denotes publication included in multiple top ten lists

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Chapter 6
Epichloë Fungal Endophytes for Grassland Ecosystems

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Abstract The *Epichloë* fungal endophytes that inhabit grasses have potentially large-scale consequences for macro- and micro-organisms and food chains in agriculture. Over 40 years of study on the benefits of symbiotic *Epichloë* fungal endophytes for host grasses, investigations have focused on the major agricultural species, tall fescue and perennial ryegrass. However, many other grass species remain to be evaluated for the effects of *Epichloë* endophytes. Animal toxicity due to accumulation of nitrogenous compounds, e.g. endophyte-derived alkaloids, particularly in areas and periods under abiotic stress, still prevent widespread application of endophyte-infected grasses in agroecosystems.

Here we review *Epichloë* endophyte-ecosystem relationships. The major points are: (1) *Epichloë* endophytes protect their host plants from vertebrate and invertebrate herbivory and allow plants to persist under water shortage, salinity, low light, mineral deficiencies and metal toxicity. Data suggests that the concentration of endophyte-derived anti-herbivore compounds increases with rising temperatures. This trend thus suggests that the strength of mutualistic interactions may increase in future climates with possible consequences for animal toxicity. (2) The benefits of endophyte infection for the host grass are context-dependent, varying with environmental conditions, grass species and cultivar, and are also highly influenced

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by interactions between both host and endophyte genetic combinations. (3) The benefits of fungal endophytes extend to neighboring grass species with respect to their protection from diseases and herbivores. (4) Novel grass-endophyte associations that produce alkaloids reducing herbivory insects, but do not produce alkaloids that are toxic to grazing vertebrates have been found. Such associations are therefore useful to control plant pests and diseases.

6.1   Introduction

Cool-season grasses are widely grown in temperate regions of the world as forage for cattle, sheep and horses, as well as for turf and conservation exploitations. They may harbor internal fungal components that until recent decades were largely unknown and their significance not recognized. Fungi belonging to the *Epichloë* (Ascomycetes) genera, have both sexual and asexual (formerly *Neotyphodium* genera) forms, and confer many benefits to the grass hosts including persistence/fitness attributes; resistance/deterrence to insect feeding, drought and salinity tolerance, resistance to nematodes and fungal pathogens, and improved mineral nutrition. The production of anti-herbivore compounds by endophytes protects host plants from herbivory, however, they may have detrimental effects, causing production losses in livestock. With these complex functions, fungal endophytes can affect the economic value of forage production in natural rangelands and sown pastures, and deserve further study to explore possible applications.

Fungal endophytes have changed our past knowledge of plant physiology, phytochemistry and ecology of grasses and the factors determining plant fitness within grassland ecosystems. It is believed that after nitrogen fixing bacteria (*Rhizobium* spp.) and mycorrhizal root fungi, systemic and aerial fungal species of the genus *Epichloë* are among the most common and highly diverse microorganisms which infect many grass species of the Poaceae family.

At present, what we know about the symbiotic associations between grass and *Epichloë* endophytes is predominantly based on two model grasses: tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort. syn. *Festuca arundinacea* (Schreb.)) and perennial ryegrass (*Lolium perenne* L.) containing the obligatory fungal endophytes *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*) and *Epichloë festucae* var. *lolii* (formerly *N. lolii*), respectively (Christensen et al.1993; Leuchtmann et al. 2014). In fact, the literature is strongly biased toward studies on tall fescue and perennial ryegrass while other species, especially annual and native species, of many countries have been seldom studied (Saikkonen et al. 2004, 2006; Omacini et al. 2012). Infection with *Epichloë* endophytes in these two species has been generally proven to benefit host grasses by conferring increased resistance to a wide spectra of herbivores, abiotic stress (e.g. drought, salinity) tolerance, and

enhanced growth and competitive ability of host plants (Clay and Schardl 2002). Some of these characteristics are conferred to host grasses via synthesis of fungal metabolites including the alkaloids peramine, lolines, lolitrems, and ergot alkaloids (Saikkonen et al. 2013a).

More than 100 grass species are reportedly infected with one or more fungal strains of *Epichloë* species and it is estimated that approximately 900 pooid grass species may harbor this fungal endophyte (Faeth 2002). Therefore, many previously unexamined grass species remain to be evaluated for *Epichloë* endophytes. In fact, there is a diversity of *Epichloë* strains in nature that may potentially be inoculated into new hosts. Moreover, the consequences of using common toxic strains which are found in widely grown grasses with high concentrations of toxic alkaloids versus modified and selected strains (novel endophytes) of known and newly-identified fungal symbionts in agricultural ecosystems, and possibly in strategic crop species of wheat and barley, could be significant in the future (Simpson et al. 2014).

Detecting new species and strains of fungal endophytes may also have applied value in uncovering those factors that can be manipulated to achieve more dynamic, sustainable and productive agroecosystems. Recent studies have revealed high levels of variation in the effects of endophytes on host plants, ranging from strongly positive to neutral and in some cases strongly negative (see next sections). This may suggest that these vertically transmitted endophytes and their functions are highly influenced by genetic elements including plant and *Epichloë* genotypes and their interaction, and non-genetic elements that are not currently fully known for grass-*Epichloë* endophytism.

Early research on grass endophytes was tasked with deciding whether endophyte infection was a benefit or a detriment to grasses and forage crops (Joost 1995). In fact, more than 40 years of investigation clarified that endophyte-infected plants exhibit several adaptive and resistance morphological and physiological responses to environmental stresses including insects, nematodes, drought and salinity. However, poor performance of beef cattle, sheep and horses grazing tall fescue and ryegrass infected with *Epichloë* endophytes still challenges the utilization of endophytes in grassland ecosystems. Consequently, in contrast to the idea of establishing endophyte-free pastures, other options of pasture management include minimizing the volume of toxic alkaloids ingested by domesticated animals and/or finding benign strains of *Epichloë* endophyte with minimal toxicity (Gundel et al. 2013; Young et al. 2013) to establish widely cultivated stands. Although the adoption of endophyte-free pastures may provide farmers with fast relief from animal disorders, it is not a long term economic decision. Therefore, considering the potential offered by variation revealed among endophytic isolates and their interaction with host genetic background, endophyte infection is likely to be of great utility in the management of grassland ecosystems.

A close connection between fungal endophytes and grass hosts results in mutualistic interactions between the two partners with outcomes not only for the symbiotum but also sometimes the whole ecosystem that comprises many vertebrate and invertebrate organisms. Although there are many published papers on grass-*Epichloë* endophyte interactions, there are also undiscovered mechanisms with respect to

how endophyte alters grass physiology, herbivory, neighboring competitors, soils, and ultimately the whole ecosystem. In other words, the consequences of grass endophyte infection at the host level have been considered in many controlled experiments but progress has been made more slowly at the community and agro-ecosystem levels where benefits conferred to the host by fungal endophytes may be extended to neighboring species (Garcia Parisi et al. 2014). The aim of this review is to synthesize the literature on the present and future potential ability of *Epichloë* fungal endophyte to affect different biological components of agroecosystems and to be used for further development of grasslands around the world.

6.2   The Risk for Animal Health and Productivity in Grassland Ecosystems

Livestock consuming forage containing cool-season grasses can be affected in two ways by the presence of *Epichloë* fungal endophytes. Firstly, these endophytes can negatively impact animals consuming endophyte-infected grasses through anti-quality factors. Secondly, pasture productivity and botanical composition can be affected by endophytes, and as a result, impact on quantity and quality of forage available to livestock. Research has focused on understanding and manipulating these effects in order to achieve optimal outcomes for the sustainability, productivity and profitability of livestock industries that are dependent on grass-based forage systems (Aiken and Strickland 2013; Young et al. 2013; Johnson et al. 2013). In this section, the anti-quality aspects of *Epichloë* endophytes are reviewed. For change in pasture productivity and botanical composition, see Sects. 6.5 and 6.7.

Researchers have been able to identify a range of secondary metabolites produced when *Epichloë* endophytes are in association with cool-season grasses. The significant compounds are primarily alkaloids; peramine, lolines, indole-diterpenes and ergot alkaloids (Panaccione et al. 2014). These alkaloids convey to infected plants important economic resistance to herbivory; invertebrate pasture pests such as insects (Sect. 6.3), and grazing vertebrates such as cattle and sheep. Only the indole-diterpenes and ergot alkaloids elicit toxic responses in livestock (anti-quality factors). Their presence and concentrations can be used to predict clinical toxicity when only herbage can be analyzed and animal trials are not possible (Tor-Agbidye et al. 2001). These alkaloids can cause a range of detrimental effects to animals, both clinical and sub-clinical. These are most well-known and best described in pastures in the New World (Australia, New Zealand and USA) where endophyte-infected grass species introduced from Europe, such as ryegrass and tall fescue, dominate sown pastures.

6.2.1 Ryegrass

Perennial and long-term hybrid ryegrasses, *Lolium perenne* and *L. boucheanum* (syn. *L. hybridum*), respectively, can be infected with *Epichloë festucae* var. *lolii*. In terms of endophyte toxicity, the ryegrass-endophyte associations that became naturalized in large areas of New Zealand and southern Australia in the nineteenth century are most widely known for causing ryegrass staggers in livestock (Fig. 6.1). Ryegrass staggers is a neuromuscular disorder which occurs in late spring, summer and autumn, caused by the common toxic strain of endophyte (di Menna et al. 2012). This disorder affects a wide range of ruminants and monogastrics, including sheep, cattle, horses, donkeys, deer, goats, llamas, camels, alpacas, and rhinoceros, and has been documented to occur around the world. It has been most well studied in New Zealand where the association between ryegrass staggers and *Epichloë* endophyte was first discovered in the summer of 1980–1981 (Fletcher and Harvey

Fig. 6.1 Condition of cattle and sheep that have been grazing tall fescue (*Schedonorus arundinaceus*) or perennial ryegrass (*Lolium perenne*) pastures in the USA and New Zealand. (**a**) Steer that has been grazing endophyte-free tall fescue; (**b**) Steer that has been grazing tall fescue infected with the common toxic endophyte strain and is suffering from fescue toxicosis (Photos courtesy of John Waller, University of Tennessee, Knoxville, USA). (**c**) A 7 month old sheep in summer exhibiting ryegrass staggers, a neuro-muscular disorder caused by the common toxic strain of endophyte in perennial ryegrass (Photo courtesy of Lester Fletcher, AgResearch Ltd., New Zealand). (**d**) Bull cooling off in water due to hyperthermia as a result of grazing common toxic endophyte-infected tall fescue pasture in Arkansas (Photo courtesy of Glen Aiken, USDA-ARS, Lexington, Kentucky, USA)

1981). Pastoral agriculture in New Zealand is highly reliant on this ryegrass, and due to intense insect predation, endophyte-infected ryegrass dominates improved pastures. In some regions of New Zealand, the climate and grazing management can combine resulting in frequent and severe toxicity to livestock.

Researchers in New Zealand found that ryegrass staggers was not the only disorder caused by the common toxic endophyte strain in ryegrass (Prestidge 1993; Fletcher 1999; Fletcher et al. 1999; Watson et al. 1999) (Table 6.1). Sheep grazing common toxic endophyte typically have higher death rates (due to staggers), more fecal soiling in the breech area (termed 'dags') leading to higher incidence of myiasis (flystrike), increased rectal temperatures and respiration rates when under heat load, reduced feed intake, and most critically, lower live weight gains. It is one of the likely contributors to livestock failing to thrive in summer. This is likely to be linked to the common toxic endophyte not only producing tremorgenic indolediterpenes, most notably lolitrem B which is the prime causative alkaloid of ryegrass staggers (Gallagher et al. 1981), but also ergot alkaloids which are most commonly quantified through concentration of ergovaline in herbage. The toxicity of ergot alkaloids in ryegrass-endophyte associations has parallels to the ergot alkaloid toxicity seen predominately in the USA when tall fescue is infected with its common toxic endophyte. In pasture-based dairy systems, the most substantive evidence of a toxic endophyte effect was seen in a 3-year systems experiment in New Zealand (Bluett et al. 2005). In this study, cows grazing common toxic endophyte-infected ryegrass, grown in association with white clover (*Trifolium repens*), suffered ryegrass staggers in 1 year, and over all the years, produced an average of 9 % less milk solids than cows grazing the equivalent ryegrass infected with the non-toxic AR1 endophyte which does not produce lolitrem B or ergovaline. Comparisons with endophyte-free pastures were not made as such pastures fail to survive in this region.

In Australia, livestock consuming perennial ryegrass infected with the common toxic endophyte suffer from what has been termed 'perennial ryegrass toxicosis (PRGT)'. Livestock exhibit ryegrass staggers, heat stress, loss of productivity, and mortality to a level which can reach epidemic proportions in some years e.g. greater than 100,000 in 2002 (Reed et al. 2005; Reed et al. 2011). The phenomenon of high mortality is characteristic of Australia, and is not seen in New Zealand. While these epidemics are sporadic, ryegrass staggers in sheep can be observed every year in some regions, while subclinical losses are also likely in most years and are widespread.

The economic impact of the common toxic endophyte strain in ryegrass is substantial in New Zealand and Australia. For example, based on data from a grazing systems trial with high fertility sheep in New Zealand, Fletcher (1999) calculated common toxic endophyte-infected ryegrass had annual gross financial returns 16–18 % lower than a ryegrass system based on endophyte-free or the AR1-selected endophyte. On a national basis, PRGT in Australia in 2006 was conservatively estimated to be causing financial losses of AU \$72 million year[-1] (Sackett and Francis 2006), with a more recent 2012 estimate placing this at AU \$100 million year[-1] (Leury et al. 2014).

Table 6.1 Effects of the common toxic strain of fungal endophyte on lambs grazing only perennial ryegrass (*Lolium perenne*) in summer and autumn in Canterbury, New Zealand

Infection status of ryegrass	Ryegrass staggers score (0–5 scale, 0=nil)	Deaths due to ryegrass staggers (%)	Fecal soiling of the breech (dags) (0–5 scale, 0=nil)	Incidence of myiasis (flystrike) (%)	Liveweight gain (g head^{-1} day^{-1})	Rectal temperature (°C)	Respiration rate (breaths/minute)
Common toxic endophyte	3.1 a	5–11	1.5 a	22 a	40 b	40.6 a	99 a
No endophyte	0.4 b	0	0.4 b	2 b	110 a	40.2 b	73 b

Data is the mean of five trials over 3 years (Fletcher et al. 1999), except for deaths which is from a grazing systems trial over 3 years with ewes and lambs (Fletcher 1999). Means within a column with the same letter are not significantly different ($P<0.05$)

Table 6.2 Threshold concentration ranges of endophyte alkaloids for the expression of clinical toxicity when fed to horses, cattle, sheep and camels

	Threshold concentration range in forage (ppm)	
Animal species	Ergovaline	Lolitrem B
Horse	0.30–0.50[a]	0.8–1.2
Cattle	0.40–0.75	1.8–2.0
Sheep	0.50–0.80	1.8–2.5
Camel	–	≤1.1

Ergovaline causes a range of adverse effects on livestock, including fescue foot and heat stress, and is produced by both tall fescue (*Schedonorus arundinaceus*) and perennial ryegrass (*Lolium perenne*) when infected with their common toxic endophytes. Lolitrem B causes ryegrass staggers and is produced by ryegrass infected with its common toxic endophyte. For ergovaline, the threshold level is lower when animals are in cold temperatures. Compiled from di Menna et al. (1992), Tor-Agbidye et al. (2001), Aldrich-Markham et al. (2003), Fink-Gremmels (2005) and Alabdouli et al. (2014)
[a]Threshold is zero for mares in late pregnancy

Ryegrass staggers in cattle has been reported in Chile (Butendieck et al. 1994), Argentina (Odriozola et al. 1993) and South Africa (Kellerman et al. 1993), all occurring on pastures sown with ryegrass seed containing high levels of common toxic endophyte. Coastal California is the only region in the USA where ryegrass staggers has been reported for cattle and sheep grazing ryegrass pastures (Galey et al. 1993), which is not surprising given the low use of this species in the USA and most US pasture cultivars are endophyte-free (Young et al. 2013). Ryegrass staggers has been recorded for endophyte-infected straw from seed production fields for turf cultivars in Oregon. This straw has also caused staggers, along with concerns over food safety, when shipped and fed to cattle and horses in Japan (Craig 2009). This toxicity in Japan led to the development of threshold alkaloid levels for toxicity of straw fed to horses, cattle and sheep (Table 6.2), and certificates for alkaloid concentrations that can be issued for straw exported to Asian countries. A limit of 2 ppm lolitrem B was set for export of straw (Young III and Silberstein 2012) based on data from the USA (Tor-Agbidye et al. 2001) and New Zealand (di Menna et al. 1992) which had established 1.8–2.5 ppm as the threshold range above which staggers could occur.

While the ryegrass endophyte is well known for its toxicity in the New World, cases in Europe of ryegrass staggers are sporadic and are small in scale (di Menna et al. 2012), and only one controlled field study has reported effects on live weight gain in sheep (Oldenburg 1998). It appears counter intuitive that Europe has low occurrence of ryegrass staggers and toxicosis, as the ryegrass endophytes causing toxicity elsewhere in the world were originally unknowingly exported from Europe by early colonizers of the New World. It is probably for this reason that endophyte studies have been reported from many European countries and have been well summarized in regular reviews (Lewis 1997, 2001; Bony and Delatour 2001; Zabalgogeazcoa and Bony 2005). Ryegrass staggers has been recorded in sheep, cattle and horses in Germany, the Netherlands, Belgium, France and the United

Kingdom. It is often associated with dry summers particularly in years of severe drought, the feeding of hay or straw, and feeding turf cultivars which are generally highly infected with common toxic endophyte. In the United Kingdom and France, ryegrass endophyte toxicoses appear to be less well-recognized, not well-documented, or on occasions misdiagnosed. Lolitrem B and ergovaline have been reported in ryegrass herbage from a range of European countries and on occasions at concentrations high enough to elicit subclinical or clinical effects in livestock if fed at a high proportion of the diet. The overall low incidence of toxicosis in Europe may be due to a range of factors. Sown pasture cultivars have no or low levels of endophyte, and while old permanent pastures may have high incidence of endophyte they are botanically diverse which would dilute endophyte toxins in the diet of grazing animals. In the drier southern regions where staggers may be expected, ryegrass is seldom the dominant species in pastures. In intensive production systems, grass is a relatively small component of the diet with high energy supplements being fed, and indoor feeding systems do not harvest the high alkaloid concentrations at the base of infected ryegrass plants. In addition, climatic conditions are only occasionally conducive (e.g. drought temporarily) to the production of high levels of toxic alkaloids.

6.2.2 Tall Fescue

The association of an *Epichloë* endophyte with livestock toxicity in tall fescue was first made in the southern USA in 1976 for grazing cattle (Bacon et al. 1977). In the USA, this toxicity is widespread and can be severe, being the major grass-induced toxicity in the country (Cheeke 1995). The prevalence of this toxicity can be attributed to the development and widespread use of the cultivar Kentucky 31 from the early 1940s which is infected with common toxic endophyte (Bacon 1995). In 1993, losses to the US beef industry alone were conservatively estimated at US\$ 609 million year^{-1} (Hoveland 1993). Based on 2013 cattle prices, and a known wider effect on live weight gains, losses are now estimated to be US\$ 1.0–1.5 billion year^{-1} (Aiken and Strickland 2013). Toxicity in livestock is characterized by three syndromes; fescue foot, bovine fat necrosis, and fescue toxicosis (summer slump or syndrome) (Strickland et al. 2009; Waller 2009), with fescue toxicosis having the greatest economic impact.

Fescue foot occurs when cattle graze endophyte-infected tall fescue in the cold temperatures of winter. As a result of a number of blood circulatory disorders, reduced peripheral blood flow results in animals developing lameness. The disorder can progress to gangrene in extremities, leading to necrosis of affected tissues with sloughing of the hooves and loss of the tips of ears and tails. When bovine fat necrosis (liptomatosis) occurs, a mass of necrotic fat builds up and occupies important space in the abdominal cavity restricting internal organs causing digestive problems and reduced reproductive capacity, all of which contribute to the symptoms of fescue toxicosis. While fescue foot and bovine fat necrosis are not generally

considered to be widespread problems, economic losses can however be significant for individual farmers when their cattle are afflicted by these syndromes.

Fescue toxicosis is most evident in the late spring and summer, when cattle fail to thrive and suffer heat stress when grazing endophyte-infected tall fescue that is otherwise of good nutritive value (Fig. 6.1). Cattle have poor summer/autumn growth rate and milk production, lowered dry matter intakes and reproduction, rough hair coat, excessive salivation, and elevated body temperature and respiration rate. Animals show an intolerance of heat, often seeking shade, forming wallows and standing in water such as dams in order to cool themselves (Fig. 6.1). The effects on live weight gains can be significant. In controlled studies, weight gains of steers and lambs have been 30–100 % greater when grazing tall fescue which is endophyte-free or infected with a non-toxic endophyte strain, compared with the equivalent tall fescue cultivar infected with the common toxic strain of endophyte (Stuedemann and Hoveland 1998; Bouton et al. 2002; Parish et al. 2003a, b). In one controlled study in Kentucky, dairy cows fed endophyte-infected tall fescue produced 20 % less milk than cows fed endophyte-free tall fescue (Strahan et al. 1987).

Growth rate of horses is reduced to a similar degree to that of cattle when ingesting endophyte-infected tall fescue (Aiken et al. 1993). However, reduced reproductive performance in mares is much greater and more important both economically and from a welfare perspective (Cross 2009). Pregnant mares grazing common toxic endophyte-infected tall fescue late in gestation suffer a range of serious symptoms, including prolonged gestations, increased abortions and foaling difficulties, high rates of agalactia (no milk), thickened and retained placentas, low pregnancy rates, and sometimes deaths during foaling. There is a high rate of stillborn foals, and live foals are weak, malformed and have low growth rates. These reproductive effects are seen widely in the eastern USA, where close to 700,000 horses, some of very high value, graze endophyte-infected tall fescue (Porter and Thompson 1992; Hoveland 1993).

The toxic symptoms seen in livestock consuming *Epichloë coenophiala*-infected tall fescue are similar to those of ergot toxicity caused by the *Claviceps* fungus that infects grass seed heads (ergots). Both fungi belong to the family Clavicipitaceae, in which ergot alkaloid production is common (Schardl et al. 2013a). It was therefore not surprising that ergot alkaloids, and more specifically ergovaline, were determined to be the primary cause of tall fescue endophyte toxicity (Bacon 1995). The impact of varying concentrations of ergot alkaloids on productivity of cattle and sheep ingesting endophyte-infected tall fescue, and perennial ryegrass, is linear or curvilinear and appears to have no threshold below which animal live weight gains or dairy cow milk production are unaffected (Fribourg et al. 1991; Schmidt and Osborn 1993; Fletcher et al. 1999; Layton et al. 2004). In the USA, the rule of thumb is that for each 10 % increase in endophyte infection in tall fescue there is a reduced potential weight gain in yearling cattle of 45 g day^{-1} (0.1 lb per day^{-1}). Studies with sheep grazing ryegrass in New Zealand have found a reduction in

growth rate of 3–4 g day^{-1} in spring and up to 10 g day^{-1} in summer-autumn for each 10 % increase in endophyte infection in ryegrass infected with an endophyte that produced ergovaline (and peramine) but no lolitrem B. Threshold concentrations have been established for expression of clinical effects, with sheep being less sensitive than cattle and horses, and thresholds being lower for ergovaline when animals are under cold temperatures and are more likely to suffer fescue foot (Tor-Agbidye et al. 2001; Craig 2009) (Table 6.2).

In Chile, Argentina and Uruguay, endophyte infection of tall fescue is widespread and can occur at high frequencies, with fescue toxicosis occurring in dairy and beef cattle (Sepulveda et al. 1996; De Battista et al. 1997; De Battista 2005). Fescue toxicosis is of greatest concern and well recognized in Uruguay and Argentina where tall fescue is the most widely sown grass species. The economic impact of toxicity can be significant, with estimated losses in Argentina in 1995 of US\$ 54 million year^{-1} due to lower weight gains. To reduce the incidence of toxicity, current regulations in Argentina prevent the sale of seed which contains greater than or equal to 5 % endophyte infection. The greatest risk of fescue toxicosis occurs when animals graze old pastures as these can be highly infected with endophyte (Petigrosso et al. 2013), and those sown with US-imported tall fescues, mainly cultivar Kentucky 31 which also contains a toxic endophyte. The overall impact of fescue toxicosis on animal production systems is, however, considered to be somewhat less than in the USA, due to South American pastures having; greater contents of legumes which dilute the intake of endophyte toxins, a faster rate of pasture renewal, and use of locally bred or maintained cultivars that are now predominately endophyte-free.

Only a few cases of fescue toxicosis have been reported elsewhere in the world; however, when toxicity does occur it can be severe. In Australia and New Zealand, heat stress and fescue foot have been recorded in cattle, but this is confined to small areas and isolated cases for naturalized populations of tall fescue (Easton et al. 1994; Hume and Sewell 2014). In these countries, pasture cultivars of tall fescue have largely been locally bred and are free of endophyte or are infected with nontoxic endophyte strains. A single case of fescue foot in cattle grazing tall fescue has been reported in South Africa, which was most likely due to the presence of *E. coenophiala* although ergotised (*Claviceps purpurea*) seed heads were also present (Botha et al. 2004). There have been reports of fescue toxicosis in Japan when cattle have been fed tall fescue straw imported from the seed production fields of Oregon (Craig 2009). An alkaloid limit for ergovaline of 0.5 ppm has been set for tall fescue and ryegrass straw exported from Oregon (Young III and Silberstein 2012) based on clinical thresholds established by Tor-Agbidye et al. (2001) (Table 6.2). Reports of tall fescue toxicity in Europe are rare, despite reports of concentrations of ergovaline being beyond the threshold to induce clinical toxicosis (Bony and Delatour 2001). There have been a few cases of tall fescue toxicity reported in France and some suspected in Spain, while in a hay feeding experiment, Emile et al. (2000) reported that endophyte reduced weight gains of dairy heifers in France.

6.2.3   Selected Endophytes of Ryegrass and Tall Fescue

Considerable natural variation exists in *Epichloë* endophytes of perennial ryegrass and tall fescue (van Zijll de Jong et al. 2008; Ekanayake et al. 2012). This variation has been utilized to develop and commercialize cultivars infected with 'selected' endophytes (Thom et al. 2012; Johnson et al. 2013; Young et al. 2013). This has been possible as strains varied in production of the four classes of alkaloids, and intense research had quantified the bioactive properties of these alkaloids. The aim was to find endophytes that protected the grass plant from biotic and abiotic stresses, but had minimal clinical and subclinical toxicity to livestock. This has largely been achieved, but trade-offs between plant performance and animal performance may occur in some situations (Fletcher 2012). Despite this, there has been considerable uptake of selected endophyte technology by grassland farmers in New Zealand, Australia and USA, particularly so in New Zealand where selected endophytes in ryegrass dominate market sales (Caradus et al. 2013).

Selected endophytes have either fewer classes or lower concentrations of animal toxic alkaloids, or are completely free of animal toxic alkaloids such as lolitrems and ergot alkaloids (Fletcher 2012; Johnson et al. 2013; Young et al. 2013). These strains still produce bioprotective alkaloids which provide the host grass with resistance to invertebrate pests. For example, tall fescue infected with the selected AR542 endophyte produces peramine and N-acetylnorloline, which have insecticidal activity, but no ergot alkaloids or lolitrems. Ryegrass infected with the Endo5 (AR5) strain produces peramine and reduced levels of the animal toxic ergovaline, but no lolitrem B thereby eliminating the risk of ryegrass staggers. Grazing studies in New Zealand, USA and Australia of ryegrass and tall fescue infected with selected strains of endophyte have shown reduced or no toxicity to sheep, cattle and horses, compared with the common toxic strains, greatly enhancing livestock health and productivity (Fletcher 1999; Bouton et al. 2002; Parish et al. 2003b; Nihsen et al. 2004; Al Rashed 2009; Beck et al. 2009; Fletcher and Sutherland 2009; Moate et al. 2012).

Exploration of this endophyte diversity and development of new grass-endophyte associations has resulted in the discovery of new bioactives. Studies of the AR37 endophyte strain in ryegrass have revealed a previously unknown group of alkaloids, epoxy-janthitrems (Tapper and Lane 2004; Fletcher and Sutherland 2009). Epoxy-janthitrems are indole-diterpenes, so it was not unexpected that sheep grazing AR37-infected ryegrass could suffer ryegrass staggers. Staggers can be as severe as that occurring on ryegrass infected with the common toxic endophyte, but for AR37, staggers are generally less severe and less frequent and other animal health performance factors are unaffected. These animal responses, combined with better agronomic performance than common toxic endophyte, have resulted in considerable uptake of AR37-infected ryegrasses in the New Zealand market with significant financial benefits to New Zealand's pastoral industries (Caradus et al. 2013). Some unexpected consequences for animal health have occurred for some novel combinations but these have been quickly withdrawn from the market (Bourke et al. 2009; Fletcher 2012).

6.2.4   Toxicity Beyond Ryegrass and Tall Fescue

While the majority of research and economic focus has been on *Epichloë* endophyte toxicity of grasses originating from Europe, viz. perennial ryegrass and tall fescue, strong endophyte-driven toxicoses have been described for at least some grasses native to other continents of the world that are grazed in natural ecosystems (Faeth 2002). In some cases, toxicity corresponds with increased presence of the infected grass in the landscape, particularly in cases where overgrazing has occurred, and consequently important economic impacts. Other toxicosis cannot be ruled out if effects are sub-clinical and are yet to be identified.

In South Africa, cattle, horses, donkeys and, to a lesser extent sheep, consuming *Melica decumbens* 'dronkgras' ('drunk grass' in English) exhibit a drunken-like behavior, similar to ryegrass staggers (Gibbs Russell and Ellis 1982; Hoare 2014). This has been linked to infection with *Epichloë* and production of tremorgenic compounds found in other grass-endophyte associations where staggers has been reported (Miles et al. 1995a). Its presence increases in overgrazed rangelands, and its rough leaves and toxicity prevents its use in sown pastures (Hoare 2014). It is of interest that *M. decumbens* is endophyte-infected even in regions of South Africa where no staggers have been observed indicating a number of factors may be involved in the occurrence of clinical toxicity.

In grasses native to Australia and New Zealand, *Epichloë* endophytes are rare with only *Poa matthewsii* and *Echingopogon* species being identified to date as harboring this species of endophyte (Miles et al. 1998; Stewart et al. 2004). When endophyte-infected, *P. matthewsii* and *Echingopogon ovatus* produce compounds that are likely to cause toxicity to livestock, but only in Australia has a ryegrass staggers-like disorder been recorded for *Echingopogon* (Seddon and Carne 1926). The rare occurrence of clinical toxicity may be related to types of secondary metabolites produced, variation in the endophyte species that infect a particular grass species (Moon et al. 2002), and in the New Zealand landscapes due to these grasses occurring in non-grazed woodland habitats.

Achnatherum inebrians (drunken horse grass), a rangeland grass of Mongolia and northwestern China, has long been known to cause intoxication and narcosis in horses, along with donkeys, sheep, goats and cattle (Hance 1876; Miles et al. 1996). Generally most animals recover within a few days but mortality can occur in severely affected animals. *A. inebrians* has been found to be infected with *E. gansuensis* var. *inebrians* (Li et al. 2004), and as a result, very high concentrations of ergot alkaloids have been detected and are most likely responsible for the drunken symptoms in livestock, and possibly along with stipatoxin (Dang et al. 1992; Miles et al. 1996). Incidence of toxicity is minor for animals indigenous to the region as they avoid grazing this grass, with intoxication generally occurring in animals recently imported from regions free of *A. inebrians*. The major problem for livestock farming is that *A. inebrians* is increasing in dominance due to overgrazing and other environmental factors, restricting the development of livestock farming in some regions (Li et al. 1997; Zhang et al. 2012a).

Some of the animal toxic effects of endophyte-infected *A. inebrians* in China are similar to those seen in animals grazing the closely related grass species *A. robustum* (syn. *Stipa robusta*) in south western USA. *A. robustum* is infected with the endophyte *E. funkii* and another *Epichloë* species (Faeth et al. 2006; Moon et al. 2007; Shymanovich et al. 2015), and has a common name of sleepy grass due to the narcosis of animals but other toxicities are also recorded (Jones et al. 2000). The narcotic effect is variable, which may be related to high variance in, the occurrence of endophyte in a population, *Epichloë* strain, and production of endophyte alkaloids (Jones et al. 2000; Faeth et al. 2006). In studies where ergot alkaloids have been detected, high levels have been recorded particularly of lysergic acid amide which is likely to be responsible for the narcosis in grazing animals (Petroski et al. 1992). The primary economic impact is the strong avoidance of this grass by cattle, rather than the infrequent narcosis.

In Argentina, several species of *Festuca* and *Poa* cause 'huecu' ('drunk' or 'uncoordinated' in English) or 'tembaldera' ('tremble' in English) toxicosis in sheep, horses and cattle (Pomilio et al. 1989), similar to symptoms seen for animals consuming *Epichloë*-infected grasses elsewhere in the world. These grasses are infected with *Epichloë tembladerae* (Cabral et al. 1999), and although not proven through incisive experimentation, it is likely these 'huecu/tembaldera' are at least in part associated with *Epichloë*-produced indole-diterpenoid tremorgens and ergot alkaloids (Miles et al. 1995b).

6.2.5   Toxicity in Grasses Infected with Sexual Epichloë Endophytes

The *Epichloë*-grass toxicoses described so far are those that occur for the asexual species of *Epichloë* (formerly classified as *Neotyphodium*), fungi which are totally reliant on vertical transmission within the grass host as no sexual recombination or spread occurs (Schardl et al. 2004). Sexual *Epichloë* species, that are able to spread by horizontal transmission, produce the same classes of alkaloids as the asexual forms, including the mammalian toxins indole-diterpenes and ergot alkaloids (Lane et al. 2000; Leuchtmann et al. 2000; Schardl et al. 2013b). While there is therefore a similar potential for endophyte-toxicoses to occur in grasses infected with sexual *Epichloë*, there is a tendency for this type of endophyte to have fewer and lower concentrations of known alkaloids (Siegel et al. 1990; Leuchtmann et al. 2000). From an evolutionary point of view, asexual *Epichloë* are totally dependent on the fitness of the grass host for survival and propagation, so high expression of defensive alkaloids contributes strongly to the grass-endophyte mutualism (Bush et al. 1997), while sexual forms are less dependent.

There appears to be a lack of reports of livestock toxicity attributed to grazing asexual *Epichloë*-infected grasses. For example, *Festuca rubra* in the dehesa grasslands of western Spain is commonly infected to high levels with the sexual *Epichloë*

festucae (Zabalgogeazcoa et al. 1999), and at least some of these endophyte-infected plants have the potential to express ergovaline above threshold levels to be toxic to livestock (Table 6.2) (Vázquez de Aldana et al. 2003). Other ergovaline producing *Epichloë*-grass associations also occur in this ecosystem but no toxicity is reported in grazing cattle. It is postulated that the high diversity of plant species dilutes the intake of ergovaline in grazing animals in these natural ecosystems.

There are some indications that at least animal grazing behavior and preference may be affected by asexual *Epichloë* endophytes. Bazely et al. (1997) reported a positive association of grazing pressure with incidence of endophyte infection in a study with feral sheep grazing *F. rubra* on three Scottish Islands, possibly through inducible plant defense (ergovaline). A similar finding has also been reported by Koh and Hik (2007) for an asexual *Epichloë* in a subarctic alpine ecosystem for an asexual *Epichloë* infecting *Festuca altaica*.

6.2.6   Overcoming the Economic Impact of Endophyte Toxicoses

The animal toxicoses caused by some *Epichloë*-grass associations have stimulated considerable international research interest. Focus has primarily been on the associations and environments where both clinical and subclinical effects have been greatest and most widespread and therefore of greatest economic impact, namely the endophyte-infected sown pastures of tall fescue and ryegrass in USA, New Zealand and Australia. For many of the toxicoses reported around the world in a range of *Epichloë*-infected grasses, farmer awareness of endophyte toxicity is mostly driven by clinical effects, such as narcosis, staggers and fescue foot. Farmers may however not recognize subclinical effects or attribute them to *Epichloë* endophytes. For example, in the USA in 2004, half the farmers did not recognize that tall fescue-endophyte toxicity was a problem on their farms (Roberts and Andrae 2005) despite significant knowledge amongst research and extension personnel, and fescue toxicity costing the US cattle industry US\$ 1.0–1.5 billion year^{-1}.

Various options are available for farmers to combat the adverse effects of common toxic endophytes on animal performance and health, each option having limitations which reflect in the level of use (Aiken and Strickland 2013; Young et al. 2013). These options include, using grazing management to minimize exposure to toxic endophyte alkaloids, manipulating pasture composition to dilute alkaloids in forage, and administering treatments to livestock. Eradicating endophyte-infected tall fescue and ryegrass in cultivatable grasslands and resowing with endophyte-free cultivars removes the endophyte alkaloids that are toxic to livestock but can greatly reduce the persistence and productivity of the endophyte-free grass, which is not a tenable option in large areas of USA, New Zealand and Australia (Bouton et al. 1993; Popay et al. 1999; Hume and Sewell 2014). The option of deploying selected endophytes in pastures that can be resown is considered to be the most promising

option for capturing the endophyte-induced bioprotective and environmental stress advantages to the grass plant along with reduced or no adverse effects on livestock (Young et al. 2013). The uptake of technology has been outstanding in New Zealand for perennial ryegrass (Caradus et al. 2013).

The vertebrate-deterrent property of some endophytes has been captured in a novel application of endophytes in a non-agricultural context for the management of birds at recreational areas and airfields (Pennell and Rolston 2013). In these situations, high ergovaline-producing endophyte associations in tall fescue and ryegrass induce post digestion feedback in herbivorous birds, reducing the attractiveness of grassed areas to birds. In recreational areas, reduced grazing by large birds has decreased fecal soiling by these birds and associated risk to human health, while limiting grazing damage to turf. Where this technology has been deployed at airfields, bird numbers have potentially declined reducing the risk of bird strike on aircraft.

6.2.7 Summary of Risk for Animal Health and Productivity

The prevalence and severity of toxicity to animals consuming endophyte-infected grasses differs widely between ecosystems and farming systems, and can be dependent on weather conditions, as these affect the expression of the endophyte alkaloids. Toxicoses occur in all continents and affect a wide range of animal species. This can present a significant challenge to farmers in terms of lost productivity and animal welfare. When toxicity manifests as clinical symptoms, such as staggers, fescue foot, and narcosis, research has been relatively intense and awareness is high. Subclinical toxicity has been less well recognized but can be substantial, particularly where endophyte-infected grasses are the dominant source of feed. To varying extents, mitigation options are understood and applied, but further research is needed to understand and optimize sustainability, productivity and profitability outcomes for farmers. A challenge for researchers is to further investigate the occurrence of endophyte-infected grasses in natural and managed ecosystems, and understand their impact on animals through anti-quality factors and altering the botanical composition and productivity of pastures.

6.3 Effects of Endophyte Infection on Invertebrate Herbivory

Endophyte-infected grasses may negatively affect a wide range of herbivores from small rodents to large birds. However, most research on anti-herbivore effects of endophyte infection has focused on invertebrates, particularly insects, due to their species richness, propensity for economic damage in agroecosystems, and usefulness as ecological models. As grasses are relatively free of anti-herbivore

chemicals, endophytes and their associated mycotoxins have become a useful pest management tool for forage crops.

6.3.1 Mechanisms of Endophyte-Associated Insect Resistance

The defensive mutualism hypothesis was first used by Clay (1988) to describe the grass-fungal endophyte symbiosis. This framework posits that the production of defensive metabolites by fungal symbionts formed the basis of the evolution of the grass-endophyte mutualism (Clay 2009). Evolutionary models predict that vertically transmitted symbionts, such as asexual *Epichloë* endophytes, should form mutualistic interactions with host plants due to overlapping fitness (Clay 2009). It is thought that Clavicipitaceaus endophytes were derived from fungal pathogens of insects that produced biologically active secondary metabolites involved in insect pathogenicity, and later host-shifted (Spatafora et al. 2007). Empirical evidence supports the idea that endophyte-derived anti-herbivore alkaloids have a major role in enhancing host plant performance (Clay 2009); however, the majority of studies within this framework are centered around a few species of agronomic grasses (Saikkonen et al. 2010a) with relatively few studies carried out in native environments. While some studies have shown that endophyte infection can have effects on herbivores outside of alkaloid toxicity (Rasmussen et al. 2009), both artificial diet experiments (Ball et al. 1997; Yates et al. 1989) and genetic knock-out studies (Tanaka et al. 2005; Potter et al. 2008) have confirmed a causal role for endophyte-derived alkaloids in insect resistance.

Endophyte-associated resistance to invertebrate herbivores is predominantly due to the production of four groups of alkaloids: peramine, lolines, ergot alkaloids, and lolitrems (Bush et al. 1997). Peramine is a pyrrolopyrazine alkaloid and is a known insect feeding deterrent with no known activity against mammalian herbivores (Bush et al. 1997). Loline alkaloids are also potent anti-invertebrate metabolites. Depending on the insect species in question, lolines can act as overt metabolic toxins (antibiosis) or feeding deterrents (antixenosis) (Bush et al. 1997). Ergot alkaloids are also deterrent and/or toxic to an array of insect groups (Popay 2009a). While not widely associated with resistance to invertebrates, there is some limited evidence that the tremorgenic indole diterpenoid, lolitrem B, may reduce the growth and development of some invertebrates (Prestidge and Gallagher 1985). For the major agronomic grasses, lolines, peramine, and ergovaline are found in tall fescue infected with the common toxic strain of *Epichloë coenophiala*, while peramine, lolitrem B, and ergovaline are produced in *Epichloë festucae var. lolii*-infected perennial ryegrass (see Sect. 6.2). Some strains of *Epichloë* that contain a class of alkaloids known as janthitrems have been also shown to have species-specific and life stage-specific effects on insect performance (Tapper and Lane 2004).

Several studies have demonstrated that alkaloid concentration is linearly correlated with endophyte concentration (Rasmussen et al. 2007; Liu et al. 2011; Ryan et al. 2014a) and has been shown to depend on factors such as plant and fungal

Fig. 6.2 Endophyte-mediated resistance to insect herbivory showing endophyte-infected (*left*) and endophyte-free (*right*) meadow fescue (*Schedonorus pratensis*) plots in early spring at Lincoln, New Zealand. Damage to the endophyte-free plot is due to herbivory of roots by the larvae of grass grub *Costelytra zealandica* (Photo courtesy of Alison Popay, AgResearch Ltd., New Zealand)

genotypes (Ball et al. 2006; Rasmussen et al. 2007), soil fertility (Arechavaleta et al. 1992; Lehtonen et al. 2005a; Hunt et al. 2005; Rasmussen et al. 2007; Ryan et al. 2014a), temperature (see Sect. 6.9), CO_2 concentration (see Sect. 6.9), soil moisture (Belesky et al. 1989; Brosi et al. 2011; Kennedy and Bush 1983) and competition from other fungal symbionts (Liu et al. 2011). There is some evidence that alkaloid concentration is linearly correlated with the degree of resistance to insect herbivores (Wilkinson et al. 2000), and as such, factors that alter endophyte and alkaloid concentrations are likely to impact host plant resistance to invertebrates.

6.3.2 Direct Effects of Endophyte Infection on Invertebrate Herbivores

The effects of endophyte infection on insect herbivores, and tests of the defensive mutualism hypothesis, have been extensively reviewed both qualitatively (See Popay 2009a and references therein) and quantitatively (Saikkonen et al. 2010a). To date, endophyte infection from different species of *Epichloë* has been associated with resistance to more than 40 species of insect herbivores (Popay 2009b). Effects are often dramatic, as seen in Fig. 6.2, which shows the impact of endophyte infection on resistance to the grass grub *Costelytra zealandica* in meadow fescue (*Festuca*

pratensis, syn. *Schedonorus pratensis*) plots. The majority of studies have concentrated on leaf chewing and sap sucking insects (Saikkonen et al. 2010a). Endophyte-associated resistance occurs to a similar extent in these two feeding guilds, while effects on detritivorous and stem-boring insects remain inconclusive (Saikkonen et al. 2010a). Some sap sucking species, such as the aphid *Rhopalosiphum padi*, are highly sensitive to endophyte infection (Cheplick and Faeth 2009) and population abundances have been shown to decrease by up to 95 % in endophyte-infected tall fescue relative to uninfected plants (Ryan et al. 2014b). Despite the fact that fungal hyphae grow only in above-ground tissues, low concentrations of alkaloids can be found in the roots of endophyte-infected plants (Bush et al. 1993) and this has been associated with resistance to invertebrate herbivores. For example, endophyte infection has been shown to lower the performance of the parasitic plant nematodes *Pratylenchus scribneri* and *Meloidogyne maryland* in plant root tissue (Kimmons et al. 1990).

There is some evidence to suggest that endophyte-associated resistance to invertebrates can be induced, analogous to plant-derived inducible defenses found in many plant groups. Mock herbivory experiments have shown that clipping tall fescue plants infected with *Epichloë coenophiala* results in an increase in the production of loline alkaloids (Bultman et al. 2004). Induction by invertebrate herbivores may have impacts for other herbivorous insects feeding on the same plants. For example, Bultman and Ganey (1995) showed that fall armyworm larvae fed damaged endophyte-infected perennial ryegrass had lower pupal weights than those fed undamaged tissues.

The effects of endophytes on invertebrate herbivores have been variable and there are many exceptions to endophyte-associated resistance. For example, the performance of the aphid *Metopolophium festucae* on perennial ryegrass is unaffected by the presence of the common toxic strain of *Epichloë festucae* var. *lolii* (Krauss et al. 2007). While many parasitic nematodes are impacted by endophyte infection, the abundance of *Helicotylenchus pseudorobustus*, an ectoparasitic nematode, was not significantly impacted by endophyte infection in tall fescue (Kimmons et al. 1990). In a vote-counting study of the literature, Saikkonen et al. (2006) found that of 118 studies on endophyte-associated resistance to herbivores 32 % found positive, 15 % found neutral and 53 % found variable effects of endophyte on herbivore resistance. A growing body of evidence suggests that endophyte-associated resistance is stronger in agronomic grasses than natural populations (Cheplick and Faeth 2009), though natural populations have been less widely studied.

6.3.3 Indirect Effects of Endophyte Infection on Invertebrate Herbivores

Endophyte-associated toxicity has been shown to have broader ecosystem consequences, affecting higher trophic levels, which can feed back to indirectly impact herbivores feeding on endophyte-infected grasses. For example, endophyte

infection has been shown to cause bottom-up ecosystem effects that may alter rates of parasitism and predation on insect herbivores. Studies have shown that parasitoids of insect herbivores fed endophyte-infected plant tissue had reduced pupal mass (Bultman et al. 1997; Härri et al. 2009) though these results did not always impact survival. It has further been demonstrated that consumption of endophyte-infected tissue by insect consumers can have consequences for hyperparasitoids (parasitoids of parasitoids). Omacini et al. (2001) found that the rate of hyperparasitism in a grass-aphid-parasitoid-hyperparasitoid model was reduced when aphids were fed endophyte-infected grasses. Studies have also demonstrated endophyte effects on insect predators. For example de Sassi et al. (2006) found that the survival of the ladybird *Coccinella septempunctata* decreased when fed on *Rhopalosiphum padi* aphids on *Epichloë*-infected perennial ryegrass. Bultman et al. (1997) found that the survival of the parasitoids *Euplectrus comstockii* and *Euplectrus plathypenae* were reduced in artificial diets containing lolines, suggesting alkaloid toxicity in non-herbivore higher trophic levels. Lehtonen et al. (2005b) demonstrated that a hemiparasitic plant acquires defending mycotoxins produced by the endophytic fungus living within their shared host grass. The uptake of defensive mycotoxins from the endophyte-infected host grass enhanced the resistance of the hemiparasitic plant to a generalist aphid herbivore. These results suggest that endophytes may have complex direct and indirect effects on several trophic levels with consequences for ecological food webs (Omacini et al. 2001).

6.3.4 Novel Grass-Fungal Associations

Endophyte infection in forage crops poses benefits, by way of enhanced resistance against insects and tolerance to abiotic stress, and simultaneous challenges, due to animal toxicity. To address this, novel grass-endophyte associations have been developed to retain those alkaloids that confer insect resistance to infected plants but do not produce those alkaloids toxic to grazing mammals (see Sect. 6.2). Such associations are produced by clearing the common toxic strain of fungus from agronomic grass cultivars and artificially inoculating with less toxic strains, or by artificially inoculating endophyte-free grasses. As described in Sect. 6.2, the *E. festucae* var. *lolii* strain 'AR1' contains peramine only, while 'AR37' produces only epoxyjanthitrems. Similarly, the *E. coenophiala* strain 'AR542' produces peramine and N-acetylnorloline only. Several other novel associations have been generated with various cultivars of perennial ryegrass (the major pasture grass in New Zealand) and tall fescue (the major pasture grass in North America). In field experiments, both AR1 and AR37 were shown to vastly reduce populations of the mealybug *Balanococcus poae* (Pennell et al. 2005) and the Argentine stem weevil *Listronotus bonariensis* (Popay and Thom 2009) relative to endophyte-free ryegrass. These reductions were similar to those observed in the common toxic strain. However, AR1 has been less successful in controlling African black beetle *Heteronychus arator*, root aphid *Aploneura lentisci* and porina *Wiseana cervinata* infestations

compared with AR37 and common toxic endophyte (Popay and Hume 2011). In AR542-infected tall fescue, mealybug infestation was reduced to levels observed in common toxic strains (Pennell and Ball 1999). The AR542 strain has also been shown to control the Argentine stem weevil and African black beetle, though the extent of reductions in herbivory were shown to be grass cultivar-specific (Popay et al. 2005).

Studies have shown that the behavior of endophytes in novel associations is dependent on host cultivar. For example, Rasmussen et al. (2007) found that the concentration of several alkaloids in AR1 and AR37 was significantly reduced in a ryegrass cultivar selectively bred to produce high levels of water-soluble carbohydrates (so-called "high sugar grasses") relative to a "normal sugar" cultivar. Despite the clear benefits with respect to lowered insect infestation, some novel associations continued to experience animal health issues, particularly first-generation perennial ryegrass associations (Fletcher 2012). As such, the success of novel associations in agricultural systems is likely to depend on a functional understanding of metabolite profiles, toxicity responses to environmental variables, and cultivar/strain compatibility.

6.3.5 Summary of Endophyte Effects on Invertebrates

One of the most notable benefits of endophyte infection to host plants is the ability to confer resistance to herbivores. Several classes of endophyte-derived alkaloids can directly affect invertebrates through antibiosis or antixenosis, and their concentrations are dependent on the environmental context in which the host plant grows. More than 40 species of insects including sap, leaf, and root feeders, have lower performance when feeding on endophyte-infected grasses, and in some cases, alkaloid production can be induced by feeding. Endophyte infection has also been shown to cause changes in the performance of invertebrates at higher trophic levels, including predators and parasitoids of insect herbivores. Novel grass-endophyte associations have been developed for pest management and are marketed for use in agriculture. In these associations, endophyte strains are used that produce alkaloids associated with reduced insect herbivory, but do not produce those that toxic to grazing vertebrates. While research in this area is largely focused on agricultural grasses, much less is known about the effects of native grass-endophyte associations on invertebrates.

6.4 Involvement of *Epichloë* in Microbial Interactions

It has been found that plants infected with fungal endophytes produce substances that inhibit growth of some pathogens (Christensen 1996). Endophytes may counteract pathogen development indirectly through induction of plant defense

mechanisms or produce antibiotic chemicals which restrain host pathogen activity. However, compared with some other aspects of *Epichloë* endophyte effects (like resistance to insect herbivory), its influence on plant diseases have not been broadly studied. Reports on the resistance of endophyte-infected grasses to diseases and pathogens are often conflicting. Unconvincing and conflicting results may have attributed significantly to the lack of interest shown towards research concerning endophyte and pathogen interactions. Inconsistent effects of endophyte infection on host pathogens may be ascribed to different factors. The complexity of disease resistance mechanisms may be a factor (Bacon et al. 1997), and depends on both host and pathogen characteristics and their interactions, as well as their individual and collective interactions with environmental conditions (Wäli et al. 2006). Reasonably, the presence of endophyte must be considered in this complex system and in relation to the other roles of endophyte that may contribute to the ecological fitness of hosts, especially those related to abiotic stress tolerance. The large biological diversity among endophytes, and the presence of more than one *Epichloë* or other endophytic species along with *Epichloë* in a single host (as a coexisting or hybrid endophyte) may also complicate the situation, making assessment of endophyte effect on host pathogens even more difficult. Studying identical clones of a single plant genotype with and without endophyte may be a solution for finding more consistent results regarding the role of endophytes in plant-disease interactions. This approach is limited to only a few reports concerning endophyte-pathogen relations in the literature.

6.4.1 **Epichloë *Endophytes Versus Fungal Pathogens***

Although *Epichloë* endophytes constitute a small proportion of the endophytic fungi connected to grass species, they are the best known and most intensively studied among the fungal endophytes discovered in grasses so far. The first report on an endophyte affecting pathogenic fungi of grasses goes back to an *in planta* study in 1983 by Shimanuki and Sato who observed that *Epichloë typhina* confers resistance to its host timothy (*Phleum pratense*) against the pathogenic fungus *Cladosporium phlei*. This suggested the possibility of mycotoxin secretion by endophyte against pathogenic fungi and triggered in vitro studies on the inhibiting effects of fungal endophytes. In many instances, endophyte infection depressed the growth of plant fungal pathogens (White and Cole 1985; Siegel and Latch 1991; Li et al. 2007). Similarly, liquid extracts from endophyte cultures showed inhibiting effects on a range of plant pathogenic fungi, indicating the production of antifungal compounds by the endophytes which consistently inhibit the in vitro growth of pathogens. For instance, three types of inhibitors isolated from a batch culture of *E. festucae*, including indole derivatives (indole-3-acetic acid and indole-3-ethanol), a sesquiterpene, and a diacetamide, were shown to have a role in disease resistance against fungal pathogens (Yue et al. 2000).

However, *in planta* effects were not completely in agreement with the in vitro studies. For example, Cromey and Cole (1984) reported no significant effect of the *Epichloë* endophyte on *Drechslera* leaf spot fungus, while Schmidt (1990) reported the antagonistic effect of *Epichloë* endophytes of perennial ryegrass against the same pathogen. Wheatley et al. (2001) also reported that infection of ryegrass by a leaf spot fungus (*Pyrenophora semeniperda*) was greater on endophyte-free than endophyte harboring plants for three cultivars. In another study, Clarke et al. (2006) found that infection of fine fescue by *E. festucae* enhanced resistance to dollar spot disease caused by *Sclerotinia homeocarpa*.

Gwinn and Gavin (1992) found that in a soilless medium amended with *Rhizoctonia zeae*, survival of tall fescue seedlings increased with an increasing percentage in endophyte-infected seeds in the greenhouse condition. However, in the field, endophyte infection could not increase tall fescue resistance against blight disease caused by *Rhizoctonia solani* (Burpee and Bouton 1993). Severity of crown rust (*Puccinia coronata*) in tall fescue was reduced in endophyte-infected plants compared with endophyte-free counterparts (West et al. 1989) but there was no effect of endophyte on pathogenesis of tall fescue stem rust, *Puccinia graminis* subsp. *graminicola* (Welty et al. 1991). The effect of endophyte on crown rust infection of ryegrass was also inconsistent in Queensland, Australia (Lowe et al. 2008). In reaction to powdery mildew fungus, *Blumeria graminis*, Sabzalian et al. (2012) reported that endophyte-infected and endophyte-free tall and meadow fescues were not significantly different although endophyte-infected clones had fewer powdery mildew infected leaves and lower disease indices when compared with endophyte-free clones.

It is not clear if the same fungal-depressing compounds that are produced by endophyte in vitro are produced *in planta* to prevent pathogen growth and development. The other possibility is that under in vitro conditions where the mycelial growth of endophyte is high and no interaction exists, the endophyte produces antifungal chemicals in adequate amounts to depress the pathogen, but *in planta* with complex interactions present, they may not be able to produce these compounds in sufficient quantities to effectively protect the host plant from fungal diseases (Latch 2009). Certainly, there are many aspects of tripartite host-endophyte-pathogen interactions to be discovered and applied to the future breeding of grasses in their continuous battle against pathogens.

There is evidence to suggest that plant association with both *Epichloë* endophytes and mycorrhizal fungi may be regarded as mutualist–parasitist interaction and infection of grass species with *Epichloë* endophytes can suppress mycorrhizal infection. This has been shown in endophyte-infected tall fescue and ryegrass (Chu-Chou et al. 1992; Mller 2003; Omacini et al. 2006; Mack and Rudgers 2008); however, it seems that competitive interaction between the two fungal symbionts could be modified by resource supply, plant genotype and *Epichloë* endophyte strain (Liu et al. 2011).

6.4.2   Epichloë *Endophytes Versus Viruses*

Epichloë endophyte may affect host pathogenic viruses either directly, through induction of plant-derived metabolites, or indirectly by deterring pathogen-transmitting insects via chemicals produced *in planta* by the endophyte itself.

In a greenhouse experiment, Lewis and Day (1993) found that when ryegrass plants were infected with ryegrass mosaic virus and barley yellow dwarf virus (BYDV), cumulative total biomass was greater for *Epichloë*-infected plants than endophyte-free versions. The authors concluded that this may show reduction in the effect of virus infection when the plant is also infected by *Epichloë* endophyte. Comparing a common toxic and a non-toxic fungal endophyte infecting tall fescue, Rúa et al. (2013) showed that endophyte increased overall plant biomass, reduced the negative effect of virus infection on the root fraction, and stimulated tiller production, possibly by increasing plant stimulating regulators compared with endophyte-free plants. This may enhance host tolerance to viral infection by reducing viral titre in plant tissues. Also, plants infected with the common toxic endophyte supported less aphid production, abundance of adult aphids and total number of aphids and thereby enhanced host tolerance to viral infection.

Compared with the direct effect of endophytes on pathogenic viruses, the indirect effects have been more widely investigated. An indirect effect of *Epichloë* endophytes on pathogenic viruses is through deterrence or detrimental influence on virus-transmitting insects, especially where endophyte infection interferes with survival of aphids (Mahmood et al. 1993; Rúa et al. 2013). *Epichloë* endophytes are well known to produce different classes of biologically active chemicals including various lolines and peramine, both harmful and effective in reducing aphid population size, feeding time and the resulting damage to the host plant (Schardl et al. 2004).

The importance of endophytes in deterring aphids from infected hosts was recognized as early as 1985 by Johnson et al. who reported that loline alkaloids produced by the endophyte inside tall fescue can deter *Rhopalosiphum padi* aphids, the vector for barley yellow dwarf virus which is one of the most important viruses infecting small grains. The virus is transferred by aphids, which may be deterred by endophyte-derived alkaloids within the plant. However, similar to interactions between endophyte and fungal pathogens, there are also some inconsistent results on endophyte-virus interactions. In roadside tall fescue in Tasmania, neither the incidence of barley yellow dwarf virus nor the occurrence of the virus vector, *R. padi* differed between endophyte-infected and endophyte-free plants; though, some endophyte-infected tall fescue plants were more resistant to BYDV than the others (Guy and Davis 2002). Also, no effects of endophyte infection on incidence of barley yellow dwarf virus in ryegrass (Hesse and Latch 1999) or on growth response of ryegrass plants infected with virus (either BYDV or ryegrass mosaic virus) (Lewis 2004) have been found. In contrast, Lehtonen et al. (2006) showed that when aphid vectors were released on endophyte-infected and endophyte-free plants, the number of aphids and the percentage of barley yellow dwarf virus infection were lower in endophyte-

infected plants compared with endophyte-free counterparts. They also concluded that the low infection rate of barley yellow dwarf virus in endophyte containing plants may protect neighboring plants from the virus as a result of lower population sizes of aphids. This may have applications in agroecosystems by sowing endophyte-containing plants next to cereals defenseless against heavy infection by barley yellow dwarf virus, and thereby reducing grain yield losses caused by the virus.

6.4.3 Epichloë *Endophytes Versus Bacterial Populations*

The effects of *Epichloë* endophyte on plant tolerance to diseases caused by bacteria have not been assessed to the same extent as those of pathogenic fungi. It is remarkable that this aspect of *Epichloë* endophytes have so far been neglected in the research, particularly at the level of pastoral and grassland systems where endophyte-infected plants could affect neighboring species and their associated pathogens. This is despite reports that mycorrhizal plants have increased their host's resistance to virulent bacterial pathogens (Liu et al. 2007).

In soil, it seems that endophyte infection of grasses may alter micro-environmental conditions so that decomposition is slower for endophyte-infected fescue litter than for endophyte-free one (Lemons et al. 2005), though this may not be due to the change in bacterial activity (Casas et al. 2011). Recent research also showed that several bacterial species recovered from tall fescue could use N-formyl loline as a carbon and nitrogen source (Roberts and Lindow 2014) meaning that bacterial populations may be resistant to alkaloids and/or other *Epichloë* fungal metabolites. The lack of information suggests that future research at in vitro and *in planta* levels are needed to elucidate how bacterial pathogens could be influenced by fungal endophyte of grasses.

6.4.4 *Summary of Endophyte Involvement in Microbial Interactions*

Epichloë fungal endophytes produce some antimicrobial compounds which may inhibit the in vitro growth of pathogens. However, the dynamics of pathogenicity involve complex interactions between variables including environmental condition, plant genotype, *Epichloë* endophyte and pathogen strains, and as such *in planta* observations do not consistently point to prevention of pathogen growth and development. This complexity may suppress secretion of sufficient quantities of antibiotic compounds and increase resistance of pathogens to *Epichloë* fungal metabolites. Nevertheless, fungal endophytes may enhance resistance to pathogens indirectly through deterring insect vectors of plant pathogens, and by also improving the general health condition of plants via enhanced growth, improved nutritional status and abiotic stress tolerance.

6.5   *Epichloë* Endophyte Affects the Competitive Ability and Persistence of Host Plants

Inter- and intraspecific plant competition is one of the major driving forces of natural and agricultural grassland communities. Because systemic grass endophytes have been found to increase plant tolerance in stressful abiotic environments and affect all types of plant-plant, plant-herbivore and plant pathogen interactions (Clay and Holah 1999; Saikkonen et al. 2006; Wäli et al. 2006), they may potentially shape both natural grassland communities and agroecosystems. Here, the known features of plant-plant communications and competitive ability of grasses affected by *Epichloë* endophyte are briefly reviewed.

6.5.1   *Competitive Ability Depends on Environmental Conditions*

The outcome of competition is conditional and depends on both biotic and abiotic factors (Callaway et al. 1996), which are potentially affected by endophyte-mediated interactions in grasses. In various studies, endophyte-infected grasses showed increased growth vigor, and become stronger competitors compared with uninfected counterparts and co-occurring plant species (Clay and Holah 1999; Saikkonen 2000; Rudgers et al. 2005; Saikkonen et al. 2006). It is believed that, their superiority is a result of increased fitness under harsh environments such as drought and flooding, and increased resistance to invertebrate and vertebrate herbivores, seed predators and plant pathogens (Clay and Schardl 2002; Saikkonen et al. 2006). Many effects of the grass-endophyte partnership, which has often been regarded as a mutualistic interaction in many cool-season grasses, are directly influenced by the endophytic partner. As a result of this capability, endophyte-infected grasses have even been claimed to threaten native plant diversity and associated food webs (Clay and Holah 1999).

Nutrient availability of soils plays a critical role in strengthening endophyte-plant associations because in low nutrient conditions, the costs associated with harboring the systemic endophyte may override its benefits to the host grass. At the same time, the majority of studies on endophyte-mediated competitive ability and grass persistence have used agricultural settings with high nutrient availability. However, in experiments comparing low and high fertilized soils, the competitive ability of endophyte-infected plants was shown to be dependent on nutrient availability (Dirihan et al. 2014). Dirihan et al. (2014) reported that during the early phase of establishment, neither meadow fescue nor tall fescue gained instant endophyte-promoted competitive advantage over red clover when sown together. In nutrient limited soils, plant competition or the cost of endophyte infection even decreased the yield of meadow fescue. There are several reports indicating that in agricultural soils with high nutrient availability, the endophyte infection can increase the performance of the host grass but the positive effects still depend on the host species and the species composition of the grassland (Table 6.3).

Table 6.3 Effects of *Epichloë*-infected grasses on competitive neighboring species

Grass infected by *Epichloë* endophyte	Endophyte species	Competing species	Competitive effects	Reference
Schedonorus arundinaceus	*Epichloë coenophiala*	*Trifolium pratense T. repens Medicago sativa*	Negative and neutral effects on legume yield	Hoveland et al. (1999)
Schedonorus arundinaceus	*Epichloë coenophiala*	*Trifolium pratense*	Positive and negative effects on yield	Malinowski et al. (1999)
Festuca arizonica	*Epichloë sp.*	*F. arizonica*	Higher biomass in uninfected plants	Faeth et al. (2004)
Schedonorus arundinaceus	*Epichloë coenophiala*	Native prairie plants	Increased invasiveness to high diversity communities	Rudgers et al. (2005)
Schedonorus pratensis	*Epichloë uncinata*	*Lolium perenne Trifolium repens Dactylis glomerata*	Increased persistence and biomass of infected *S. pratensis*	Takai et al. (2010)
Schedonorus arundinaceus	*Epichloë coenophiala*	Invading weeds	Plant and endophyte genotype affect plant composition	Rudgers et al. (2010)
Festuca rubra	*Epichloë festucae*	*Trifolium pratense, T. repens, Lotus corniculatus, Plantago lanceolata*	Negative allelopathic effects on seed germination and radicle growth of competing plants	Vazquez de Aldana et al. (2011)
Schedonorus pratensis	*Epichloë uncinata*	Invading weeds	Infected plants hindered weed invasion	Saikkonen et al. (2013b)
Festuca rubra	*Epichloë festucae*	*Trifolium pratense*	Decrease in shoot and root biomass of *T. pratense*	Vazquez de Aldana et al. (2013b)
Lolium perenne Schedonorus arundinaceus Schedonorus pratensis	*Epichloë festucae var lolii Epichloë coenophiala Epichloë uncinata*	*Lolium perenne Bromus catharticus Trifolium repens*	Positive and negative growth in *Trifolium*, depending on fungal and grass species	Cripps et al. (2013)

<div align="right">(continued)</div>

Table 6.3 (continued)

Grass infected by *Epichloë* endophyte	Endophyte species	Competing species	Competitive effects	Reference
Schedonorus arundinaceus *Schedonorus pratensis*	*Epichloë coenophiala* *Epichloë uncinata*	*Trifolium pratense* *Schedonorus arundinaceus* *Schedonorus pratensis*	Negative effects on biomass of infected *S. pratensis* in low nutrient soils	Dirihan et al. (2014)

On the other hand, on many occasions the effects of endophyte infection on the competitive ability of grasses turn out to be neutral or negative. In fact, the outcome is dependent on the abiotic and biotic environmental factors, grass species, grass and fungal genotype, and genetic combination of the plant and the fungus (Saikkonen 2000; Faeth et al. 2004; Rudgers et al. 2010; Saikkonen et al. 2006, 2010b). Several studies have demonstrated that endophyte-promoted competitive superiority of host plants may be most pronounced in selectively bred grass cultivars growing in nutrient rich agroecosystems and in environments where grazing pressure is high (Cheplick and Faeth 2009; Saikkonen et al. 2004, 2006, 2010a). However, in natural grasslands and other more variable environments, the grass-endophyte symbiosis may form a continuation of interactions from mutualism to antagonism depending on the grass species and/or genotype and environmental conditions. Studies using native grass species to study competitive abilities of endophyte-infected and uninfected grasses have shown that in some environments, endophyte-free grasses outperform their infected counterparts (Faeth et al. 2004).

6.5.2 Species Diversity in Ecosystem as Affected by Endophyte Infection

Recent evidence suggests that endophytic fungi can strongly affect grassland plant community composition and productivity both in natural and agroecosystems (Clay and Holah 1999; Rudgers et al. 2010; Saikkonen et al. 2013b). Increased plant growth, reproduction and resistance to various biotic and abiotic factors give infected grasses the ability to invade and compete in fields and grasslands.

The competitive ability of endophyte-infected tall fescue cultivar 'Kentucky 31' is observed to be high compared with uninfected conspecifics of the same cultivar. After a 4-year field study, Clay and Holah (1999) suggested that endophyte-infected plants were reducing species diversity in successional fields by outcompeting native plant species. The competitive superiority of endophyte-infected Kentucky 31 may, however, be more related to a lack of genetic diversity of the cultivar in the new environment and the systemic endophyte, rather than a common phenomenon of the

grass species (Saikkonen 2000). This is because tall fescue is a species originating from Europe, where it has not shown competitive superiority but is rather occurring in competition-free environments in river banks, sea shores and waste lands (Saari et al. 2010). Noteworthy is that these native European tall fescue populations are highly infected with systemic endophytes (Saari et al. 2010).

In a northern European agronomic field, a long-term study using endophyte-infected and endophyte-free meadow fescue cultivar 'Kasper' monocultures demonstrated that endophyte infection promoted competitive dominance of the grass and retarded weed invasion to the field (Saikkonen et al. 2013b). The maintenance of a high frequency of endophyte infections and superior productivity of infected grasses was shown to be a result of higher survival, growth and reproduction of the infected plants and not mediated by endophyte-origin substances inhibiting seed germination of endophyte-free plants. After 6 years, the percentage cover and biomass of uninfected meadow fescue monocultures was significantly reduced compared with infected meadow fescue monocultures and the species richness, percentage cover and biomass of the weed species were markedly higher.

6.5.3 Allelopathic Interaction between Infected and Non-infected Species

It has been suggested that the competitive superiority of endophyte-infected grasses, in addition to enhanced growth and reproduction, is based on allelopathy i.e. endophyte-mediated chemical effects on the abiotic and biotic properties of soils, which in turn may influence the composition and persistence of plant communities. The effect of plant-soil feedback has important implications both in natural and managed ecosystems. The allelopathic potential of endophyte-infected grasses has recently received increasing attention, with researchers aiming to explain mechanisms behind observed competitive superiority of endophyte-infected grasses (Antunes et al. 2008; Cripps et al. 2013). Allelochemical by-products of infected grasses, which are mostly alkaloids, could enter the soil through plant roots or from decomposing plant material (Siegrist et al. 2010). Such endophyte-derived compounds are suggested to directly inhibit the growth of other plants or suppress mutualistic microbes e.g. mycorrhizal fungi of neighboring plants (Antunes et al. 2008). Experiments using species mixtures to study allelopathic effects of endophytes have shown both negative and positive effects of endophyte on plant performance (Table 6.3). Cripps et al. (2013) found that the outcome of these allelopathic effects via soil was conditional on both endophyte-infected grass species and competing plant species studied. The negative conditioning effects of endophytes could be also due to reduced nutrient availability, altered microbial composition, endophyte-derived alkaloids in the soils and/or alterations to other root exudates (McNear and McCulley 2012).

6.5.4 Weed Management Using Endophyte-Infected Grasses

Overall benefits associated with endophytes in managed fields are compelling (Saikkonen et al. 2006, 2010a). On the other hand, the competitive superiority of endophyte-infected cultivars may cause negative side-effects when grown in mixtures with desirable pasture species e.g. legumes. A decrease in the abundance of legumes when grown in mixtures with endophyte-infected grasses compared with uninfected conspecifics has been reported in several studies (Hoveland et al. 1999; Malinowski et al. 1999; Takai et al. 2010). However, these endophyte-mediated impacts are context-dependent varying in environmental conditions, grass species and cultivars and genetic combinations of the fungus and the host grass (Dirihan et al. 2014).

The competitive superiority of endophyte-infected cultivars in nutrient rich agro-ecosystems should be regarded as having the potential for biological control of weeds (Saikkonen et al. 2013b). The breeding and use of endophyte-infected cultivars that do not produce mycotoxins but increase plant growth, seed production, seed germination rate and stress tolerance can increase productivity and prevent the use of herbicides in forage production (Gundel et al. 2013). Furthermore, possible allelopathic effects of endophyte-infected grasses on weeds could be used to prevent weed invasion. In set-aside fields, the use of endophyte-infected cultivars with high competitive ability has been demonstrated to slow weed invasion. Thus, in the long term, endophyte-infected grasses could be used to reduce the application of synthetic herbicides in grass and pastoral ecosystems and neighboring agricultural fields.

6.5.5 Summary of Endophyte Effects on Host Competitive Ability and Persistence

There is a continuum of interactions between grass and fungal endophytes from mutualism to antagonism depending on the grass species, plant-fungal genetic combination, and abiotic and biotic environmental factors. In mutualistic relations, endophyte-derived chemical compounds secreted into the soil by endophyte infected grasses may directly inhibit the growth of other species and neighboring plants. Higher survival, growth and reproduction of infected plants may also increase the superior productivity of infected grasses in ecosystems and maintain a high frequency of endophyte-infected grasses. This in turn may influence the composition of plant communities in natural- and agro-ecosystems. Although our knowledge on allelopathic effects of endophyte-infected grasses on weeds and volunteer species is low, it seems that there is the potential to use infected grasses for biological control of weeds in agroecosytems with minimum or no herbicide application.

6.6 Fungal Endophytes Support Host Plants in Overcoming Abiotic Ecological Constraints

Fungal endophytes of grasses are known to induce resistance in host plants to a range of abiotic stresses including drought, high soil salinity, heat, cold, oxidative stress, heavy metal toxicity and nutrient deficiency. It is this enormous diversity of effects, coupled with its vertical transmission, which makes fungal endophytes of grasses a stimulating field of study in agronomy and ecology. Several reviews and books dedicated to fungal endophytes of grasses and recording in detail their effects against abiotic stresses, have been published during the last 20 years (see Schardl 1996; Malinowski and Belesky 2000; Malinowski et al. 2005a; Cheplick and Faeth 2009). In this section, we feature recent progress in research (mainly between 2004 and 2015), and address its implications for the utilization of fungal endophytes of grasses in the ecological intensification of agriculture.

6.6.1 Water Availability and Drought Stress

Water scarcity due to drought and salinity stress affects more than 10 % of the total arable land areas of the world (Bartels and Sunkar 2005), and unsurprisingly it is the most documented abiotic stress in the grass-endophyte literature. Endophyte effects have been mainly investigated on the ability of two agronomically important grass species (perennial ryegrass and tall fescue) to grow and produce satisfactory yields under periodic drought (Saikkonen et al. 2006). An extensive body of research was produced on these symbioses for the purposes of characterizing the beneficial outcomes of endophytes on plants that could be used in grass improvement. Two decades of research show that endophyte effects on plants are more versatile than initially thought (Cheplick 2004; Müller and Krauss 2005; Saikkonen et al. 2006). The recent research on endophyte effects on native grasses also revealed that the effects of endophyte on host performance under water-limited conditions varied from one plant species and/or genotype to another (Morse et al. 2002; Ahlholm et al. 2002; Kannadan and Rudgers 2008; Rudgers and Swafford 2009). In fact, the endophyte effects appear to be dependent on environmental conditions and host-endophyte genotypic combinations.

In a recent meta-analysis, Chamberlain et al. (2014) highlighted the fact that biotic interactions are very often context-dependent, and suggested that the focus should be moved from 'mean outcomes' to the factors contributing to 'variation in outcomes'. It remains unclear what factors contribute to the context-dependency in grass-endophyte symbioses (see Cheplick and Faeth 2009; Davitt et al. 2011), but field observations suggest a role for water availability in some species. Several surveys of native grasses have documented a higher frequency of symbiosis in drier

habitats in *Lolium perenne* (Lewis et al. 1997; Gibert et al. 2012), *Bromus setifolius* (Novas et al. 2007), *Festuca rubra* (Saona et al. 2010), the alpine grass *Festuca eskia* (Gonzalo-Turpin et al. 2010), and in many other *Lolium* and *Festuca* species in Europe (Leyronas and Raynal 2001). Therefore, water shortage in such grassland ecosystems may lead to higher infection rates among populations and a greater connection between growth and production in ecosystems to endophyte infection. In contrast, some other surveys show no correlation between infection rate and water availability in *Poa spiciformis* and *Phleum alpinum* in south Patagonia (Novas et al. 2007) or in native grasses from the California province (Afkhami 2012). These contrasting patterns suggest that the responsiveness of the endophyte-grass symbiosis may not depend only on environmental factors.

To expand our understanding of the variable effects of endophytes on their hosts, several authors have called for a better awareness of the origin of symbiotic plants in controlled experiments (Hesse et al. 2003; Kane 2011). Indeed, in grassland species (regardless of endophyte presence), adaptation of plants to drought has been consistently shown to depend on the origin of the genotypes (Pecetti et al. 2011; Annicchiarico et al. 2011), suggesting that drought resistance is associated with intra-specific variability. Experimental evidence of a positive effect of endophyte under drought have been highlighted by studies focusing on plants originating from dry environments and populations with different endophyte frequencies (Hesse et al. 2003; Gibert and Hazard 2011; Kane 2011; Gibert et al. 2012). For instance, Kane (2011) assessed the effects of the endophyte *Epichloë festucae* var. *lolii* on growth under stress of native *Lolium perenne* accessions originally collected from Italy, Morocco, Tunisia, and Turkey, areas where drought is known to occur. In four accessions, out of the six studied, endophyte-infected plants had more tillers, greater tiller lengths, total dry mass and green shoot mass under drought than endophyte-free plants, suggesting a positive effect of endophyte infection on host growth.

The occurrence of a high-infected population at a dry site is not evidence *per se* that the main advantage of endophyte occurs under drought. Indeed, Rudgers and Swafford (2009) tested the hypothesis that fungal endophyte *Epichloë elymi*, in the native grass species *Elymus virginicus*, would promote host growth under drought. In a growth chamber experiment, they imposed a 67 % reduction in water, corresponding to the very high end of drought predictions for the Midwestern US, where *E. virginicus* is common. Contrary to their initial prediction, they found that the positive effects of endophyte were stronger under the daily watering treatment than under drought: non-infected plants had 45 % less aboveground biomass than endophyte-infected plants under daily watering, and only 23 % less under drought. Similarly, Vázquez de Aldana et al. (2013a) showed no significant differences in response to the water treatment between endophyte-infected and endophyte-free plants in two populations of *Festuca rubra* from grasslands of western Spain originating from two contrasting habitat in terms of drought severity. Their results showed that *Epichloë festucae* did not increase the resistance of *Festuca rubra* plants to drought; there was no effect neither on plant biomass nor on proline content (a solute involved in osmotic adjustment, a strategy of drought tolerance). Instead,

differences in plant nutrition in response to water availability occurred, with endophyte-infected plants showing a significant increase in N, P and Zn in their shoot tissues, and Zn in roots relative to endophyte-free plants.

It seems that the intensity of drought experienced by the plant is an important parameter to consider when responses of perennial herbaceous species to drought are analyzed (Volaire et al. 2014). Different plant species are highly different with respect to their optimum environmental conditions, and a harsh event which is harmful for one plant species, might not be stressful for another. However, symbiotic plant performances under drought is usually characterized by drought resistance, *i.e.* the ability of plants to maintain a certain level of living biomass and growth under water shortage (Volaire and Lelièvre 2001). Drought resistance includes both drought stress avoidance and drought tolerance mechanisms, which both may be affected by fungal endophytes of grasses (for review see West 1994; Malinowski and Belesky 2000; Malinowski et al. 2005a; or more recently Swarthout et al. 2009). This criterion is relevant for most species of agronomic interest subjected to short and moderate periods of drought. However, when plants experience successive and severe summer droughts, their persistence is mainly determined by survival *i.e.* the ability of plants to remain alive during summer and recover when rehydration occurs (Volaire et al. 2001). It is now known that plant persistence during severe drought is governed by mechanisms different from those conferring resistance to moderate drought (Milbau et al. 2005).

Although drought survival in the grass-endophyte symbiosis has been much less explored than drought resistance, the few studies focusing on survival or recovery after stress support the view that endophyte benefits are greater during recovery from drought or during survival than during moderate water deficit (Malinowski and Belesky 2000; Hesse et al. 2003; Gibert and Hazard 2011). For instance, Gibert et al. (2012) estimated growth during drought stress and survival after severe stress for five populations of *Lolium perenne* along a gradient of water availability. Plant survival rates were highest in driest populations reinforced by fungal endophyte. Plants with a higher survival had lower growth, suggesting a trade-off between growth during stress and survival after stress. Consequently, the characterization of symbiotic plant stress responses should consider the intensity of stress experienced by the plants in the wild, and focus on the plant strategy potentially involved (resistance versus survival).

More and more studies have focused on the role of hybridization on the grass-endophyte symbiosis. Hybrid endophytes presumably result from somatic fusion of distinct *Epichloë* species infecting the same host individual (Schardl and Craven 2003). The common idea is that hybrids may express traits from both ancestral species (Clay and Schardl 2002), and thus may have an advantage in a larger range of environments than non-hybrid endophytes (Schardl and Craven 2003). Several studies highlighted the abundance of hybrid endophytes among host species and populations (e.g. Iannone et al. 2009; Oberhofer and Leuchtmann 2012; Iannone et al. 2012), but the ecological consequences of endophyte hybridization have not been widely explored. In the native grass *Festuca arizonica*, hybrid endophytes are prevalent in habitats with low nutrients and moisture, whereas non-hybrid endophytes

are more prevalent in sites with higher nutrients and moisture (Sullivan and Faeth 2007; Hamilton et al. 2009). Under controlled experiments, hybrid endophytes of *F. arizonica* produced higher volume/mass ratio (Sullivan and Faeth 2007), higher biomass (Saari and Faeth 2012) and better competition and survival (Hamilton et al. 2010) than those having non-hybrid endophytes in stressful habitats (nutrients and water were manipulated). However, *F. arizonica* appears to be an exception among cool-season grasses by hosting mainly both hybrid and non-hybrid endophytes. In *Hordelymus europeaus*, a European woodland grass hosting preferentially hybrid endophyte, Oberhofer et al. (2014) found that non-hybrid endophytes prevailed at dry sites. In a controlled experiment, the authors showed no effect of hybridization on drought tolerance and plant survival and both hybrid and non-hybrid endophytes increased biomass and tiller production after recovery from stress. Yet, in this experiment, hybrid endophytes reduced or completely prevented plant reproduction compared with non-hybrid endophytes.

The last 5 years have been spent seeking evidence of water availability as a main cause of variability in the outcomes of grass-endophyte symbioses. This goal has been partially reached and water availability appears to be a driver of endophyte-grass symbiosis outcomes in some species, but the pattern of functioning appears undeniably more complex for other species. Beyond this unsatisfying conclusion, an interesting result is the effect of endophyte on both plant strategies of resistance and survival which is observed sometimes for the same host species (e.g. for *Lolium perenne*, Gibert et al. 2012, and Kane 2011). Since grasslands are expected to have production over several years, their sustainability is associated with both productivity and long-term persistence (Volaire et al. 2014). Consequently, short-term adaptation in natural grasslands should include changes in species with greater resistance under periodic stress and/or greater survival under extreme events, two processes that may be improved by fungal endophytes of grasses (Fig. 6.3). The challenge is now to identify the most advantageous grass/endophyte combinations for local conditions.

6.6.2   Light Availability

In contrast with our current knowledge on drought, little is known about the effect of endophyte on grasses in response to shade. Yet, the response of plants to light availability is a key element for plant growth in mixed vegetation of tall and short plant species. Previous studies have recorded positive effects of endophytes on host plant growth under shade (Lewis 2004), and a higher concentration of alkaloids and phenolics under shade than under open sites in infected plants (Belesky et al. 2009). In a recent meta-analysis, Davitt et al. (2010) reported that approximately 25 % of symbiotic grasses were restricted to shady habitats versus only 12 % of non-symbiotic grasses. They also performed greenhouse experiments to study the effects of shade on the growth and traits of six perennial grass species (*Elymus villosus*, *Poa alsodes* and *Festuca subverticilliata* occurring in shade, and tall fescue, *Poa*

Drought stress intensity
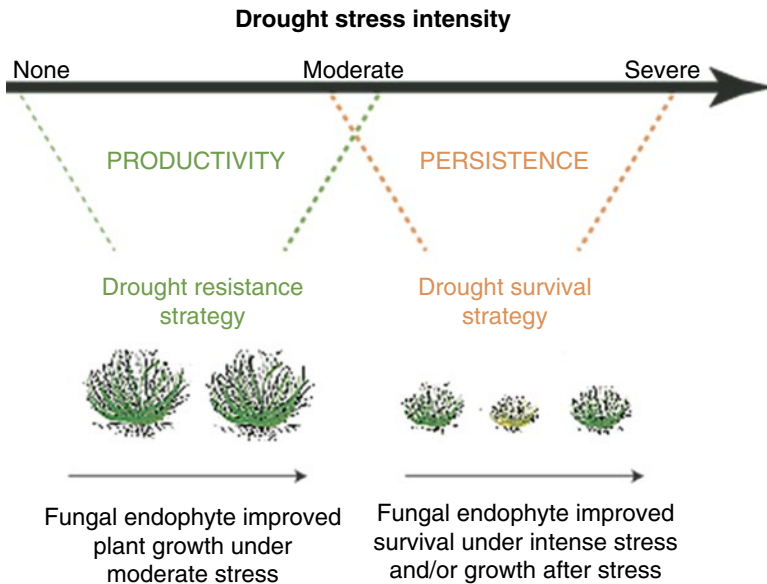
Fig. 6.3 Plant strategies associated with drought stress intensity, and the role of fungal endophyte (Adapted from Volaire et al. (2014))

autumnalis and *Agrostis perennans* occurring in light). They reported no effect of endophyte on plant growth under shade in all species, but endophyte had different effects on other traits such as spike production or specific leaf area depending on the host species. Altogether, these results suggest that depending on host species, endophyte-infected plants may be better adapted to the low-light intensity prevalent in some agro-forest systems. However, the context-dependency of endophyte effects on plant responses to light availability requires more detailed study.

6.6.3 Nutrient Availability and Mineral/Metal Stresses

The influence of fungal endophytes on plant responses to mineral stresses has interested researchers for some time, but the evidence remains anecdotal compared with those showing an impact of endophyte on herbivore resistance or drought stress. Several studies have highlighted the role of fungal endophytes in N and P metabolism, particularly in N-use efficiency (for a review see Malinowski et al. 2005a). However, some observational and experimental evidence suggests that endophytes have a higher positive effect under water-stress than under N-stress in host plants. Indeed, relatively few observational studies suggested a correlation between N-availability and fungal endophyte incidence (Lewis et al. 1997; Ravel et al. 1997), compared with those highlighting the role of water-availability (as discussed above). In addition, in a recent study explicitly comparing these two stresses, Ren et al.

(2013) showed that the beneficial effect of endophyte infection was less dependent on fertilizer supply than on water availability in *Leymus chinensis*, a dominant species native to the Inner Mongolia steppe. However, since nitrogen-availability appears to be a driver of alkaloid production in endophyte-infected grasses (Bylin et al. 2014), nitrogen-stress might be accompanied by herbivory stress in grasses, and should be studied in this context. On the other hand, in some dry land and overgrazed grass ecosystems, drought stress is accompanied by high concentrations of nitrogen; however the ways in which these conditions interact with *Epichloë* endophyte to alter grass growth and survival remains to be studied.

Whereas it is difficult to draw a clear and definite conclusion on endophyte functioning under metal stresses, studies have highlighted a large range of action of fungal endophytes on grasses (Table 6.4). For instance, fungal endophytes have been recently shown to increase tolerance to cadmium stress in *Achnatherum inebrians* (Zhang et al. 2010), salt-stress in *Hordeum brevisubulatum* (Wang et al. 2009) and in tall fescue (Sabzalian and Mirlohi 2010; Yin et al. 2014), Ni stress in tall fescue (Mirzahosseini et al. 2014) and arsenic stress in seedlings of *Festuca rubra* (Vázquez de Aldana et al. 2013b) (see Table 6.4). Unsurprisingly, the effect of endophyte on host plants is variable and has been shown to depend on the

Table 6.4 Effects of *Epichloë* fungal endophytes on host plants under some mineral/metal stresses

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Aluminum	*Schedonorus arundinaceus*	*Epichloë coenophiala*	No effect on root and shoot dry matter More Al (47 %) and P (49 %) desorbed from root surfaces More Al (35 %) and P (10 %) concentrations in roots No differences in mineral concentrations in shoots	Malinowski and Belesky(1999)
	Festuca rubra fallax, *Festuca rubra rubra*,	*Epichloë festucae*,	Variable effect on root and shoot dry weight (positive to negative)	Zaurov et al. (2001)
	Poa ampla	*Epichloë sp.*		
Arsenic	*Festuc arubra*	*Epichloë festucae*	No effect on germination response Longer radicles	Vazquez de Aldana et al. (2013c)

Table 6.4 (continued)

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Cadmium	*Schedonorus arundinaceus*	*Epichloë sp.*	Increased tiller number and biomass under both control and Cd-stress conditions Increased Cd accumulation in host Improved Cd transport from the root to the shoot Higher phytoextraction efficiency (2.41-fold higher)	Ren et al. (2006)
	Schedonorus arundinaceus, *Schedonorus pratensis*	*Epichloë sp.*	Higher biomass production (12–24 %) Higher potential to accumulate Cd in roots (6–16 %) and shoots (6–20 %)	Soleimani et al. (2010)
	Achnatherum sinebrians	*Epichloë gansuensis*	More biomass and higher values for plant height and tiller number Increased antioxidative enzyme activities, H_2O_2 concentration, and chlorophylls "a" and "b" levels Decline in proline and malondialdehyde content	Zhang et al. (2010)
	Elymus dahuricus	*Epichloë sp.*	Higher germination rate and index, and higher values for shoot length, root length and dry biomass	Zhang et al. (2012 b)
Copper	*Schedonorus arundinaceus*	*Epichloë coenophiala*	Higher Cu concentrations in plants under greenhouse and field plot and pasture experiments	Dennis et al. (1998)

(continued)

Table 6.4 (continued)

Mineral/Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Nickel	*Schedonorus arundinaceus*	*Epichloë coenophiala*	Higher plant growth in one genotype Improve in the antioxidative system in one genotype Reduction of Ni accumulation in the shoots of one genotype	Mirzahosseini et al. (2014)
Salt	*Hordeum brevisubulatum*	*Epichloë sp.*	Higher number of tiller, biomass accumulation Higher soluble sugar and proline contents, and superoxide dismutase isozyme activity Lower malondialdehyde content	Wang et al. (2009)
	Schedonorus pratensis	*Epichloë uncinata*	Higher leaf survival rates, root dry matter Reduced Na+ and Cl– concentrations in roots Increased K+ concentrations in the shoots	Sabzalian and Mirlohi (2010)
	Achnatherum inebrians	*Epichloë gansuensis*	Higher alkaloid levels	Zhang et al. (2011)
	Schedonorus arundinaceus	*Epichloë coenophiala*	Higher Na accumulation and improved Na transport from the roots to the shoots Higher phytoextraction efficiency (2.34-fold higher) Higher tiller number, shoot height and total biomass	Yin et al. (2014)

Table 6.4 (continued)

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Zinc	*Lolium perenne*	*Epichloë festucae var lolii*	Decrease of growth less pronounced Lower Zn concentration (24–32 %) in leaves Greater quantum yield of electron flow through the photosystem II (PSII) Normal level of internal CO_2 concentration Higher total dry weight, and tiller number	Monnet et al. (2001)
	Schedonorus arundinaceus	*Epichloë coenophiala*	Higher chlorophyll fluorescence at high concentrations of Zn Greater concentration of Zn in shoots	Zamani et al. (2015)

(continued)

genotype of both partners (Vázquez de Aldana et al. 2013b; Mirzahosseini et al. 2014), or the level of stress experienced (Zhang et al. 2010). Despite this variability in the symbiosis outcomes, it seems that fungal endophyte will open up new alternative strategies for phytoremediation of heavy metals and desalinization processes in the future of agroecosystem management.

6.6.4 Summary of Endophyte Support under Abiotic Ecological Constraints

Fungal endophytes support host plants and allow them to overcome abiotic stresses including drought, light, mineral and metal stresses. Recent evidence about the effect of fungal endophyte on drought survival, suggest that this symbiosis would be used for both productivity and stability and long-term persistence of grasslands. In addition, evidence of the role of fungal endophytes on plant resistance to metal/ mineral stress suggest their large potential for phytoremediation of heavy metals and desalinization processes. However, the context dependency of the

grass-endophyte symbiosis is still problematic for a general use of these symbioses across different plant species or environmental conditions. The challenge is now to identify the combination of grass-endophyte that will be the most advantageous for local conditions (environmental and cultural conditions).

6.7 Endophyte Modifies Biomass and Energy in a Grassland Ecosystem

The ability of *Epichloë* endophytes to impart beneficial properties to the host grass, resulting in improved plant growth and persistence, has been most intensely utilized in the mesic and managed grassland ecosystems of USA, New Zealand and Australia. In these regions, tall fescue and/or perennial ryegrass introduced from Europe have become naturalized in large areas and are the preferred species in many sown pastures as they display a wide range of adaption to climates, soils and management (Jung et al. 1996; Fribourg et al. 2009). Depending on the grass species and region, endophyte infection can be ubiquitous in naturalized populations and is preferred in sown seed due to the agronomic benefits. Unlike some other aspects of *Epichloë* endophyte infection which have been studied in controlled conditions, the effect on plant biomass and the subsequent energy input into the ecosystem has been extensively studied in field experiments.

6.7.1 USA

6.7.1.1 Continental-Type Tall Fescue

Continental-type tall fescue (Hand et al. 2010) is the most widely used temperate grass species in the USA, occupying some 14 million ha of pasture lands in parts of the Pacific northwest but mostly in the eastern USA (Young et al. 2013). While its range of adaptation is large, its predominate use is in the transition zone of the upper South and lower Midwest, an area commonly termed the 'fescue belt' reflecting the dominance of this grass in pastures, roadsides and amenity areas. Within the fescue belt, it is generally accepted that endophyte is necessary for tall fescue persistence in the southern half of this area. Abiotic stresses occur widely in this region, as droughts and high temperatures occur in summer on soils of low water holding capacity and poor nutrient status as the soils are highly weathered, eroded and shallow (Belesky and West 2009). Through a variety of morphological, biochemical and physiological mechanisms, endophyte in tall fescue has been shown to confer tolerance to both soil water deficit and some nutrient stresses (see Sect. 6.6). In addition, endophyte-enhanced growth in this region is likely due to protection from biotic stresses such as insects and nematodes (Popay 2009a; Timper 2009 and for more information see Sect. 6.3) and reduction in overgrazing in the case of common toxic

endophyte (Waller 2009). Even in unmanaged grassland ecosystems that are free of grazing livestock, endophyte has been shown to have a beneficial effect on persistence of infected grasses (Clay et al. 2012).

Increases in persistence and productivity due to endophyte infection of tall fescue have been demonstrated widely across the south eastern states of the US (Belesky and West 2009). In northeast Texas, Read and Camp (1986) measured 35 % less available forage on low endophyte (8 % infection) than highly endophyte-infected (95 % infection) paddocks of tall fescue over 2 years of cattle grazing, and failure of two out of three low endophyte paddocks to survive the final summer. In Georgia in a 3-year cutting experiment with four tall fescue populations, Bouton et al. (1993) reported a range of endophyte effects depending in location. In the most extreme case at Americus, endophyte-infected populations (greater than or equal to 85 % infection) in year 1 produced twice the herbage yield of endophyte-free and by autumn, endophyte-free plots had failed to persist (Table 6.5). Endophyte effects were more moderate at Tifton, where agronomic advantages to endophyte infection became more consistent with increasing time, and by autumn of year 3 endophyte-infected plots had twice the yield and ground cover of endophyte-free plots. At Watkinsville, no differences in yield and persistence were observed. These differences between locations in Georgia corresponded with the severity of summer drought, being greatest at Americus. Summer soil water deficit was also important in determining the advantage to endophyte infection in northwest Arkansas (West et al. 1993). At this location, in a dry year under no irrigation, tiller numbers in endophyte-free plots only recovered to 62 % of the numbers in endophyte-infected (80 % infected) plots, and these differences continued into the following year. While herbage yields did not consistently exhibit the same endophyte differences, possibly due to greater tiller size in endophyte-free plants, the weed contents in non-irrigated plots free of endophyte were twice as high as endophyte-infected. Bouton et al. (2002) also reported selected non-toxic endophytes largely enhanced the productivity and persistence of tall fescue comparable with what is seen for the common toxic endophyte.

6.7.1.2 Mediterranean-Type Tall Fescue

Use of tall fescue of Mediterranean origin and growth pattern (Hand et al. 2010) has the potential to extend the range of tall fescue into southern California and the sub-humid to semiarid transition zone of the southern Great Plains of Oklahoma and Texas (Young et al. 2013). Through summer dormancy, this type of tall fescue can survive hot, arid summers where Continental-type tall fescue fails (Malinowski et al. 2005b). Given the positive effects that endophyte has in enhancing the agronomic performance of Continental-type tall fescue, particularly in the southern USA, the role endophyte may have in expression of this summer dormancy, and the overall productivity of the host grass, is of interest.

Mediterranean-type tall fescues collected from countries of the Mediterranean basin are commonly highly infected with endophyte. In three studies of 211 seed

accessions collected from Morocco, Tunisia, Sardinia and Greece, endophyte was present in 89 % of accessions, with mean plant infection rates of greater than 57 % (Clement et al. 2001; Piano et al. 2005; Takach et al. 2012). High infection rates are a strong indication that under the abiotic and biotic stresses of these environments, endophyte infection enhances persistence of the host tall fescue plant. In addition, bioactivity has been demonstrated by Clement et al. (2001) with resistance to the bird cherry-oat aphid (*Rhopalosiphum padi*) occurring in most endophyte-infected accessions. However, mammalian toxic alkaloids may be produced by many of these associations (Takach et al. 2012; Piano et al. 2005).

Field testing of Mediterranean-type tall fescue at Vernon, Texas has shown variable agronomic effects of endophyte depending on year and plant population. At this site, Malinowski et al. (2005b) reported no effect of endophyte on dry matter yield of cv. Grasslands Flecha over 3 years, but a 22 % increase due to endophyte in a year following a year of extreme drought, and 21–50 % greater numbers of tillers surviving drought in 2 years. Furthermore, Malinowski et al. (2012) found endophyte responses for the TX06V population for plant persistence, herbage yield, dormancy rating, and competitive ability, but this was not the case for Flecha. In contrast, at this site Thomas et al. (2013) found no effect of endophyte presence in summer on the survival, shoot dry matter yield, leaf senescence, tiller-base water content, or a range of biochemical parameters for TX06V-B-FA and Flecha tall fescues.

Despite these variable field results in Texas, indoor studies have shown endophyte presence can improve mineral nutrition in both Mediterranean and Continental-type tall fescues (Malinowski et al. 2012) which may result in better agronomic performance in situations of limited soil nutrients. In addition, extensive testing in Australia has shown infection with the selected, non-toxic 'AR542' endophyte improves agronomic performance relative to endophyte-free in a majority of experiments for a range of cultivars of both Mediterranean- and Continental-type tall fescues (Hume and Sewell 2014). There is a need for a greater understanding of the environmental drivers for endophyte-enhanced growth and persistence of tall fescue and how these differ between regions/countries and years.

Table 6.5 Effects of common toxic endophyte on yield and persistence in the first year of production (1988) of tall fescue (*Schedonorus arundinaceus*) in the southern zone of the 'fescue belt' at Americus, Georgia, USA

Infection status of tall fescue	Yield (kg DM/ha)				Stand persistence (% ground cover)	
	Winter	Spring	Autumn	Total	Late spring	Late autumn
Common toxic endophyte	378 a	5792 a	3510 a	9680 a	75 a	91 a
No endophyte	260 b	4601 b	0 b	4861 b	61 b	0 b

Plots were sown in October 1987. Data presented is pooled data for four populations. Within columns, means followed by the same letter are not significantly different (*P* < 0.05). Adapted from Bouton et al. (1993)

6.7.1.3 Ryegrass

In contrast to tall fescue, use of perennial ryegrass in pastures is limited to only 110,000 ha, being grown in the cooler, moister or irrigated northern latitudes of the USA (Young et al. 2013). Cultivars for use in forage are mostly endophyte-free, with no claims being made by US seed companies about endophyte levels or strain. There appears to be an absence of published information on agronomic field experiments to test if endophyte enhances persistence and production in these environments. However, within the amenity turf industry, it is widely accepted by breeders and practitioners that endophyte enhances persistence and performance of perennial ryegrass (and tall fescue) largely through enhanced insect tolerance (Brilman 2005; Young III and Silberstein 2012). Although level of endophyte in seed is not regulated, in 2006 over 73 % of turf perennial ryegrass cultivars in the USA had high (greater than 60 % infected seeds) infection levels (Young III and Silberstein 2012). It would therefore seem reasonable that endophyte could enhance the agronomic performance of ryegrass in pastures in the USA, dependent on the severity, frequency and type of biotic and abiotic stresses. If this is the case, selected endophytes should be used with low or no toxicity to livestock (see Sect. 6.2) (Young et al. 2013; Johnson et al. 2013).

6.7.2 Australia and New Zealand

6.7.2.1 Tall Fescue

In Australia and New Zealand, tall fescue utilized in sown pastures has been largely free of endophyte (Easton et al. 1994). It has long been known that endophyte-infected plants that occur on occasions in pastures, waste lands and alongside water ways can cause toxicity to livestock due to infection with common toxic endophyte (see Sect. 6.2). Endophytes to enhance agronomic performance have been therefore selected with no toxicity to sheep and cattle, and these are currently available to farmers (Young et al. 2013; Hume and Sewell 2014).

In New Zealand, the role endophyte may have in improving agronomic performance of tall fescue was first seen in Northland in soils that are free draining, suffer from summer drought and harbor damaging populations of African black beetle (Hume and Barker 2005) (Fig. 6.4). In a small plot agronomic experiment, endophyte infection increased from 30 % at sowing to 80 % of tillers infected after 4 years. Further small plot testing over 10 years in Northland with a range of Continental- and Mediterranean-type tall fescue cultivars showed increases of up to 66 % in plant numbers and annual dry matter yields, with differences being greatest in late summer-autumn (Hume et al. 2009). Greater persistence of endophyte-infected tall fescue which is more competitive than lower quality C4 grasses, compared with endophyte-free, has been seen on farms in this region under cattle grazing. Similar levels of endophyte-enhanced growth of tall fescue have been

Fig. 6.4 Perennial ryegrass (*Lolium perenne*) and tall fescue (*Schedonorus arundinaceus*) small plots in autumn near Bega, New South Wales, Australia (4 years post sowing). Differences in persistence predominately due to damage from African black beetle (*Heteronychus arator*) (Photo courtesy of David Hume, AgResearch Ltd., New Zealand)

recorded in other regions of New Zealand, with the advantage to endophyte varying between regions corresponding closely to the severity of both soil water deficit and insect pest pressure particularly in the summer-autumn period (DE Hume unpublished data).

Agronomic evaluation of selected endophytes has occurred throughout the south eastern states of Australia where within the same cultivar, endophyte-free has been compared with the selected 'AR542' endophyte in more than 30 experiments since 2000 (Fig. 6.4) (Hume and Sewell 2014). Over 80 % of these experiments showed agronomic advantages to the AR542 endophyte, which were typically in the range of +8 % to +100 % (mean +38 %, median +30 %) for both Continental- and Mediterranean-types of tall fescue. In the most extreme case in the Hunter Valley of New South Wales, endophyte-free cultivars had completely failed to survive 1 year after sowing while those infected with AR542 were dense and productive. In many cases, the specific environmental drivers behind these differences were unclear, but in general, differences occurred and were largest when soil, insect, and climate/weather stresses were at their greatest, similar to what has been seen in New Zealand and the USA. When these biotic and abiotic stresses were lowest, tall fescue cultivar rather than endophyte was the major determinant of agronomic performance.

Despite the useful agronomic attributes of tall fescue, particularly under harsh soil and climatic conditions where perennial ryegrass struggles, its use in the past has been very limited in New Zealand and restricted in Australia (Easton et al. 1994). For example, tall fescue in Australia is grown on 1.1 million ha, only 7 % of its potential adaptive area. Given the agronomic evidence from comparisons of endophyte-infected and endophyte-free cultivars, it is highly likely that elite cultivars with non-toxic selected endophytes will broaden the useful range of tall fescue, increasing pasture and overall farm productivity in both Australia and New Zealand (Young et al. 2013). Models to predict where and when endophyte infection would be of agronomic and financial benefit to farmers may assist in uptake of selected endophyte technology. In the case where new pastures are being sown due to crop rotation or pasture renewal, selected endophyte technology can be deployed for only a small marginal increase to the overall cost of establishment.

6.7.2.2   Ryegrass

The situation in Australia and New Zealand for perennial ryegrass parallels that of the USA for tall fescue, as endophyte-infection is essentially the norm and research focused initially on the agronomic impact of removing the common toxic endophyte due to its clinical and subclinical toxicity to livestock (see Sect. 6.2). The agronomic importance of endophyte infection in New Zealand was first determined 1 year after the link had been established between endophyte and ryegrass staggers in sheep. In early 1982 in a field experiment at Hamilton in northern New Zealand, endophyte-infected plots produced threefold more dry matter in late summer-early autumn than endophyte-free plots (Mortimer et al. 1982). Endophyte-free ryegrass had been severely damaged by Argentine stem weevil, a major insect pest of ryegrass. Unlike the USA, this dispelled the concept of deploying endophyte-free seed as the solution to endophyte livestock toxicity in ryegrass, as the negative impact on yield and persistence of ryegrass and so ultimately the total productivity of the pasture was considered to be too great.

Further field experiments examining the impact of endophyte on agronomic performance continued to demonstrate the important role endophyte had in ensuring the high yield and persistence of ryegrass pastures in many but not all cases, in both New Zealand and Australia. For example, in a national series of 10 small plot experiments sown in four regions of New Zealand, endophyte infection enhanced yields in all experiments, predominately in the period of mid-summer to mid-autumn period, in some cases by up to 88 % (Popay et al. 1999). Under dairy grazing in Waikato, New Zealand, endophyte-free ryegrass pastures were so severely depleted that they needed to be resown to restore them to an agronomically productive level (Thom et al. 2014). In Australia, over half of 18 experiments had advantages of +7 % to +212 % (mean +44 %, median +29 %) to endophyte-infected plots (Fig. 6.4) (Hume and Sewell 2014). However, in areas or years of low insect damage and cool moist summers (and/or irrigation) there may be no agronomic advantage in the short term to endophyte infection.

Selected endophytes of no or low toxicity to livestock (see Sect. 6.2) have varying impacts on agronomic performance (Popay and Hume 2011). This has been most clearly demonstrated in New Zealand, but similar evidence is accumulating in Australia. The key driver behind these differences is the breath of protection each endophyte strain imparts to the ryegrass from damage by up to five of the six major exotic and native insect pests in New Zealand. Strain differences are most pronounced when damage from high pest populations occurs when ryegrass is under stress from heat and soil water deficits in summer/autumn (Popay et al. 1999; Popay and Hume 2011; Thom et al. 2014). Under these conditions, AR37-infected ryegrasses have the best agronomic performance in both New Zealand and Australia (Hume et al. 2009; Popay and Hume 2011; Hume and Sewell 2014).

Effects of endophyte infection on overall agronomic performance of the pasture are less well documented than the effects solely on the endophyte-infected host grass. This is primarily due to the sown grass component of a mixed sward often being the major driver of total yields and to a large extent quality of the pasture. In some cases, catastrophic collapse of endophyte-free ryegrass in mixed pastures necessitates the resowing of these fields, e.g. Thom et al. (2014). In other cases, endophyte infection of ryegrass may have relatively little impact on total pasture production, but undesirable changes occur in species composition due to ingress of broad-leaf weed species and grasses species of poor feed quality and/or agronomic performance. For example, in sub-tropical, south-east Queensland, Australia, irrigated-plots sown as pure ryegrass differed little in total dry matter yields over 3 years between endophyte-free (40.03 t DM/ha) and common toxic endophyte-infected ryegrass (41.02 t DM/ha) (Table 6.6) (Lowe et al. 2008). However, undesirable C_4 grasses were 55 % higher in endophyte-free than endophyte-infected plots, reducing the forage quality of the sward.

White and subterranean clovers (*Trifolium repens* and *T. subterraneum*, respectively) are important components of managed grasslands in Australia and New Zealand. While their overall contribution to total sward production is small relative to the sown grass, clovers produce highly nutritious feed for livestock and provide nitrogen to the pasture through symbiotic rhizobia that fix nitrogen from the atmo-

Table 6.6 Effects of common toxic endophyte on plant persistence and yields of irrigated perennial ryegrass (*Lolium perenne*) in subtropical, southeast Queensland, Australia

Infection status of ryegrass	Plant persistence (% frequency)				Yield (t DM/ha)	
	End of summer			End of autumn	Total 3-year ryegrass	Total 3-year weeds
	Year 1	Year 2	Year 3	Year 3		
Common toxic endophyte	61.2 a	49.0 a	16.7 a	15.6 a	33.69 a	7.33 b
No endophyte	48.1 b	40.1 a	5.6 b	5.0 b	28.67 b	11.36 a

Within columns, means followed by the same letter are not significantly different ($P<0.05$). Adapted from Lowe et al. (2008)

sphere. In general, any factor that enhances ryegrass growth, such as nitrogen fertilizer and grazing management, results in ryegrass being more competitive for limited soil and light resources, so clover growth and content may decline in the short term. Endophyte-infected ryegrass, compared with endophyte-free ryegrass, has on occasions been shown to reduce clover contents in the field, most likely through greater competitive ability but also possibly through allelopathic effects that endophyte-infected grasses have over other plant species (see Sect. 6.5) (Sutherland et al. 1999; Thom et al. 2014). In some cases this can be substantial, with Cunningham et al. (1993) reporting subterranean clover contributing 30 % of the yield in endophyte-free ryegrass pastures but close to zero in endophyte-infected pastures in southwest Victoria, Australia. However at the same site over 3 years, Quigley (2000) reported no effect of endophyte infection in ryegrass on subterranean clover plant numbers despite 37 % and 7 % more ryegrass plant and tiller numbers and 10 % more total dry matter yields in endophyte-infected pastures.

6.7.3 Summary for Endophyte Effects on Biomass and Energy in a Grassland

There is compelling evidence that suggests *Epichloë* endophytes improve the medium to long term agronomic performance of tall fescue and perennial ryegrass pastures in large areas of the mesic, managed grasslands of the USA, New Zealand and Australia. The advantages of endophyte infection is greatest with edaphic stresses (e.g. drought) combined with biotic stresses (e.g. insect pests). These data support the widespread commercial use of endophyte-infected cultivars in these countries. While endophyte-infected cultivars have enhanced persistence and yield, and weed contents of swards are reduced, in comparison with equivalent endophyte-free cultivars, farmers need to be aware that the performance of companion clovers could be compromised on occasions.

There are intriguing differences between these countries as to whether tall fescue and ryegrass have naturalized as infected with animal-toxic endophyte strains, or are endophyte-free. In the case where endophytes are toxic to animals, cultivars with selected endophytes can be utilized. These selected endophyte-infected cultivars may have varying agronomic performance relative to the common toxic endophyte-infected cultivars, but they represent a significant economic gain for farmers through good agronomic performance with no or reduced adverse effects on grazing animals. Where in the past endophyte-free cultivars have been used, for example tall fescue in New Zealand and Australia and ryegrass in USA, use of selected endophytes provided an opportunity to enhance agronomic performance and also broaden the useful range of these valuable pasture grasses. The deployment of selected endophytes in Mediterranean-type tall fescue cultivars in Australia and USA provides an opportunity to use endophytes to boost the agronomic performance of tall fescue in regions with hot, arid summers. There is a need for a greater understanding of the environmental drivers for endophyte-enhanced growth and

persistence of Mediterranean tall fescue and how these differ between regions/countries and years. Opportunities exist beyond these countries to improve the performance of tall fescue and ryegrass through the use of selected endophytes in cultivars adapted to these regions.

6.8 Global Change Impacts on Grass-Endophyte Interactions

6.8.1 Global Change Variables

Atmospheric CO_2 is expected to increase to between 450 and 950 ppm by the year 2100 with subsequent radiative forcing expected to increase global mean surface temperature by 1–3.7 °C (IPCC 2013). Altered precipitation and temperature-induced reductions in soil water content are likely to enhance drought in some regions (IPCC 2013). Climate variables are expected to alter the physiology and phenology of plants, animals and fungi, with broader consequences for species interactions such as herbivory, competition and mutualism.

6.8.2 Global Change Impacts on the Ecology of Grass-Endophyte Interactions

The effects of climate variables such as CO_2 and warming have not been widely studied in *Epichloë* endophytes, though effects on other fungal mutualists of plants have been well documented. Studies have shown that global change variables can impact the ecology of plant-fungal symbiont interactions both by directly altering fungal growth (Compant et al. 2010) and through fungal symbiont-mediated changes in plant growth responses (Kivlin et al. 2013). In a review of plant mutualisms and climate change, Compant et al. (2010) found that both temperature and CO_2 enhanced the colonization of arbuscular and ectomycorrhizal fungi. For CO_2, this is likely due to enhanced carbon resources from higher photosynthetic output, and a similar observation might be expected in other carbon-limited fungal mutualists. Changes in plant nutrient allocation may be especially important for plant-fungal mutualisms where nutrient exchange forms the basis of species interactions.

Table 6.7 shows the literature to date on changes in endophyte and alkaloid concentrations in response to experimental warming and CO_2 increase. In most cases, experimental warming resulted in increased endophyte prevalence and concomitant increase in alkaloid concentration, and this is consistent with evidence from field studies. di Menna and Waller (1986) found that pastures of *Epichloë*-infected *Lolium perenne* had higher hyphal density in mid-summer when temperatures were highest. Similar seasonal fluctuations were noted for *Epichloë*-infected tall fescue

Table 6.7 Studies that have investigated the effects of elevated atmospheric CO_2 or temperature on grass-endophyte interactions in controlled environments

CO₂ studies

Plant species	Epichloë endophyte species	Treatment levels		% Concentration change					Reference
				Lolines	Ergots	Lolitrem B	Peramine	Endophyte[a]	
Schedonorus arundinaceus	*Epichloë coenophiala*	396 ppm	+300 ppm	−25.7	−31.4			+12.3	Brosi et al. (2011)
Bromus erectus	*Epichloë bromicola*	360 ppm	600 ppm					NS	Groppe et al. (1999)
Lolium perenne	*Epichloë festucae var. lolii*	368 ppm	466 ppm		+819.5		+196.8		Hunt et al. (2005)[b]
Lolium perenne	*Epichloë festucae var. lolii*	368 ppm	466 ppm		NS		NS		Hunt et al. (2005)[c]
Bromus erectus	*Epichloë bromicola*	350 ppm	600 ppm					+53.1	Meijer and Leuchtmann (2000)[d]
Bromus erectus	*Epichloë bromicola*	350 ppm	600 ppm					−37.2	Meijer and Leuchtmann (2000)[e]
Bromus erectus	*Epichloë bromicola*	350 ppm	600 ppm					NS	Meijer and Leuchtmann (2000)[f]
Schedonorus arundinaceus	*Epichloë coenophiala*	390 ppm	800 ppm	−18.2	−13.4		NS	+36.6	Ryan et al. (2014a)
Schedonorus arundinaceus	*Epichloë coenophiala*	390 ppm	1000 ppm	NS	NS		NS	NS	Ryan et al. (2014a)

(continued)

Table 6.7 (continued)

Temperature studies		Treatment levels		% Concentration change					
Plant species	Epichloë endophyte species			Lolines	Ergots	Lolitrem B	Peramine	Endophyte[a]	Reference
Lolium perenne	Epichloë festucae var. lolii	10 °C	20 °C					+53.2	Breen (1992)
Lolium perenne	Epichloë festucae var. lolii	14 °C	21 °C					NS	Breen (1992)
Lolium perenne	Epichloë festucae var. lolii	21 °C	28 °C					NS	Breen (1992)
Schedonorus arundinaceus	Epichloë coenophiala	ambient	+3 °C	+25.7	NS			NS	Brosi et al. (2011)
Lolium perenne	Epichloë festucae var. lolii	20 °C	25 °C		+105.3	NS	NS		Eerens et al. (1998)
Schedonorus arundinaceus	Epichloë coenophiala	21/15 °C	32/27 °C	−63.5					Kennedy and Bush (1983)
Schedonorus arundinaceus	Epichloë coenophiala	10 °C	15 °C					+288.2	Ju et al. (2006)
Schedonorus arundinaceus	Epichloë coenophiala	15 °C	20 °C					+306.1	Ju et al. (2006)
Schedonorus arundinaceus	Epichloë coenophiala	12/6 °C	25/19 °C					+3578.9	Ju et al. (2006)

Schedonorus arundinaceus	*Epichloë coenophiala*	ambient	+3 °C	NS	NS			NS	McCulley et al. (2014)[g]
Schedonorus arundinaceus	*Epichloë coenophiala*	ambient	+3 °C	+30.9	+40.7			NS	McCulley et al. (2014)[h]
Lolium perenne	*Epichloë festucae var. lolii*	10/10 °C	20/10 °C		NS	+3293.1	+128.8	+191.3	Ryan et al. (2015)

Changes in alkaloid and endophyte concentrations were extracted from the listed references and are expressed here as % concentration change relative to controls. In cases where there were two-way interactions between CO_2/temperature and other variables, effects are reported separately for each treatment level of the other variable (e.g. year). Otherwise, the main effects are reported

[a]Endophyte concentration studies included those measuring infection frequency (Brosi et al. 2011; Ju et al. 2006; McCulley et al. 2014), number of endophytic cells using qPCR (Groppe et al. 1999; Ryan et al. 2014a; Ryan et al. 2015), hyphal counts (Breen 1992), and choke rates in parasitic *Epichloë* species (Meijer and Leuchtmann 2000).
[b]Measured at 40 mol/m³ nitrogen
[c]Measured at 10 mol/m³ nitrogen
[d]No fertilizer 1996
[e]High fertilizer 1996
[f]1997
[g]Measured in Spring
[h]Measured in Fall; NS = not significant; Blank cells = parameter not measured

where both infection frequency (Ju et al. 2006) and alkaloids (Kennedy and Bush 1983) increased in mid-summer.

Two studies found that *Epichloë*-derived alkaloids decreased in tall fescue grown in elevated CO_2 (Table 6.7). In contrast, Hunt et al. (2005) found increases in alkaloid concentrations in perennial ryegrass grown in high CO_2 and high nitrogen fertilization, though those grown in nitrogen poor conditions showed no change in alkaloids. As Table 6.7 suggests, the disparities within the CO_2 literature may be due to plant and fungal species, CO_2 treatment levels, and plant nitrogen status. Endophyte concentration tended to increase under elevated CO_2 (Table 6.7) though several studies have found no change and one study found decreased concentrations in a high fertilization treatment. The observation that endophyte growth and transmission may increase while alkaloid production decreases may have consequences for the strength of mutualistic interactions in the future.

6.8.3 Impacts of Global Change on the Utility of Fungal Endophytes in Agroecosystems

Epichloë-infected cool-season grasses have high agronomic importance due to increased resistance to stresses such as drought and insect herbivory. As such, novel associations (see Sects. 6.2 and 6.3) containing alkaloid profiles that reduce toxicity to grazing vertebrate herbivores, but maintain invertebrate toxicity, have been widely marketed for pest control in forage crops. Insect herbivore performance is generally reduced in high CO_2 due to lower plant concentrations of limiting nutrients such as nitrogen, though responses have been shown to depend on insect feeding guild (Robinson et al. 2012). Few studies have examined grass-endophyte-insect interactions in the context of climate change. Ryan et al. (2014a, b) found that while alkaloid concentration was reduced in high CO_2, aphid colonization on endophyte-infected tall fescue was consistently low, regardless of CO_2 concentration. Similarly, Marks and Lincoln (1996) found that while the growth rate of fall armyworm was reduced by endophyte infection, there was no $CO_2 \times$ endophyte interaction. This may be due to simultaneous endophyte-mediated changes in both primary and secondary plant metabolism, which can alter host quality for insects in complex ways. Hunt et al. (2005) found that a CO_2-induced decrease in soluble proteins in uninfected plants was not observed in infected conspecifics, suggesting that endophyte infection may mediate changes in plant primary metabolism in ways that can affect insect diet quality.

Insect pests are likely to respond to warming through both direct (i.e. physiological) and indirect (i.e. plant-mediated) mechanisms and indirect changes in host plant quality can be mediated by endophytes. Salminen et al. (2005) found that fall armyworm performance was lower when fed endophyte-infected grass tissue that had been grown in higher temperatures relative to material that had been grown in lower temperatures, suggesting that warming may provide enhanced resistance in

endophyte-infected hosts. However, direct effects of warming are likely to increase the number of generations of multivoltine insects in a given year due to increased development rate and longer growing season (Bale et al. 2002). Thus, increased insect herbivore loads in some regions may increase the competitive advantage of endophyte-infected grasses, particularly in light of preliminary observations that warming may increase endophyte and alkaloid concentrations (Table 6.7).

Endophyte-infection and climate variables can interact in ways that affect forage crop yields. Marks and Clay (1990) found that biomass increased by 80 % under elevated CO_2 in perennial ryegrass infected with *Epichloë festucae* var. *lolii* compared with only a 21 % increase in uninfected plants. However, no $CO_2 \times$ endophyte interaction was found for yield responses in tall fescue (Chen et al. 2007; Brosi et al. 2011; Ryan et al. 2014b). The advantages of endophyte infection for drought avoidance, tolerance, and recovery in host plants have been extensively documented (Malinowski and Belesky 2000). A recent meta-analysis by Kivlin et al. (2013) found that fungal symbiont associations in general (leaf endophytes, arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and dark septate endophytes) were able to ameliorate the negative effects of drought on host plants associated with climate change. In future climates, such adaptations could be beneficial for plant growth and persistence in soils where water is a growth-limiting factor. In addition, some alkaloids have previously been shown to increase under drought stress (Kennedy and Bush 1983; Belesky et al. 1989; Brosi et al. 2011), which may result in increased protection against insect herbivory in future climates when insect outbreaks are hypothesized to increase (Bale et al. 2002).

6.8.4 Risks, Opportunities, and Future Directions

Table 6.7 highlights the paucity of work that has been done in the area of grass-endophyte responses to climate change. Most studies on endophyte responses to temperature were not designed to directly address hypotheses related to climate change though information about potential responses might be inferred from such work. Only two studies (Brosi et al. 2011; McCulley et al. 2014) have examined endophyte response to warming treatments in the range of projected global mean temperature increases (in both cases ambient +3 °C). These studies found that while endophyte infection frequency was similar between ambient and elevated temperatures, alkaloids tended to increase in response to warming, though such increases were dependent on alkaloid group (Brosi et al. 2011), season (McCulley et al. 2014), and precipitation level (McCulley et al. 2013). Thus while some general responses are beginning to emerge, it is not yet clear how these suite of changing variables may interact. There is also evidence that climate change effects on grass-endophyte interactions may also interact with variables such as UVB (Newsham et al. 1998) and mineral fertilization (Hunt et al. 2005; Kivlin et al. 2013).

More research is needed to identify potential risks and opportunities associated with grass-endophyte interactions in a changing climate and to inform future

management practices and breeding programs. Increased alkaloid toxicity in warmer climates may benefit pest management in forage crops. However, alkaloid toxicity has been associated with poor animal performance during summer grazing when alkaloid concentrations tend to increase (Kennedy and Bush 1983) and this phenomenon is likely to be exacerbated by climate change. For example, recent outbreaks of severe ryegrass toxicosis in Australia have in part been attributed to warmer springtime temperatures (Reed et al. 2011). As such, breeding for low toxicity strains and/or novel associations will likely form a critical part of adaptive responses. Selection of grass-endophyte combinations that maximize drought tolerance and recovery will likely benefit regions of higher drought incidence in future climates. It has been suggested that breeding and bioengineering programs could be undertaken to take advantage of high CO_2 (Ainsworth et al. 2008) and such strategies could also be used for pasture productivity. In addition, research has shown that high endophyte pastures are able to sequester more carbon than endophyte-free pastures, suggesting that endophytes may even have a role in climate mitigation (Iqbal et al. 2012).

6.8.5 Summary of Grass-Endophyte Responses to Global Change

Changes in CO_2 concentration, water availability, and temperature are likely to affect grass-fungal mutualism interactions in the future. While the effects of drought responses on grass-endophyte interactions have been widely studied, much less is known about how these species will respond to rising CO_2 and temperature. Research to date suggests that endophyte growth (tiller infection frequency and endophyte concentration) and alkaloid concentrations may increase in response to temperature, and this is consistent with field studies showing increased endophyte-associated toxicity in summer months. Alkaloid and endophyte responses to CO_2 have been less consistent, and preliminary research suggests that responses are dependent on plant and fungal species, and plant nitrogen status. The effects on agroecosystems are likely to be complex. Some research suggests that endophyte-infected grasses have higher yield responses to CO_2 than endophyte-free grasses, though this effect appears to be species-specific. Additionally, endophyte infection may buffer yield losses associated with drought in some regions. However, such benefits will need to be weighed against the possibility of increased toxicity for grazing animals, a phenomenon that is already occurring in some parts of Australia.

6.9 Conclusion

Most of the information available on ecological consequences of *Epichloë* fungal endophytes pertains to two important grass species, tall fescue and ryegrass, and mainly from a few pioneer countries in endophyte research. Lower live weight gains

and neurological symptoms in grazing animals is still a common problem in plains predominantly covered by these two species infected with common toxic *Epichloë* endophytes. However, changes in management practices to utilize selected strains of *Epichloë* and searching for new isolates with no or minimum animal toxicity, not only let farmers get rid of potential animal disorders, but also help pastures and grasslands become more productive and stable over time.

In contrast to natural conditions, in agroecosystems a particular grass-endophyte combination could be propagated and maintained to increase productivity and stress resistance and to shape the growing area. This has been done to decrease the negative impact of *Epichloë* toxic alkaloids on grazing animals in sown pastures. For some environments like saline soils or environments under heavy metal stress, some other strains of endophyte may be more appropriate to be used as symbionts of grasses. Screening a wide variety of grass-endophyte genotypes is a prerequisite to exploring the combinations in pre-determined environments which could be used for specific applications in agroecosystems.

The advantages of *Epichloë* endophyte for host grasses are predominantly observed in areas or in years of severe drought and high insect damage. This may show the preference of sowing endophyte-infected tall fescue and ryegrass in drought prone areas of the world in order to extend grass plantations or rangeland rehabilitations. However, growing infected grasses under drought could be associated with higher toxic alkaloid accumulation leading to more fescue toxicosis and ryegrass staggers especially if turf cultivars are infected with common toxic endophytes.

Epichloë endophyte can improve mineral nutrition, biotic and abiotic stress tolerance and competitive ability of host grasses relative to endophyte-free counterparts, which in turn results in better agronomic performance in agroecosystems. Recent studies show that it could also have broader consequences, affecting higher trophic levels in ecosystems. However, the stability of endophyte effects over years and under different environments may not be consistent. This urges investigation on how the effects of *Epichloë* endophyte may differ between host species, regions/countries and years.

Despite the vast majority of studies focusing on drought and mineral stresses, the outcome of endophyte infection against some stressors like flood, high soil salinity, heat, cold and nutrient deficiency in ecosystems has been scarcely addressed. It is evident that a lot of variation from positive to negative impact of endophyte infection on host tolerance to biotic and abiotic stresses does exist. This is believed to originate from grass genetic background, endophyte genetics and their interactions. In a large population of grass genotypes of a given species in an ecosystem, there should be interactions from parasitism to full mutualisms and their mean effect could be neutral unless ecological constraints favor some infected grass counterparts. Therefore, there is a need to understand the environmental drivers which promote compatible combinations of endophyte-host plants resulting in superiority over non-infected or incompatible endophyte-host counterparts. As stress conditions are likely to intensify in grassland ecosystems in the future, there may be a greater reliance on endophyte infection for growth and production in ecosystems.

Grasses infected with *Epichloë* endophyte may have the potential to decrease the need for application of pesticides, help weed management and phytoremediation, and desalinization of agricultural soils. The applied consequences of *Epichloë* infection to engineer agroecosystems in these ways remain to be explored. Due to many multi-lateral interactions, selection of superior grass-endophyte combinations is difficult and deserves greater attention to develop protocols or models for rapid evaluation of variation that would be useful in agroecosystems. Also, similar to many other plant-microbe interactions, the relationship has a genetic basis and for better exploitation of this symbiosis for ecosystem purposes, the plant genes responsible for receiving or rejecting the endophytic partner should be identified.

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Chapter 7
Nanofertilisers, Nanopesticides, Nanosensors of Pest and Nanotoxicity in Agriculture

Alpna Dubey and Damodhara R. Mailapalli

Abstract Food security in the world is challenging due to the limited available resources for the rising population. Various efforts are being practiced by governments, organisations and researchers to mitigate the demand and supply gap in human food chain. Agriculture took the roots of growth prior to industrial revolution, in around 90 countries. Though nanotechnology has already found industrial applications, the use of nanotechnology in agriculture is much more recent.

Here we review nanotechnology applications in agriculture such as plant production, protection, and detection of pathogen. We also discuss the environmental risk associated with nanotechnology. The major points are: (1) research funding for nanoresearch is highest in USA, followed by Germany and Japan, whereas China published the highest number of publications, and USA obtained the highest number of patents. (2) Nanofertilizers based on carbon walls, metal and metal oxide increase germination, photosynthesis, nutrient use efficiency and plant growth (3) The metal oxide-based nanomaterials such as ZnO, TiO_2, Cu and SiO_2 are increasingly used in pesticides and fungicides to protect crops from bacterial disease and control microbial activity. (4) Silver, copper and gold nanoparticles are used as bio-nanosensors and electrical-nanosensors to detect a potential pathogen problem in plant and postharvest foods. (5) The level of nanotoxicity in soil, plant and water mainly depend on the composition, size and concentration of the nanoparticles. (6) Nanoparticles of size lower than 50 nm usually adversely affect human health and the potential routing could be through inhalation, ingestion and dermal exposure. Overall, nanotechnology has the potential to increase agricultural production, but there is very limited knowledge about its long term adverse effect on soil, plants and ultimately on human. An intelligent use of nanotechnology may help to achieve food security with the qualitative and sustainable environment.

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7.1　Introduction

Food security becomes a fundamental human right and implies that all people, at any socioeconomic and geographical level, whenever they require, must have access to enough, affordable and healthy food that suffice people dietary requirement and food choice for active and healthy life (FAO 2009). Achieving the economic food with the optimum nutritional and calorific values from upper to very last chunk of the population is a hard task for any country due to the population rise. The world's population was about 7 billion in 2013 and it is estimated to attain about 9.6 billion by 2050 and 10.9 billion by 2100 with Asia being the largest contributor, of 60 % of this population (UN World Population Prospects 2013). The net increase of 230,000 people each day in the world increases the demand of food, shelter, and other resources (UN Population Division 2007). With so much of projected population of the world, there comes a very viable social problem of food security. World cereal production including wheat, rice and coarse grains estimated at 2525.4 million tonnes in 2013-2014 and projected to reach about 3 billion tonnes by 2050 (FAO 2009). Of this, 50 % of cereals is used as animal feed to achieve the world's meat demand. The current estimation of 50 % increase in food production is essential to maintain the demand of food grains and cereals by 2050 but due to compounding damaging effects by climate change, land degradation cropland losses, water scarcity and species infestation induce 5–10 % additional food demand (UNEP 2009).

Uncertainty of food production, poor maintenance and distribution are the major challenges of food security. To control food security crises, the first priority is to increase food production by enhancing the resources and technology, and the second priority is to improve quality of available inputs (FAO 2009). Modern technologies that possibly enhance food production could be biotechnology (Ervin et al. 2010; Spiertz 2010), Deoxyribonucleic acid (DNA) (Devos et al. 2007), advance water management techniques such as micro irrigation, crop and soil sensors and modern farm mechanization. Food and agriculture organization (FAO 2000) reported that DNA and biotechnologies increase yield of sweet potato and cassava by 30 % and 27 %, respectively and income of small holder banana farmers by 25 %. Micro irrigation reduces nitrogen emissions and increases tomato crop yield by 78–119 Mg ha^{-1} (Kennedy et al. 2013). Zero tillage operations save 18–53 % energy and reduce 25–41 % cost of cultivation per hectare (Sorensen and Nielsen 2005). All these technologies improve crop production and reduce inputs, but require high skill and basic knowledge and regular monitoring. They are not sufficient to reduce the gap of demand and supply of food. Nanotechnology (Chen and Yada 2011; Sekhon 2014) has the ability to change entire agriculture and food industries and has potential solution to over-come all these problems and increase production. Scientist are working on nanotechnology to improve agriculture. Though nanotechnology can be very effective in making the agricultural production economic and resource efficient. But from the environmental point of view, it creates some unpredicted harmful effects (Bouwmeester et al. 2009; Nel et al. 2006) Therefore, it is essential to study all facets to nanotechnology from the agro-ecology perspective.

7.2 Nanotechnology

Nanotechnology is one of the rising technologies of the twenty-first century. Taniguchi (1974) first coined the term nanotechnology and stated that nanotechnology consists of the processing, separation, consolidation, and deformation of materials by one atom or one molecule. Nanoscience, nanotechnology and nanoengineering involve working under nanosize (one billionth of a meter) scale (NNI 2009). Engineering nano materials can be defined as the material that has single unit of size less than 100 nm (Taniguchi 1974). At nanoscale, the chemical and physical properties of material change and surface area of material is large compared to its volume. This makes material more chemically reactive and changes the strength and electrical properties of material compared to the bulk counterpart.

Application of nanotechnology in different fields anticipated to be beneficial for society and environment, reduce cost of input and increase production, improve quality of product, open opportunity for employment (Hansen and Maynard 2008). It covers a broad area, including medicine biology, electronics and instrumentation, cosmetics, defence, energy, environment, agriculture, information and communication technology. Nanotechnology in medicine involves application of nanoparticles in drug which is more effective than bulk material and directly attracted to the disease cell. (Ramsden 2011, 2013). Nanoparticles are used in sunscreens, abrasion-resistant coatings, barrier coatings, antimicrobial coatings, and fuel combustion catalysts (Ramsden 2013). Nanotechnology also used in high energy physics; carbon nanotube inside silica nano pores used as high resolution particle detector (Angelucci et al. 2003). Other uses of nanotechnology in electronics are to manufacture microchips and data storage devices. The small data storage device has potential to store 400–500 gigabyte/inch^2 data and can be used in wrist watches, mobiles and laptops (Mamalis 2007).

Governments of different counties have been spending millions of dollars on research and development of nanotechnology. The U.S.A government spent $862 million in 2003-2005 and proposed $1574.3 million to different agencies under national nanotechnology initiative (NNI) for year 2013-2016 (NNI budget, 2015). In Japan and china the total budget for nanotechnology were $810 and $280 million, respectively for the year 2003 (Jia 2005). According the Cientifica Ltd (2011) data source, only china spent $1.3 billion on nanotechnology research it is nearly close to U.S. budget for nanotechnology which is about $2.18 billion in 2011. India launched nano mission in 2007 with a budget of $250 million for 5 year with well-established research laboratories for nanotechnology development programme (6th Bangalore India Nano report 2013). The market of nanotechnology was around $147 billion in 2007 and predicted that would reach to $3 trillion by 2020 (Clunan and Hsueh 2014). Figure 7.1 shows the impact of nanotechnology on the economy of different countries. It is clear from the figure and data that contribution of nanotechnology to enhanced the economy of countries like U.S., China, Russia, Germany, and Japan increased. Many countries try to increase annual budget for research and development of nanotechnology to give a good competition in the market. With the
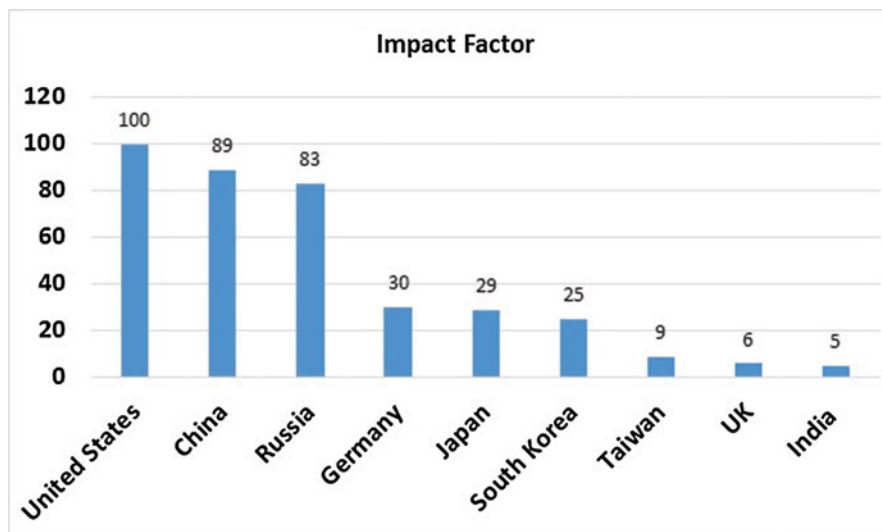
Fig. 7.1 Impact of nanotechnology on the economy of different countries (Source: Cientifica Ltd, 2011)

awareness of government of different countries, publications in nanoscience and technology are increasing rapidly. Figures 7.2 and 7.3 show the trends of research publications and patents available till date. World-wide research publications and patents in different fields of nanotechnology were about 70,000 and 30,000, respectively in the year 2012 (Figs. 7.2 and 7.3). China published large number of articles in 2012 and the USA being the second largest. European Patent Office (EPO) and United State Patent and Trademark Office (UPTO) have been registered worldwide 4994 and 35,081 patent respectively. The number of patents is continuously highest for USA during last 5 years followed by Germany, Japan, France and South Korea. The following sections explain the applications of the nanoparticles in agriculture and the associated risks to agroecology.

7.3 Nanotechnology Applications in Agriculture

Nanotechnology contribution in agriculture is increasing day by day to achieve higher and more stable yield of food grains based on optimizing water and nutrient supply. Application of nanomaterial in agriculture getting a wide space because of its positive response in food production. In agriculture two types of nanomaterials are mostly used: (1) carbon based single and multi-walled carbon nanotubes, (2) metal based aluminium, gold, zinc, and metal oxide based ZnO, TiO_2, and Al_2O_3. Single and multi-walled carbon nanotubes are used as nanosensors and plant regulator to enhance plant growth (Khodakovskaya et al. 2012). Nanosilver is used in
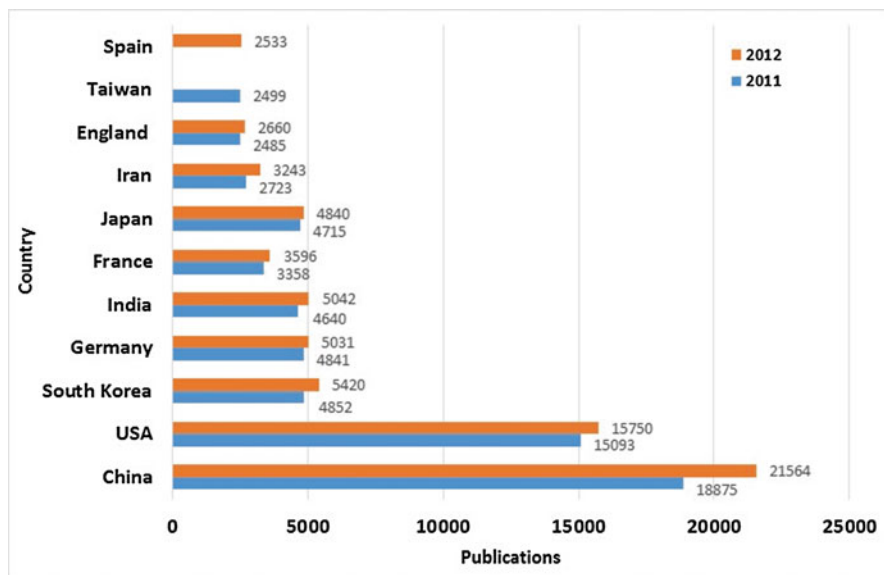
Fig. 7.2 Research publications on nanotechnology related fields during the years 2011 and 2012
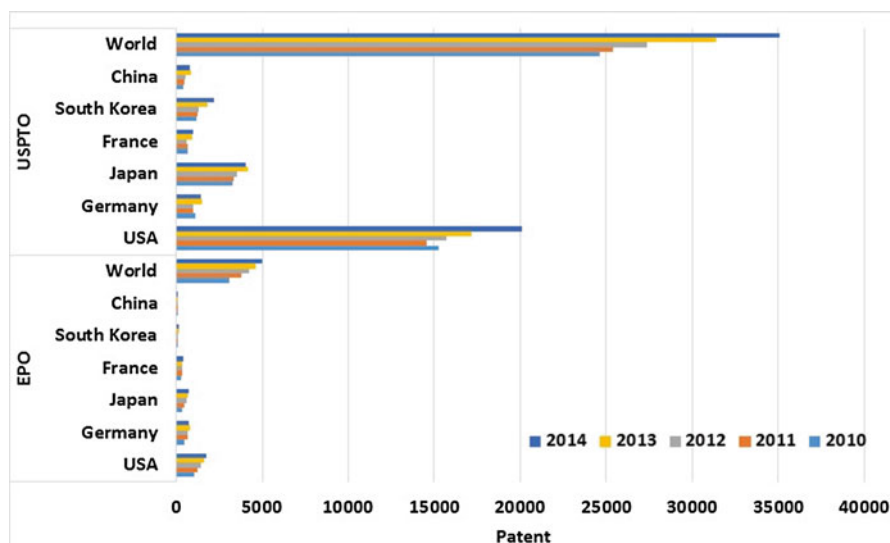
Fig. 7.3 Nanotechnology related patents at the United State Patent and Trademark Office (UPTO) and European Patent Office (EPO) (Source: http://www.statnano.com/)

packaging food material for killing bacteria from stored food (Food Safety Authority FSA of Ireland 2008). Nanosilica is used in filtration of food and beverages and packaging. Metal oxides like ZnO, TiO_2, and Al2O3 are used in nanofertilizers to boost the crop growth (Gogos et al. 2012; Sabir et al. 2014). The applications of nanomaterial as fertilizers, pesticides, sensors have been described in the following sections.

7.3.1 Crop Production

Nanomaterials like TiO_2, multi walled carbon nanotubes and ZnO are reported to be increased crop growth and quality of crop. It is also found that some nanomaterials could absorb water and nutrient more than their bulk size, it helps to enhance vigor of root system and breakdown activity of organic substance (Harrison 1996). Carbon nanotubes have the ability to augment germination and plant growth. Khodakovskaya et al. (2012) found that multi walled carbon nanotubes have potential to increase the growth of tobacco cell culture by 55–64 %. The interaction of nanoparticles with plant cell, modify the plant gene expression and biological pathways, which affect the plant growth and development (Nair et al. 2010). The carbon nanoparticles help to enhance photosynthesis process and transform plant leaves into biochemical sensors. Single walled carbon nanotubes able to monitoring of nitric oxide using near-infrared fluorescence, this function convert plat leave to a photonic chemical sensor (Giraldo et al 2014). To find the phytotoxicity of alumina nanoparticles on corn, cucumber, soybean, cabbage, and carrot, Yang and Watts (2005)) investigatedthat uncoated alumina particles reduce root elongation and the surface characteristics of the nanoparticles are very important for phytotoxicity of alumina nanoparticles. The effect of different types of nanoparticles on the growth of different crops is presented in Table 7.1.

To increase the growth of plant and control disease, huge amount of chemical fertilizers and pesticide are being used. About 90 % of the fertilizer applied is being wasted through runoff and other processes and causes downstream surface and ground water pollution. Nano fertilizers are more environmental friendly and more effective with little amounts. Kottegoda et al. (2011) used modified hydroxyapatite nanoparticles coated urea fertilizer, encapsulated into cavities of soft wood '*Gliricidiasepium*' and found that nanoferilizer releases nitrogen slowly and uniformly upto 60 days compared to commercial fertilizer which losses the fertilizer upto 30 days with uneven release rate. Milani et al. (2012) compared solubility and dissolution kinetics of nano and bulk ZnO coated monoammonium phosphate and urea fertilizers. They found that coated monoammonium phosphate granules with nano ZnO showed slow release of Zn and more solubility in sand columns and help to improve Zn use efficiency of plant. Nano sulphur coated urea fertilizer was mostly used to control nutrient release where soil is low in sulphur (Wilson et al. 2008).

The growth of crop depend upon concentration of nanomaterial used. Zheng et al. (2005) found that photosynthesis rate of spinach was increased by 3.13 times

Table 7.1 Effect of different types of nanoparticles and their size on the crop growth

NPs	Size (nm)	Crop	Effect	Reference
Titanium dioxide (TiO₂)	N.A.[a]	Spinach (*Spinaciaoleracea*)	Photosynthesis rate (~3 times), chlorophyll-a (~45 %) and chlorophyll-b (~28 %) was increased	Zheng et al. (2005)
MWCNTs	N.A.[a]	Tomato (*Solanumlycopersicum*)	Water absorption of seed was increased by 58 % and germination was increased by 90 %	Khodakovskaya et al. (2009)
Iron (Fe) and Copper (Cu)	7.5–20.5	Potato (*Solanumtuberosum*)	Weight of sprouts was increased by 50 % with Fe and it was not significant with Cu	Chalenkoa et al. (2010)
MWCNTs	~30	Mustard (*Brassica juncea*)	Seed germination was increased by 99 %	Mondal et al. (2011)
Carbon nanotubes (CNTs)	10–30	Gram (*Cicerarietinum*)	Water absorption was increased by 50 % through Xylem	Tripathi et al. (2011)
Zinc Oxide (ZnO)	~25	Peanut (*Arachishypogaea*)	Crop yield was increased by 25–30 %	Prasad et al. (2012)
ZnO, FeO, and ZnFeCu-oxide	20 (ZnO) 100 (FeO) and 40 (ZnFeCu)	Mung (*Vignaradiata*)	Root and shoot biomass ware increased by 40 % and 44 % with ZnO, 68 % and 48 % with FeO, 42 % and 84 % with ZnFeCu, respectively	Dhoke et.al. (2013)
Hydroxyapatite (Ca₅(PO₄)₃(OH))	<200	Rice (*Oryzasativa*)	Sugar level in rice straw and rice husk was reduced by 21–41 %	Dutta et.al. (2014)

[a]N.A. is not available

at 2.5 % TiO_2, and decreased beyond 4 % of TiO_2 concentration. Metal oxides like TiO_2, FeO and ZnO can be apply directly by foliar spray because it able to penetrate directly from pore spaces of leaves of plant and affect the growth, but the maximum results was shown at 50 ppm of ZnFeCu-oxide, FeO oxide and 20 ppm of ZnO concentration (Dhoke et.al. 2013). Multiwalled carbon nanotubes penetrate in seed coat and affect the biological activity mostly increased the water uptake inside the seed. But mechanism of water uptake by nanoparticle inside the tomato seed is yet not clearly understand by the researcher but in gram seed water uptake through xylem by capillary motion and increase growth of every part of plat (Khodakovskaya et al. 2009; Tripathi et al. 2011). Therefore, it is observed that carbon walled, metal and metal oxide based nanofertilizers successfully helped in increasing germination, photosynthesis, nutrient use efficiency and plant growth in the laboratory studies. However, systematic and rigorous experimentation is essential to study the nanofertilizers effect at the field scale.

7.3.2   Crop Protection

Fabrication and characterization of nanomaterials have the advantage to know the mechanism and interaction between plant and pathogen. It helps researcher to establish a relation between plant cells and plant pathogen fungi like F. oxysporum, C. lunata, A. alternata, and P. destructiva. Nanoscale material help to reduce degradation of pesticide and fungicide and increase the effectiveness of application with reduce amount. Application of nanoparticles and nanocapsules in pesticides and fertilizers distribute it in a control manner and reduce plant damage (Nair et al. 2010). Cucurbits family is very sensitive for powdery mildew disease; nano silver (100 ppm) inhibits the growth of fungal hyphae and germination of conidia (Lamsal et al. 2011). Table 7.2 describes the use of nanoparticles for protection of different crops.

The metal oxide nanomaterials like ZnO, TiO_2, Cu and SiO_2 are increasing their presence in pesticide and fungicide to protect plant from bacterial disease and control microbial activity. ZnO nanoparticle inhibit the growth of human pathogen like Escherichia coli, Listeria monocytogenes and plant pathogen like Botrytis cinerea, Penicillium expansum, and Botrytis cinerea by its antifungal and antibacterial activity, affecting the cellular function of fungi. Nanoparticles inhibit the development of conidiophores and conidia also called mitospores of fungi which causes the death of fungus hyphae. Hyphae is the main root of vegetative growth of fungi (He et al. 2011; Kairyte et al. 2013). TiO_2 photocatalysis technique is more effective to control litchi fungal disease than conventional fungicide (Lu et al. 2006). Silica nanoparticle use in drug and DNA delivery in animal cells and tissue, but its use in plant is limited because of cell wall present in plant that restrict delivery system. The current research by Torney et al. (2007) shows that mesoporous silica with end cap of nano gold particle can be used as gene gun to deliver chemicals, protein and necessary nutrient directly into the plant in control condition.

Table 7.2 Effect of different types of nanoparticles for protection of various crops

NPs	Size (nm)	Crop	Application type	Effect	Reference
TiO$_2$	32	Lychee (*Litchi chinensis*), maize (*Zea mays*), and Rice (*Oryzasativa*)	Fungicide	Litchi downy blight, rice blast and maize southern leaf spot were reduced by 80 %, 38 % and 67 %, respectively	Lu et al. (2006)
Silica (SiO$_2$)	100–200	Tobacco (*Nicotianatabacum L.*)	Gene gun	Successfully delivered Genetic material (DNA), and chemical to plant cell	Torney et al. (2007)
Imidacloprid (C9H10ClN5O2)	30	Rice (*Oryzasativa*)	Insecticide	About 95 % effective than conventional imidecloprid on Martianusdermestoides insect	Guan et al. (2008)
ZnO	70–80	N.A.	Fungicide	Growth of Botrytis cinerea and Penicilliumexpansum was reduced by 63–80 % and 61–91 %, respectively	He et al. (2011)
	~200	Gram (*Cicerarietinum*)	Fungicide	About 58 % of colony growth was inhibited with a concentration of 5×10^{-3} Moles	Kairyte et al. (2013)
Cu	3–10	N.A.	Fungicide	The activity was superior to bavistin (commercial fungicide) against plant pathogenic fungi *F.oxysporum*, *C.lunata*, *A.alternata*, and *P. destructiva*	Kanhed et al. (2014)

7.3.3 Detection

Nanosensors are powerful tools to detect nutrient deficiency, toxicity, disease of plants and animals, also control health of plant, food quality and safety. It helps to improve agriculture production with increasing efficiency of input such as minimum loss of input like irrigation, fertilizer and pesticide. Mainly two types of nanosensors are being used in agriculture: (i) bio-nanosensors and (ii) electrical-nanosensors. Biological organism has sense to identify the environmental condition; combination of biology and nanoparticles into sensors has potential to increase sensitivity and could reduce the response-time to sense a potential problem (Scott and Chen 2012). Several biosensors are developed for accurate detection of toxicity of microcystins, which are produced by cynobacteria and threat to agriculture and animal's health (Singh et al. 2012). Table 7.3 shows the various nanosensors used for agriculture safety.

Wireless nanosensors give the precise time based information including pesticide detection in food material and environment, quality control, and environmental condition. Salicylic acid is a phenolic phytohormone present in plant, help to improve plant growth, photosynthesis and transpiration. It is an important part of plant and sensitively need to detect level of salicylic acid in plant. Wang et al. (2010) use electrical nanosensor with gold electrode modified with copper nanoparticle. Copper nanoparticles sense the electrocatalytic oxidation of salicylic acid and detect the electrochemical behaviour of salicylic acid. Nano gold electrode with copper nanoparticle accurately detect salicylic acid levels in oilseed rape infected with the fungal pathogen sclerotinia sclerotiorum (Wang et al. 2010). Electrochemical sensor with carbon nanotube electrode modified with deposition of gold nanoparticle used to detect triazophos insecticide present in postharvest vegetables (Li et al. 2012). Gold and silver nanoparticle also used in biosensor to detect level of organophorous pesticide in environment and postharvest food (Simonian et al. 2005; Wu et al. 2011). Surface enhanced Raman scattering (SERS) spectrum used in analytical chemistry and also used in agriculture to detect pesticide in food and environment. In a new study fabricated silver nanoparticle monolayer used to enhance sensitivity for Raman detection and help to detect concentration of methylparathion (Zhang 2013).

7.4 Agroecological Risks

Application of nanomaterials in agriculture is not always effective. It has number of negative effects on soil, plant, and aquatic life and most importantly human because of long food chain and easy motion of nanoparticles. Study of behaviour of nanoparticles at different size with different concentration in soil, plant and water are necessary to understand the agroecological toxicity.

Table 7.3 Application of nanosensors in agricultural safety

NPs	Size (nm)	Sensor type	Target	Detection limit	Reference
Gold (Au)	N.A.	Biosensor	Neurotoxic organophorous pesticide in environment	Not available	Simonian et al. (2005)
Liposome (enzyme)	300	Biosensor	Organophorous pesticide	10^{-6} mole	Vamvakaki and Chaniotakis (2007)
Calcium carbonate-chitosan ($CaCO_3$-chi)	N.A.	Biosensor	Methyl parathion pesticide	1 ng mL^{-1}	Gong et al. (2009)
Cu and Au	<100	Electrochemical	Fungal pathogen *Sclerotiniasclerotiorum*in oilseed rape	0.1 μM	Wang et al. (2010)
Au	4–7	Biosensor	Organophorous pesticide and immobilization of protein in food	0.8 pg/mL	Wu et al. (2011)
Au coated Carbon-nanotube (CNT)	30–60	Electrochemical biosensor	Triazophos in vegetables and food sample	9.3×10^{-8} M	Li et al. (2012)
Iron oxide (Fe_3O_4) coated CNT	N.A.	Biosensor	Organochlorine and organophosphate insecticide in food and environment	0.1 nmol L^{-1}	Chauhan and Pundir (2012)
Silver (Ag)	<50	Raman detector	Monolayer film to detect pesticide	10^{-7} M	Zhang (2013)
Cu	N.A.	Biosensor	Propineb fungicide detection in river and sea water	1 μM	Abbacia et al. (2014)
Ag	~16	Electrochemical	Organophorous pesticide, and dipterexfungiside	0.18 ng mL^{-1}	Li et al. (2014)

Table 7.4 Adverse effects of nanoparticles on soil health

NPs	Size (nm)	Effect	Reference
C_{60} fullerene	50	Fast growing bacteria and protozoa were reduced by 20–30 %	Johansen et al. (2008)
Ag, CeO_2 and TiO_2	7–45	Growth (9–21 %), fertility (11–28 %) and survival (20–30 %) of Caenorhabditiselegans (species of nematode) was reduced	Roh et al. (2009, 2010)
TiO_2 and ZnO	10–20	Traces of ZnO (~50 µg g^{-1} weight) and TiO_2 (~32 µg g^{-1} weight) were found inside the earthworm	Hu et al. (2010)
ZnO, Zn and Zn^{2+}	50	Soil enzymes (dehydrogenase, phosphatise, and β-glucosidase) were reduced by 17–80 %	Kim et al. (2011)
Ag	9–21	The activity of nitrifying bacteria was reduced by 50 %	Okkyoung and Zhiqiang (2008)
	10	Culturability of beneficial soil bacterium Pseudomonas chlororaphis O6 was reduced	Calder et al. (2012)
Zero-valent iron (nZVI)	20–100	Mortality of eiseniafetida and lumbricusrubellus species of earthworm was 100 % at 750 mg/kg	Temsah and Joner (2012)
CeO_2, Fe_3O_4 and SnO_2	50–105 (CeO_2), 20–30 (Fe_3O_4) and 61(SnO_2)	Microbial stress was noticed	Antisari et al. (2013)
Cr_2O_3, CuO, Ni, and ZnO	<100	The activity of Enzyme (60 %), dehydrogenase (~75 %), and urease (44 %) was reduced	Josko et al. (2014)

7.4.1 Soil

Soil is prima facie receiver of fertilizers with nano-particles. There is harmful chemical reactions and contamination by these nanoparticles to soil ecosystem and change in soil structure due to their large surface area and Brownian motion. Nanoparticles used through fertilizers could be harmful to soil biota and fertility (Ranallo 2013). They affect microbes, micro fauna of soil and digestive system of earthworm. The properties of nanoparticles may change the structure of soil and default to detect contamination due to nanoparticles in soil and environment (Du et al. 2011; Mura et al. 2013). Table 7.4 shows the adverse effects of nanoparticles on soil health.

The potential harmful effects of nanoparticles Ag, TiO_2, ZnO, CeO_2, Fe_3O_4 include reduction in growth, fertility, survival and increase mortality of earth worm and soil bacteria. Size is the main factor for ecotoxicity. To find out the relationship between size and toxicity Roh et al. (2010) was started their investigation with TiO_2

and CeO_2 nanoparticle on Caenorhabditis elegans, it is a free-living, transparent nematode, about 1 mm in length, that lives in temperate soil environments. They found that smaller size of TiO_2 (7 nm) and CeO_2 (15 nm) nanoparticles are seems more toxic compared to larger size (TiO_2 of 20 nm and CeO_2 of 45 nm). If doses increased from certain amount ZnO nanoparticle become toxic for soil. Hu et al. (2010) were increase amount of ZnO from 1 g/kg of soil to 5 g/kg, ZnO nanoparticles were bioaccumulated inside the earthworm and causes DNA damage.

7.4.2 Plant

Toxicity of nanoparticles depends upon various factors like plant species, size and concentration of nanoparticles in different stages of crop. Toxic effect of nanoparticles also depends upon their composition and size. Small sized nanoparticles are more reactive and toxic compared to large sized and affect the respiration or photosynthesis process (Navarro et al. 2008). Hund-Rinke and Simon (2006) worked on different size of photocatalytic active TiO_2 nanoparticles and its ecotoxic effect on algae (EC50: 44 mg/L) and daphnids with maximum concentration of 50 mg/L and found that ecotoxicity of nanomaterials depend upon nature of particles. Toxicity found in algae is more than daphnids. Daohui and Xing (2007)) worked on phytotoxicity of nanomaterials. They used MWCNT, Al, Al_2O_3, Zn, and ZnO in their experiment on radish, rape, rye-grass, lettuce, corn, and cucumber and found that seed germination of corn and rye-grass are affected by nano scale ZnO and Zn, respectively. Al_2O_3 nanoparticles showed phytotoxicity only on corn, reduced the root elongation by 35 %. Al improved root growth of rape and radish and inhibited root elongation of rye-grass and lettuce but had no effect on cucumber. Some of the toxicological studies on the effect of nanomaterials are presented in Table 7.5.

The level of toxicity in plants due to nano-particles is in direct relation with size and nature of the particles. ZnO nanoparticles easily dissolve in soil and uptake by plant and TiO_2 nanoparticles accumulate in soil and retain for log time and stick with the cell wall of wheat plant. Both are reduced the biomass of wheat crop (Du et al. 2011). Phytotoxicity was studied by Mazumdar and Ahmed (2011) on rice crop. They found that silver nanoparticle accumulated inside the root cell and damage the cell wall during penetration of particles due to complex mechanism and small size of particles, it was damaged the external and internal portion of cell wall. The other factor for plant toxicity is the concentration of nanoparticle because a nanoparticle of same size in different concentration change its chemical properties. ZnO nanoparticle shows great toxicity in different concentration. Boonyanitipong et al. (2011) investigate that ZnO start showing adverse effect on rice plant from 100 mg/L and fully inhabit root growth and biomass at 500–1000 mg/L concentration.

Table 7.5 Toxicological effect of nanoparticles on plant

NPs	Size (nm)	Crop	Adverse effect	Reference
TiO₂, and ZnO	20–100 (TiO₂) and 40–50 (ZnO)	Wheat (*Triticumaestivum*)	Wheat biomass was reduced by 7.6 % due to TiO₂. No significant result due to ZnO	Du et al. (2011)
Zno and TiO₂	N.A.	Rice (*Oryzasativa L.*)	75 % reduction in root as concentration of ZnO increased from 10 to 1000 mg/L. No significant reduction with TiO₂	Boonyanitipong et al. (2011)
TiO₂	<100	Corn (*Zea mays*)	Aberration index increased from 0.5 % to 2.5 % with control and 4 % concentration respectively. Inhibit root elongation by 34 %	Castiglione et al. (2011)
Au	25	Rice (*Oryzasativa L.*)	Damage of internal and external cell wall of root due to deposition of Au through xylem	Mazumdar and Ahmed (2011)
Aluminium oxide (Al₂O₃)	N.A.	Tobacco (*Nicotianatabacum*)	As concentration of Al₂O₃ increased as 0–1 %, the average root length, biomass per seedling, and germination rate significantly decreased as 93 %, 83 % and 2 %, respectively	Burklew et al. (2012)
ZnO and Fe-ZnO	18.4 (ZnO) and 13.4 (Fe-ZnO)	Green pea (*Pisumsativum L.*)	Chlorophyll and ROS (Reactive oxygen species) production were reduced by 27 % and 50 % respectively	Mukherjee et al. (2014)

7.4.3 Water

The nanoparticles can easily be released in water body or air and uptake by living organisms, create toxic effect for human, animals and also for aquatic life. TiO_2 reduced the light to entrap the algal cell and thus reduce the growth (Sharma 2009). The toxicity effect of Ag, Cu, AL, Ni, TiO_2 and Co nanomaterials on algal species, zebrafish, and daphnids revealed that Ag and Cu nanoparticles cause toxicity to all organisms (Griffitt et al. 2008) and the metal form are less toxic than soluble form of nanoparticles. Table 7.6 describe the aquatic toxicity of use of nanomaterials release in surface water body. It has been proved from different studies that nanoparticles like Ag, Cu, Al, Ni, TiO_2 and Causes unrecoverable toxic effect on aquatic ecosystem. Silver, iron oxide and copper nanoparticle adversely affected health of Zebrafish. It enhance mortality, hatching and reduce heartbeat and survival rate affected normal development (Asharani et al. 2008; Griffitt et al. 2007; Zhu et al. 2012). Therefore, the level of nanotoxicity in soil, plant and water mainly depend on the composition, size (<20 nm) and concentration (>100 ppm) of the nanoparticle.

7.4.4 Human Health

The rising field of nanotechnology has created an interest on health risk associated to nanoparticles. These particles create new challenge for researchers to understand and find risk associated with human health. Exposure of these materials occurs

Table 7.6 Adverse effects of nanoparticles on aquatic species

NPs	Size (nm)	Aquatic species	Effect	References
Fullerene (nC60)	10–200	Daphnia	Mortality was increased by 40 % and offspring production was reduced by 50 %	Oberdorster et al. (2006)
Cu	80	Zebrafish	NKA (Na/K atpase) activity was reduced by 88 %	Griffitt et al. (2007)
Ag	5–10	Zebrafish	Heartbeat (150–50 beat/min) was decreased from 150 to 50 beat/min and mortality rate was10 %	Asharani et al. (2008)
TiO_2	21	Rainbow trout	Glutathione level was reduced by 65 %	Federici et al. (2007)
	10–100	Marine phytoplankton	Toxic to the aquatic life in sunlight	Miller et al. (2012)
Ag	18	Freshwater fish Cyprinuscarpio	Mortality was 100 % at 1 ppm NP's concentration	Hedayati et al. (2012)
FeO	30	Zebrafish	About 75 % of fishes were killed at high concentration (50 mg L^{-1}) of NP	Zhu et al. (2012)

Table 7.7 Adverse effects of nanoparticles on human health

NPs	Size (nm)	Body part	Effect	Reference
MWCN and Carbon nano fibres (CNFs)	20 (MWCN) and 150 (CNFs)	In-vitro on lung tumour cells	MWCN and CNFs reduced the living cells by 33 % and 58 %, respectively	Magrez et al. (2006)
TiO$_2$, Ag, Al, Zn, and Nickel (Ni)	N.A.	Alveolar epithelial cells and apoptotic damage	Cell damage was observed in all cases	Park et al. (2007)
ZnO	30	Epidermal cells	Glutathione (51–59 %), catalase (55–64 %) and superoxide dismutase (72–75 %) were reduced	Sharma et al. (2009)
Ag	<10	Hepatoma cells	Cytotoxicity (oxidative stress) was noted	Kim et al. (2009)
CuO	<50	Lung epithelial cells A549	Cell viability was decreased by 40 %	Moschini et al. (2010)
TiO$_2$	1–200	Mammalian cell	Reactive oxygen species production, cytokines level, apoptosis and genotoxicity were increased and cell viability and proliferation were reduced	Iavicoli et al. (2011)
Cadmium Sulphide (CdS)	~3	Escherichia coli and HeLa cells	Oxidative stress in both Escherichia coli and HeLa cells. Reduced growth of E. Coli by 50 %	Hossain and Mukherjee (2013)
Ag	10–80	–	Cell viability was decreased by 20–40 %, Oxidative stress in cells	Nguyen et al. (2013)
Ag	10–50	Lung cell (via inhalation)	The Ag particles of size 10 nm were found more Cytotoxic than other size	Gliga et al. (2014)
Cu	23.5	Nerve cells and astrocyte cell	Central nervous system was damaged	Bai et al. (2014)

through inhalation, ingestion and dermal exposure during synthesis, manufacturing and application of these nanomaterials. Table 7.7 shows the adverse effects of nanomaterials on human health.

7.4.4.1   Inhalation

The most common way of exposure is inhalation of airborne nanoparticles. Greatest emission risk occurs in the manufacturing process with poor filtering and ventilation system (AFSSET 2006). Factors affecting inhaled dose are particle geometry

and physiochemical properties, lung morphology, respiration physiology, and environmental condition (Shade and Georgopoulos 2007). Nanoparticles deposit in respiratory traces after inhalation and increase the total deposition fraction (TDF) in the lungs with decreasing in particle sizes. Nanoparticles can also be uptaken in the brain through the olfactory epithelium (Borm et al. 2006; Jaques and Kim 2000). Ultrafine airborne particles may increase respiratory and cardiovascular morbidity and mortality (Shade and Georgopoulos 2007).

7.4.4.2   Ingestion

Ingestion is another source of entry of nanoparticles into human body. The nano particles entered through gastrointestinal tract directly through intentional ingestion or indirectly via water, food, animal food and fish (Bergin and Witzmann 2013). Mucociliary escalator can be excreted inhaled particles and ingested into the gastrointestinal tract, ingestion also depends upon physicochemical characteristics and size of particles (Hagens et al. 2007). Jani et al. (1990) found that particle size less or equal to 50 nm is more uptake or absorbed across gastrointestinal tract and can be passed to the liver, spleen, blood and bone marrow by the momentary lymph supply and nodes. Plants have more resistance to prevent translocation of nanoparticles than mammalian barriers (Birbaum et al. 2010).

7.4.4.3   Dermal Exposure

Dermal exposure is an import route to absorb nanoparticles via the skin. Skin contents approximately 10 % weight of body and plays an important role as barrier against environmental impurities with the protection, homeostasis maintaining, metabolism, synthesis, and deposition function (Crosera et al. 2009). Penetration of nanoparticles depends upon physicochemical characteristics of nanoparticles and medical condition of skin such as eczema, dermatitis, and skin irritation. Absorption between epidermis and dermis or permeability increase in damage skin (Nielsen et al. 2007). Dermal exposure of small size nanoparticles lower than 10 nm is more dangerous. This size of particles may cause erythema, oedema and eschar formation. Further larger size particles cannot penetrate into the skin from transappendageal routes (Gautam et al. 2011).

Nanoparticles adversely affect human health and the potential routing could be through inhalation, ingestion and dermal exposure. It is understood that the nanoparticles show significant health complications in human when exposed to the size of particles less than 50 nm.

7.5 Conclusion

Nanotechnology is in its beginning face and it provides enormous possibility to transform the way of agriculture and lure the microbiologists and other researchers to contribute to food safety with innovative green chemistry approaches. Nanotechnology can facilitate additional advantage in food processing, distribution and packaging and functional food, but it couldn't make its presence in large scale agricultural production. Academics and industrial patents are rapidly increasing in agro-chemical sector but the end products from this technology have not hit the market so far (Gogos et al. 2012; Parisi et al. 2014). After reviewing many articles related to nanotechnology, it is understand that the governments of the USA, Germany and Japan are more supportive in nanoresearch and the research publications and patents are largest for China and USA, respectively. The carbon walled, metal and metal oxide based nanofertilizers successfully helped in increasing germination, photosynthesis, nutrient use efficiency and plant growth. The metal oxide nanomaterials like ZnO, TiO_2, Cu and SiO_2 are increasing their presence in pesticide and fungicide to protect plant from bacterial disease and control microbial activity. Silver, copper and gold nanoparticles are being used as bio-nanosensors and electrical-nanosensors to detect a potential pathogen problems in plant and postharvest foods. The level of nanotoxicity in soil, plant and water mainly depend on the composition, size (< 20 nm) and concentration (>100 ppm) of the nanoparticles. Nanoparticles adversely affect human health and the potential routing could be through inhalation, ingestion and dermal exposure. It is understood that the nanoparticles show significant health complications in human when exposed to the size of particles less than 50 nm.

It is clear that nanotechnology has potential to increase production of agriculture, but there is very limited knowledge about its long term adverse effect on soil, plants and ultimately on human. It is required to study about the non-toxic limit of nanoparticles related to its size and concentration. The positive benefit of nanoparticles should be selected on the basis of their risk related to environment and human. An intelligent use of nanotechnology may help to achieve food security with the qualitative and sustainable environment.

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Chapter 8
Impact of Fertilizers and Pesticides on Soil Microflora in Agriculture

Pratibha Prashar and Shachi Shah

Abstract Soil health management is crucial for ensuring sustainable agricultural productions and maintenance of biodiversity. Fertilizers and pesticides are a necessary evil for industrial agriculture. Though, they continue to be critically important tools for global food security, their undesirable effects cannot be overlooked particularly when sustainable agriculture is the universal focus. Apart from a range of widely discussed and well-known adverse effects of chemical fertilizers and pesticides on environment and human health they have also been held responsible for strongly influencing the microbial properties of soil.

Soil microflora is a key component of agricultural ecosystems that not only plays a significant role in the basic soil processes but is also actively involved in enhancing soil fertility and crop productivity. Microbial activity in soil has a strong impact on its physical properties and at the same time it is also instrumental in pursuing eco-friendly practices like bioremediation and biocontrol of phytopathogens in agricultural soils. Soil microorganisms have thus been accepted as the bioindicators of soil health and activity.

Fertilizers and pesticides tend to have long persistence in the soil so they are bound to affect the soil micoflora thereby disturbing soil health. Amendment of soil with fertilizers and pesticides strongly influences a range of soil functions and properties like rhizodeposition, nutrient content of bulk and rhizospheric soil, soil organic carbon, pH, moisture, activities of soil enzymes and many others. All these factors indirectly lead to a shift in the population dynamics of soil microflora along with the direct effects of fertilizers and pesticides such as toxicity and altered substrate availability profile of the soil. Though such effects are variable depending on many biotic and abiotic factors ranging from soil characteristics to crop variety, still it has been well established that long term and excessive chemical inputs in soil

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undoubtedly influence the soil microbial communities in terms of their structural and functional diversity as well as the dominant soil species.

Here, we review the impact of long term usage of fertilizers and pesticides on the soil microflora of cultivated soils in relation to soil health and fertility, their persistence level in soil, factors affecting their toxicity and pesticide degradation.

Keywords Chemical pesticides and fertilizers • Sustainable agriculture • Soil microorganisms • Soil health

List of Abbreviations

ACC	1- aminocyclopropane-1-carboxylic acid
ARDRA	Amplified Ribosomal DNA Restriction Analysis
AWCD	Average Well Color Development
BOO	Bromoxynil Octanoate
CLCP	Community Level Catabolic Profiles
CLPP	Community Level Physiological Profiles
CRP	Catabolic Response Profiles
DDT	p, p-dichlorodiphenyltrichloroethane
DGGE	Denaturing Gradient Gel Electrophoresis
DHA	Dehydrogenase Activity
EPA	The United States Environmental Protection Agency
FAME	Fatty Acid Methyl Ester Analysis
FAO	The Food and Agriculture Organization of the United Nations
MBC	Microbial Biomass Carbon
MDS	Minimum Data Set
NPK	Nitrogen, Phosphorus and Potassium
PBT	Persistent Bioaccumulative and Toxic
PCR	Polymerase Chain Reaction
PLFA	Phospholoipid Fatty Acid Analysis
SOC	Soil Organic Carbon
WHO	World Health Organization

8.1 Introduction

Modern agriculture is wholly dependent on the chemicals in the form of pesticides and fertilizers. There is no denying to the fact that the much required improvement and stability in agricultural productions in the last century has largely been accomplished through the efficient control of pathogens and pests together with the adequate supply of requisite plant nutrients with the help of chemical pesticides and fertilizers only. However, currently we have reached a stage where issues such as

human and environmental health, maintenance of ecological balance and conservation of soil biodiversity also need attention at par with the goal of managing the rising food demands across the globe.

Soil microflora including bacteria, fungi, protozoa, algae and virus forms a vital component of agro-ecosystem and is responsible for many critical and fundamental soil functions such as nutrient-cycling, soil-fertility, improving plant productivity through enhanced availability of limited nutrients and decomposition of organic as well as inorganic matter. Physical soil properties such as its structure, porosity, aeration and water infiltration are also favorably affected by soil organisms through the formation and stabilization of soil aggregates (Zhong and Cai 2007). At the same time soil microbial community is instrumental in pursuing eco-friendly practices like detoxification (bioremediation) of soils contaminated with toxins and undesirable components due to human activities (Canet et al. 2001) as well as biocontrol of phytopathogens.

Apart from a range of undesirable effects on environment like the release of greenhouses gases due to N-fertilizers (Velthof et al. 1997), development of algal blooms in water bodies and development of resistance among pest, chemical fertilizers and pesticides have also been reported to strongly impact the soil biodiversity. Experimental evidences have established the fact that prolonged use of chemical fertilizers and pesticides affects the structural and functional properties of microbial communities in soil (Nicholson and Hirsch 1998; Yang et al. 2000; Bohme et al. 2005) and at the same time creates nutrient-imbalance in agricultural soils. Soil biodiversity along with other forms of agro- biodiversity i.e. plant and animal resources, is the backbone of global food security. Thus, if we wish to go ahead with the idea of sustainable agriculture it is essential to understand the link between soil biodiversity and soil functions as well as to access the effects of various anthropogenic activities on soil microbial diversity. In accordance with this, the evaluation of various effects of prolonged pesticides and fertilizers application on soil microflora of agricultural ecosystems is of critical significance.

8.2 Soil

Soil is a living, highly complex and dynamic ecosystem that harbors and support extremely rich diversity of micro and macro flora which in turn influence its properties. It primarily consists of inorganic mineral nutrients and organic matter along with huge numbers of living forms and maintains a balance between physical, chemical and biological factors (Doran and Safley 1997). Soil is the basis of agriculture and thus the universal food production. Apart from its most widely known role as a medium for plant growth soil performs many other vital functions such as mediating the exchange of gases, flow of energy, nutrients and water, detoxification of pollutants and many other (Larson and Pierce 1994). Hence, management of soil health is crucial for ensuring sustainable agricultural productions and maintenance of soil biodiversity including microbial diversity.

8.2.1 Soil Health

Soils are not only responsible for providing most of the food items consumed by mankind but are also vital in maintaining environmental quality at various levels (Glanz 1995). Hence, looking at the growing food requirements of the world it is very necessary to analyze and maintain soil health. The concept is even more important considering the development of sustainable agriculture. The term soil health has been defined as, "the capacity of the soil to function within an ecosystem and land use boundaries, to sustain biological productivity, maintain environmental quality, to promote plant and animal health and to support human health and habitation" (Doran and Parkin 1994). Another widely accepted definition of this term is, "continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water, and maintain plant, animal and human health" (Avidano et al. 2005). In a broader way it is the ability of soil to perform and function according to its potential (Doran and Safley 1997).

The terms soil health and soil quality are often used interchangeably in order to describe the capacity of soil to support plant growth while itself not undergoing degradation (Harris and Bezdicek 1994). While defining the term health, soil is treated as a living and dynamic system, thus Larson and Pierce (1991) have proposed the examination of certain basic indicators of system functions for assessment of soil health analogues to the examination of human health. Accordingly the concept of minimum data set (MDS) of soil parameters to be used in assessing the soil has been put forward. This includes physical parameters like texture, water holding capacity, chemical parameters like pH, salinity, soil organic matter content and biological parameters like microbial activity, mineralization of N and soil respiration to name some (Fig. 8.1) (Larson and Pierce 1991; Larson and Pierce 1994; Doran and Parkin 1994; Doran et al. 1996).

Ever since the beginning of practices of farming and cultivation, man has been highly instrumental in depleting the soil health in numerous ways. Extensive chemical inputs in the form of inorganic fertilizers and pesticides have turned out as one of the major causes of the same. Since biological properties of soil are influenced by the prevailing physical and chemical environment, it may be drawn that microbial activity and their functional diversity are important indicators of soil health. Thus, assessment of soil micrflora may be looked as a potential tool to provide vital insight into the health and functioning of soil.

8.2.2 Microflora of Agricultural Soils

Soil represents the black box of microbial diversity. It is the most diverse and favorable habitat for a variety of microorganisms including bacteria, fungi, protozoa, algae and virus. Cultivated soils are richer in terms of quantity and variety of
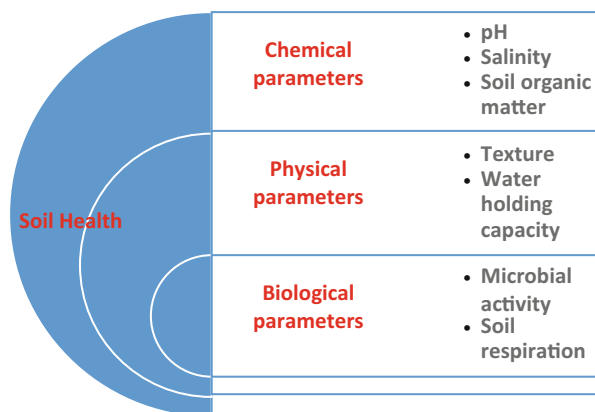
Fig. 8.1 Parameters of soil health. Complete assessment of soil health is based on three classes of soil parameters i.e. physical, chemical and biological. It may be seen that various parameters are influencing each other and thus soil microbial activity is affected by the physical and chemical health of the soil. SOM soil organic matter

microflora. Soil carries almost 10^4 microbial species per gram (Klug and Tiedje 1993) and according to a culture-independent study by Torsvik et al. (1996) there are about 6000 different bacterial genomes per gram of soil considering the genome size of *Escherichia coli* as a unit. However, on the basis of advanced analytical tools it has recently been shown that there can be as many as one million prokaryotic genomes per gram of soil (Gans et al. 2005; Handelsman and Tiedje 2007).

The microbial communities and food webs in soil are extremely complex and not fully understood. Though bacteria are generally the most abundant microbes in soil followed by actinomycetes, fungi, algae and protozoa in that order (Sylvia et al. 1998) (Fig. 8.2) variable patterns are observed in cultivated soils in terms of fungal-bacterial dominance. Microbial biomass in soil majorly consists of bacteria and fungi and it constitutes almost 1–4 % of total organic matter in soil (Brookes 2001). However, great variations in fungal/bacterial biomass ratios have been observed in arable soils and this has been established to be linked with the land management practices, nutrient content of soil, environmental factors as well as the methods used to determine the biomass content. As reviewed by de Vries et al. (2006) and Strickland and Rousk (2010) under conventional tillage system bacterial biomass is dominating whereas fungi dominate under no-tillage or untilled farming system. The explanation for this is based on the difference in structural features and growth forms of fungi and bacteria. Bacteria and actinomycetes better withstand the soil disturbances in tilled soil than the fungal populations which are not able to establish themselves easily in such conditions. Similarly, organic fertilization of soil results in higher fungal/bacterial biomass ratios while the reverse effect is observed with inorganic nutrient inputs. This establishes the fact that organic fertilization favorably affects the soil ecosystem and thus supports the concept of sustainable agriculture. Soil parameters like pH, moisture, temperature and $CO2$ levels also have
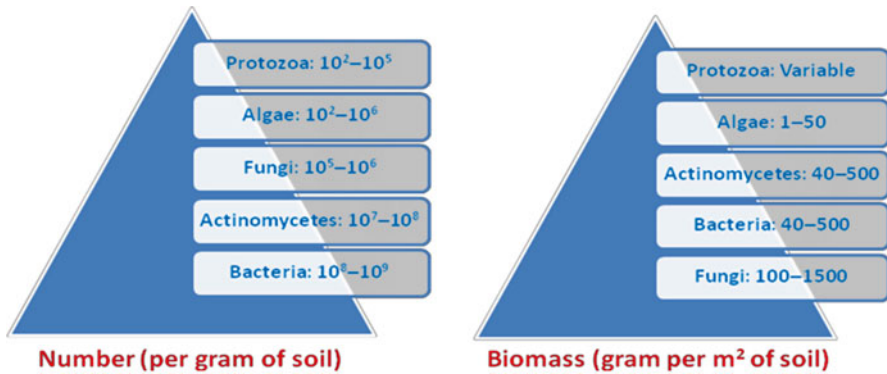
Fig. 8.2 Structural composition of soil microflora (0–15 cm depth of soil). In terms of numbers, bacteria are the most populous microbes in soil while fungi may contribute maximally towards the total microbial biomass in soil due to their large size and mycelial structure (Sylvia et al. 1998; Hoorman and Islam 2010). This however, is affected to a large extent by the soil management practices and other soil parameters ranging from nutrient content to physical conditions

variable effects on fungal/bacterial dominance in cultivated soils (Strickland and Rousk 2010). Protozoans are the most important grazers in soil which feed on bacteria, fungi and other small protozoans while they themselves serve as feed for higher organisms in soil i.e. meso and macrofauna (Griffiths et al. 2005). Thus, they affect the bacterial and fungal biomass as well their diversity in soil.

Though it has been well established that microbes form a crucial component of soil ecosystems and 80–90 % of the soil functions are mediated by microorganisms (Nannipieri and Badalucco 2003) (Fig. 8.3), till recent past most of the biodiversity studies have been focused on plant and animal resources only while microbial ecology of agricultural soils has got little attention. A possible explanation of the same may be based on the complicacies involved in the accurate estimation of soil microflora and requirement of specific techniques, different from those used for estimating macroflora. In spite of the fact that microorganisms are amongst the most diverse and large group of organisms that constitute about 60 % of the earth's biomass (Singh et al. 2009) majority of them are non-culturable and they generally have complex interactions with soil particles which adversely affect the soil sampling processes (Stotzky 1985). Moreover, though the concentration of microorganisms per gram of soil is much higher than those of other organisms in the same ecosystem but the cultivable fraction of the total number of prokaryotic species present is generally less than 1 % (Rastogi and Sani 2011). In addition to all the above stated factors another critical factor is that microbes have an indirect contribution in agricultural productions. Thus, it has served as an inhibition for the agronomists, ecologists and soil scientists to focus their research towards microbial ecology of crops.

However, in the wake of world-wide hunt for sustainable tools for agricultural practices and the recent global initiatives towards conservation and maintenance of
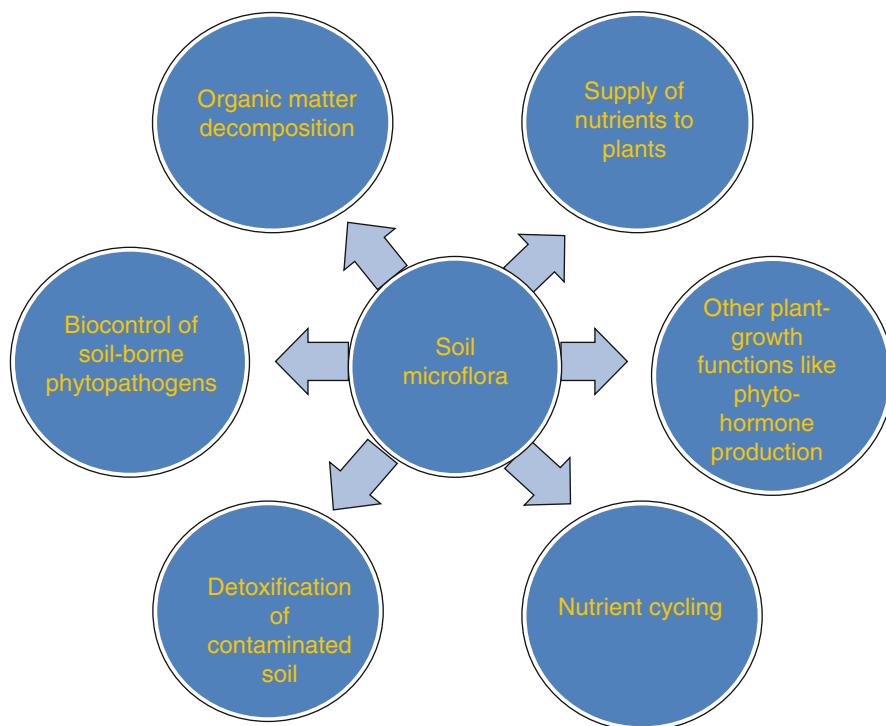
Fig. 8.3 Functions of soil microflora. Bacteria and fungi are the major decomposers of organic matter in soil (van Veen and Kuikman 1990) thus regulating carbon cycling. Soil microbes transform mineral nutrients in soil like phosphate, zinc into plant-available forms and provide other nutrients like nitrogen through symbiotic and non-symbiotic fixation processes (Glick 1995). A number of other plant growth promoting activities are carried out by soil microbes such as production of phytohormones (Ahmad et al. 2008) and ACC deaminase (Belimov et al. 2001). Inhibition of soil-borne plant pathogens through secretion of antibiotics; extracellular lytic enzymes; parasitism; competition (Prashar et al. 2013) and bioremediation of contaminated sites (inorganic or organic contaminants) in soil (Bollag et al. 1994) are the two other critical processes mediated by soil microflora. Microorganisms form a vital part of complex food webs in soil at various levels such as decomposers; parasites; saprophytes; pathogens and thus mediate the cycling of nutrients in a critical manner. ACC: 1- aminocyclopropane-1-carboxylic acid

biodiversity, microbial flora has finally received its long due attention. This has been further supported by the recent development of advanced molecular tools for detection, enumeration and characterization of soil microorganisms without cultivation. In the last two decades, a large number of studies have been reported for the assessment of structural and functional diversity of microbes in soils using culture independent methods like polymerase chain reaction (PCR) based techniques including amplified ribosomal DNA restriction analysis (ARDRA); denaturing gradient gel electrophoresis (DGGE) (Liu et al. 1997; Berg 2000; Yang et al. 2003), phospholoipid fatty acid (PLFA) analysis and catabolic response profiles (CRP) (Romaniuk et al. 2011), fatty acid methyl ester (FAME) analysis (Kozdroj and van Elsas 2001).

Such efforts have accelerated the pace for the study of unculturable microflora of soil. However, till few years back the focus was more on exploring the bacterial diversities (Borneman et al. 1996; Yang et al. 2003) but the trend is now shifting to fungal populations as well which was earlier limited (Li et al. 2008; Jumpponen et al. 2010; Orgiazzi et al. 2012). Agriculturally important microorganisms have thus been the focus of research in recent past and studies concentrating on the impact of farming practices on soil microbial diversity have gained momentum.

Microbial diversity of soil denotes the entire range of microbes residing in all the macro and micro habitats existing in soil ecosystem. It encompasses the diversity between species as well as within species originating from the genetic variations, evolutionary and ecological adaptations of species, interactions with biotic and abiotic factors and complexities of habitats. Genetic diversity of microbes has been defined as the amount and distribution of genetic information within microbial species and in a simpler manner it may be viewed in terms of richness and evenness of soil microflora (Nannipieri et al. 2003).

Shifts in microbial activity and diversity have been reported due to a number of biotic and abiotic factors including soil management practices like monotype cultivation, nutrient amendment either as organic manures or inorganic fertilizers, land use practices and environmental factors (Sun et al. 2004; Li et al. 2007; Nautiyal et al. 2010). Though there are clear evidences that chemical fertilizers and pesticides affect the soil microflora, still to a large extent a variable pattern has been observed in the limited amount of available literature. Pesticides and fertilizers can have short or long-term effects on the soil microflora brought about directly by their action on the organisms and indirectly due to undesirable changes in the environment (Seymour 2005).

Thus, it may be concluded that in light of critical role played by soil microflora in the ecological soil functions including the detoxification reactions, assessment of the structural and functional characteristics of microbial populations may be used to monitor the impact of chemical fertilizers and pesticides on soil ecosystems.

8.3 Fertilizers

Plants require 16 essential elements for their normal growth and yield, out of which 13 are provided by soil. Nitrogen, phosphorus and potassium are referred as primary nutrients because they are required by the plants in highest quantities (Hodges 1995). Continuous crop cultivation leads to depletion of these nutrient reserves in the soil and thus they need to be regularly replenished in order to maintain their optimal supply for the crops. The most common mode adopted by man for supplying the nutrients in cultivated soils has been the use of chemical fertilizers, primarily nitrogen (N), phosphorus (P) and potassium (K) fertilizers.

Fertilizer has been defined by soil science society of America as "any organic or inorganic material of natural or synthetic origin, other than liming materials that is added to soil to supply one or more plant nutrients essential to the growth of plants".

In accordance with the rising food productions, chemical fertilizer supply has been continuously increasing with time. Global fertilizer consumption of arable and permanent crop area has increased from 79.29 tones/1000 Ha in 2002 to 98.20 tones/1000 Ha in 2010 and the demand for total fertilizer nutrients has been estimated to rise further at 1.9 % per annum from 2012 to 2016. China and India are the world's leading consumers of chemical fertilizers (N, P, K) while highest production of the same is reported in China, USA and India in that order (FAO 2012). So, fertilizers may be seen as an indispensable part of modern agriculture. The effects of chemical fertilizers on soil properties and microflora have been discussed in the following paragraphs.

8.3.1 Effects of Fertilizers on Soil Properties

Long-term application of nitrogen-phosphorus-potassium (NPK) based fertilizers has a pronounced effect on the biochemical properties of soil which in turn leads to shift in microbial populations. Changes in soil organic carbon (SOC), nitrogen (N) content, pH, moisture and thus the variation in nutrient availability to microbes have been observed due to long-term fertilizer use in a variety of crops like wheat, corn and others (Bunemann and McNeill 2004; Bohme et al. 2005; Wu et al. 2012). In contrast to chemical inputs organic amendments in soil have been proven to favourably affect various soil properties and functions. For example organic inputs tend to enhance SOC and N content more significantly than chemical fertilizers and thus lead to higher microbial populations. Sradnick et al. (2013) established the variation in soil pH and SOC content due to fertilization as the basis of difference in the catabolic profiles of soil microorganisms of a sandy soil that had received long-term mineral fertilizer and cattle manure treatments. On the basis of community level physiological profile it was found that functional diversity of soil microorganisms was higher in manure treated soil as compared to mineral fertilized soil.

Activities of soil enzymes like dehydrogenase, β-glucosidases, alkaline phosphatases and proteases are important indicators of soil fertility and microbial activity (Casida et al. 1964; Nannipieri et al. 1990). Evidences are there that long-term application of organic manure enhances the dehydrogenase activity (DHA) as well as microbial biomass while NPK fertilizers do not have a positive influence on this. Further, it has been observed that copper which is a normally found contaminant in soil as a result of irrigation or application of fertilizers and pesticides, adversely affects the soil dehydrogenase activity and this effect is more pronounced in NPK treated soils as compared to organic-manure treated soils (Xie et al. 2009b). In contrast to this, application of microbial fertilizer based on *Azotobacter chroococcum* has been reported to increase the dehydrogenase activity and favourably alter the bacterial and fungal community diversity in the rhizosphere of wheat (Shengnan et al. 2011). Other soil enzymes like β-glucosidases, alkaline phosphatases and proteases have also been found to be positively affected in organically treated soils as compared to treatments with inorganic fertilizers (Bohme et al. 2005). Lazcano

et al. (2013) observed enhanced enzyme activities for β-glucosidases, phospho-monoesterase and proteases in sweet- corn cultivated soil when treated with organic manure as compared to inorganic fertilization.

Similarly, the activities of other soil enzymes like urease and saccharase have been indicated to be stimulated by organic N application as compared with mineral fertilizing. Significantly lower enzyme activities were observed in inorganically fertilized soil than in organically fertilized soils cyclically cultivated with wheat, spring cereals and clover (Balezentiene and Klimas 2009). In some cases though soil pH and activities of enzymes like dehydrogenase, catalase, invertase, urease, caseine-protease and arylsulphatase are positively affected in organically treated soils, no significant difference is observed in microbial functional activity of organically and inorganically treated soils (Lopes et al. 2011). Thus, it may be concluded that addition of NPK fertilizers generally tend to decrease the activities of most soil enzymes and also bring about undesirable changes in SOC and N concentrations.

8.3.2   Effects of Fertilizers on Soil Microflora

Since fertilizers are meant to increase the nutrient content of the soil in order to improve the crop productivity they are bound to increase the SOC as a result of enhanced root turnover, rhizodeposition and crop residue fall thereby boosting microbial activity. It has been well established that functional diversity of the soil microbial community is primarily governed by the resource (N, P and C) availability (Cruz et al. 2009; Liu et al. 2010b; Yang et al. 2011; Lupwayi et al. 2012). Thus, a significant co-relation exists between SOC and microbial populations as well as microbial activities (Bohme et al. 2005). This directly indicates that the class and composition of fertilizer applied will certainly affect the microbial community structure of the cultivated lands. However, when compared with organic amending materials, inorganic fertilizers lag behind in this feature. Though total microbial counts tend to be higher in fertilized soils in comparison to untreated soils but the effect is more pronounced in organic-compost amended soils than those treated with chemical fertilizers for long periods (Islam et al. 2009). Many studies have reported significantly higher increase in organic carbon content, microbial populations and activities in soils treated with organic manure as compared to the ones treated with inorganic fertilizers in crops like mustard, wheat, tobacco and maize-wheat rotation (Kumar et al. 2000; Kang et al. 2005; Yang et al. 2011; Chauhan et al. 2011). Further, it has been observed that bacterial community structure of organic manure treated soils are more closely related to the structure of the untreated soil than that of soils treated with inorganic NPK fertilizers for long periods of time (Sun et al. 2004) and at the same time are more evenly distributed. Moreover, the population of gram-negative bacteria which includes many plant-friendly groups like *Pseudomonas* gets adversely affected by long term application of chemical fertilizers while organic amendments results in set-up of bacterial populations more closely resembling to that of untreated soils in crops like rice and wheat (Islam et al. 2009; Wu et al. 2012).

Another important aspect of organic fertilization is reduced bioavailability of pollutants like heavy metals and pesticides in soils. Organic manures along with increasing the organic matter content in soil also tend to form complexes with such pollutants and thus decrease their bioavailability (Perez-de-Mora and Madrid 2007). The toxicity effect of heavy metals and pesticides like copper, cadmium and cypermethrin on soil microflora was examined by Xie et al. (2009b) and it was found that sensitivity of microorganisms to these pollutants was higher in soils treated with inorganic fertilizers as compared to organic-manure treated soils. Moreover, lower dissipation rate of cypermethrin was recorded in fertilizer treated soils. This establishes that inorganically treated soils exhibit more pronounced effects of contaminants like heavy- metals in contrast to organically treated soils.

Higher and functionally more diversified microbial populations have been observed in agricultural ecosystems amended with organic inputs in comparison to those having long-term treatments with inorganic fertilizers in a variety of crops (Chauhan et al. 2011; Tan et al. 2012; Sradnick et al. 2013). Moreover, fertilization regimes have pronounced effects on the community structure of total bacteria of agricultural soils. Wu et al. (2012) recorded a shift in structural diversity and the dominant bacterial groups of agricultural soils due to long-term treatment with inorganic fertilizers of different types like N, NP or organic manures as well as different growing stages of the crop.

Another aspect of chemical fertilization is that it leads to generation of nutrient channels or patches thus creating nutrient gradients in the soil that affects the microbial populations. Li et al. (2013) studied the effect of N-gradient created by chemical fertilizers like ammonium sulfate or urea on nitrogen transformation, soil microbial biomass and microbial functional diversity. Changes were observed in soil microbial biomass as well as microbial functional diversity with the N-gradient. However, the extent of changes was governed by the nitrogen concentration and the form of inorganic fertilizer. While the average well color development (AWCD) and functional diversity indices of the microbial communities were lower after application of ammonium sulfate, urea application resulted in higher AWCD and Shannon indices. These were also observed to vary with the depth of soil layers.

The effects of soil management practices primarily in the form of fertilization may also vary with crop. As discussed above, many authors have reported an increase in soil microbial biomass activity and microbial functional diversity as a result of organic treatment of soils against conventional farming in crops like mustard, wheat and maize-wheat rotation. However, contrasting results have been reported for rice cultivated land. Lopes et al. (2011) compared the effect of organic and conventional farming on soil microbial properties and also assessed the temporal variations associated with the same in paddy fields. It was observed that the total microbial count did not vary considerably over the rice cycle among the two differently treated paddy soils. The community level physiological profiles (CLPP) and denaturing gradient gel electrophoresis (DGGE) profile-based richness of the soils were similar over the rice cycle. Further the Shannon and the evenness diversity indices based on the CLPP and DGGE profiles also did not vary in each paddy over time or differed between paddies. Thus, it may be drawn that heterogeneity and

co-abundance of different organisms existed in rice soils with high functional diversity, irrespective of the farming type and management practice did not have a major influence on the functional and microbial communities of the rice soil.

It may thus be summarized that chemical fertilizers certainly disturb the soil microbial communities in terms of their structural and functional diversity as well as dominant soil species. Moreover, organic fertilizers are more favorable and soil-friendly option to enhance nutrient content of agricultural soils as compared to chemical fertilizers.

8.3.3 Positive Effects of Fertilizers

Though fertilization does not have direct positive influence on microbial activities in soil, an improvement in activities as well functional diversity of soil microflora has been reported as an indirect effect of enhanced SOC, elevated concentrations of nutrients like N, P, K and improved crop yields that affects rhizodeposition. A favorable stimulation of many soil parameters was reported by Zhong and Cai (2007) after a 13 years long treatment of paddy soil with inorganic phosphate fertilizers for flooded double rice crops. The number of cultivable microorganisms, microbial biomass and community functional diversity was notably increased as compared to those without P fertilization. At the same time it was detected that the positive effect of nitrogen application on microbial activity, diversity as well as rice crop yields was achieved only in the presence of sufficient P supply while K application had no effect on rice crop yield or on microbial parameters. Similarly, a favorable influence of 39 years of application of NPK fertilizers was observed in a tropical flooded rice field by Bhattacharyya et al. (2013). They found that while the emissions of greenhouse gases and global warming potential were increased with this continuous application of chemical fertilizers, it had positively affected the soil fertility by improving C, N pools, soil enzymatic activities and microbial populations.

It has been recorded in certain cases that long term application of chemical fertilization does not result in any significant changes in the microbial characteristics of agricultural soils. Black soils of Northeast China, when exposed to different combinations of NPK chemical fertilizers for long period did not show any marked variation in the microbial biomass and functional diversity (Kong et al. 2008). Further, it was recorded that the functional diversity tends to increase with increment in the dose of fertilization i.e. double or triple fertilizer treatments. It has also been observed that inorganic fertilization may give variable results when applied singly or in combination with organic inputs. Wu et al. (2011) did not notice any change in bacterial abundance after long-term application of inorganic fertilizers alone in paddy soil. However, rice straw incorporation combined with inorganic fertilizers appreciably increased bacterial abundance with shifts in bacterial community composition. Moreover, the bacterial phylogenetic groups also differed in their response to fertilization administration in soil. γ- proteobacteria and δ-proteobacteria were mainly affected by inorganic fertilizer, while β-proteobacteria and verrucomicrobia were influenced by rice straw incorporation.

Hence, it may be concluded that variable effects are observed for long-term applications of chemical fertilizers in agricultural soils depending on factors ranging from soil characteristics to crop variety. However, the overall performance of organic fertilizers under all circumstances is always superior to that of chemical fertilizers.

8.4 Pesticides

Plant diseases are one of the most important causes of crop-loss world over and thus impose a major threat to global food security. For the major crops of the world i.e. rice, wheat, maize and potato almost 10–15 % of the yield is lost every year due to pest-induced plant diseases (Pinstrup-Andersen 2001). So far chemical pesticides have been the method of choice to control phytopathogens of various kinds. Thus, their consumption has been on a constant rise since last many decades. In order to minimize the pest-induced crop-loss and to keep pace with the rising food demands pesticide consumption in agricultural soils has steeply increased by the end of last century. Asia is the world's largest pesticide consumer followed by Europe while in terms of countries China is the world leader in pesticide production as well as consumption and is closely followed by USA (FAO 2012).

Pesticides are bioactive, toxic substances and they directly or indirectly influence soil productivity and agro-ecosystem quality (Imfeld and Vuilleumier 2012). According to the Food and Agriculture Organization of the United Nations (FAO) pesticides include a wide range of chemicals such as insecticides, fungicides, herbicides, rodenticides, nematicides, plant growth regulators, defoliants, fruit thinning agents, desiccants, agents for preventing the premature fall of fruits, chemicals applied post-harvest to prevent crop-loss during storage or transport. Most of the currently used pesticides are synthetic organic or inorganic chemicals. Classification of pesticides may be based on various criteria such as their target pest, chemical composition, soil persistency (half-life), spectrum of activity, mode of entry in target pest, mode of formulation, toxicity of the active molecule, volatilization behavior (Anonymous 2000; Zacharia 2011; EPA 2012b) (Table 8.1). However, classification based on the chemical composition of the active molecule (Table 8.2) gives an outline of the properties, behavior and nature of the pesticide.

In principle a pesticide should not affect the non-target soil organisms, should have low persistence and should be cheap and biodegradable. However, most of them have acute and chronic toxicity and are described as biocides i.e. capable of harming all forms of life other than the target pest (Zacharia 2011). Many of them are able to penetrate the cell walls of non-target soil-microbes thus disturbing their normal metabolism leading to cell death. Apart from their well-established ill-effects like contamination of soils and water, their entry in the food chain thereby threatening the health of higher organism including man and development of resistant pest varieties, pesticides have lately been identified as a serious threat to soil biodiversity and the natural habitats in soil (Sattler et al. 2006). Effects of pesticides on non-target soil organisms is thus of major concern.

Table 8.1 Classification of agriculturally important pesticides

S. No.	Criteria	Types
1	**Target pest**	1. Algicides: Act against algae. 2. Bactericides: Act against bacteria. 3. Fungicides: Act against fungi. 4. Herbicides: Act against weeds. 5. Insecticides: Act against insects. 6. Nematocides Act against nematodes. 7. Rodenticides: Act against rodents. 8. Virucides: Act against virus.
2	**Chemical composition**	1. Organophosphate 2. Organochlorines 3. Carbamates 4. Pyrethroids
3	**Soil persistency**	1. Non-persistent: Half-life <30 days 2. Moderately persistent: Half-life $=30$ to 100 days 3. Persistent: Half-life >100 days
4	**Spectrum**	1. Broad spectrum pesticides 2. Selective pesticides
5	**Mode of entry**	1. Contact pesticides: Must come into physical contact with the pest. 2. Systemic pesticides: Applied to either plant or soil and translocated throughout the plant. 3. Stomach poisons: Must be eaten by the pest. 4. Repellents: Distasteful preparations that keep the pests away from treated sites.
6	**Mode of formulation**	1. Emulsifiable concentrates: Suspensions of oil based substance in water. Active ingredient is a liquid. 2. Wettable powders: Dry, finely ground material that is suspended in water and agitated before each application as a spray. 3. Soluble powders: Dry, finely ground material which when mixed with water dissolve readily and form a true solution. Agitation is not required. 4. Fumigants: Form poisonous gases when applied. Active ingredient may be a liquid packaged under high pressure, a volatile liquid or a solid that release gas when mixed with water. 5. Dusts: Ready to use formulations that are not mixed with water and used dry. The active ingredient is mixed with a very fine dry inert carrier like talc, chalk and many others. 6. Granules: Similar to dusts but in this case the carrier particles are larger and heavier e.g. clay. Active ingredient is either coated on the surface of the granules or is absorbed into them. 7. Baits: Prepared by mixing an active ingredient with a food based or another attractive substance.

Table 8.1 (continued)

S. No.	Criteria	Types
7	**Toxicity**	1. Class Ia: Extremely hazardous
		2. Class Ib: Highly hazardous
		3. Class II: Moderately hazardous
		4. Class III: Slightly hazardous
		5. Class IV: Unlikely to present acute hazard in normal use
8.	**Volatilization behavior**	1. High volatile
		2. Medium volatile
		3. Low volatile
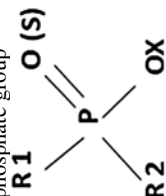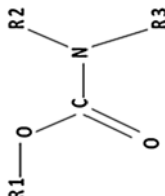
Source: (Anonymous 2000; Zacharia 2011; The United States Environmental Protection Agency (EPA) 2012b)

Though it has been established that pesticides when applied at the recommended dose have minor or transient effects on soil microflora, still the accurate assessment of their toxicity is challenging either due to low-level contamination and diffusion in case of continuous use of poorly degradable pesticides or high-level in case of disposal or accidental release (Imfeld and Vuilleumier 2012). Transformation of pesticides in soil may be accomplished through many physical, chemical and biological processes, but enzyme-catalyzed biological mechanisms such as oxidation, hydrolysis, reduction, conjugation are being considered as primary means for the same (Chowdhury et al. 2008). Consequently, soil microbes may be seen as biological agents who are majorly responsible for transformation of the accumulated toxic pesticides in soil ecosystem. Hence, in the light of soil clean-up capacity of soil microorganisms along with their significant role in a number of key soil-processes, it becomes a matter of concern to access the effect of long-term and continuous use of chemical pesticides on the structural and functional make up of microflora of agricultural lands.

8.4.1 Persistence of Pesticides in Soil

Pesticides tend to persist for longer periods in soil as compared to that in plants or animals because the chemical residues are rapidly metabolized or diluted in actively growing living system than in relatively static soil system (Edwards 1975). A range of factors related to soil, environment and the pesticides themselves affect their persistency in soil. Some of these properties of the pesticide include its chemical structure, volatility, solubility in water, method of formulation and application. Similarly, many soil related factors such as types of soil, content of organic matter and clay in soil, hydrogen ion concentration, diversity of soil microflora and invertebrates affect the behavior and fate of pesticide. Apart from these, environmental factors like temperature, precipitation and ultra-violet radiations of sunlight may also influence the degradation of chemical pesticides in soil (Edwards 1975).

Table 8.2 Chemical classification of pesticides

Class	Structure	Used as	Persistence in environment	Some commercial products	Mode of entry in target pest
Organochlorines	Organic compounds with five or more chlorine atoms	Either insecticides or fungicides	Long	**Insecticides:** Aldrin, Heptachlor, Endosulfan **Fungicides:** Captan, Chloroneb, Hexachlorobenzene	Contact
Organophosphates	Basic structure contains a phosphate group	Either insecticides or herbicides	Moderate to Long	**Insecticides:** Chlorpyrifos, Diazinon, Parathion, Malathion, Phenthoate **Herbicides:** Glyphosate, Fosamine	Contact/stomach poison
Carbamates	Nitrogenous compounds. Carbamic acid derivatives	Insecticides, fungicides or herbicides	Moderate	**Insecticides:** Carbaryl, Mexacarbate, Carbofuran **Fungicides:** Thiram, Ferbam, Mancozeb **Herbicides:** Asulam, Terbucarb	Contact/stomach poison
Pyrethroids	Esters derived from *trans*-chrysanthemic acid and *trans*- pyrethroic acid	Insecticides	Moderate	Bifenthrin, Cypermethrin, Cyphenothrin, Permethrin	Contact

On the basis of their chemical composition pesticides have been categorized into four classes. Chemical composition of the pesticide affects its toxicity, persistence, target pest and many such properties. In case of organophosphates R1 and R2 are methyl or ethyl groups, O in the OX group can be substituted by S in certain cases (fungicides) and X may take different forms. In carbamates R1 is an alcohol group, R2 is a methyl group and R3 is hydrogen. Chemical structure of pyrethroids is based on a class of naturally occurring insecticides called pyrethrins which are derived from chrysanthemum flowers

Source: Anonymous (2000), Zacharia (2011), EPA (2012b)

C Carbon, *DDT* Dichlorodiphenyltrichloroethane, *O* Oxygen, *P* Phosphate, *S* Sulphur

According to EPA (2012a) half-life is a measure of rate at which the pesticide breaks down in soil (soil half-life) or water (hydrolysis half-life). The longer it will stay in water or soil in its original form, the more likely it is to leach through the soil. Depending on their half-lives, pesticides have been assigned various levels of soil-persistence ranging from low persistence (half-life <30 days) to very high persistence (half-life >100 days). Organochlorines are the most persistent pesticides in the environment as they contain five or more chlorine atoms per molecule thus making their degradation process very slow. EPA has classified many organochlorine pesticides including aldrin, dieldrin, chlordane, p,p-dichlorodiphenyltrichloroethane (DDT), mirex, and toxaphene as persistent bioaccumulative and toxic (PBT) chemicals. PBT pollutants are chemicals that are toxic, persist in the environment and bioaccumulate in food chains and thus pose risk to human health and ecosystems. These pesticides generally bind strongly to soil particles and may remain in surface soils from a few months to many years (EPA 2000).

While persistent pesticides tend to have long term effectiveness in pest control they have toxic and harmful effects on soil flora and fauna and at the same time contaminate the environment. Thus, pesticides which persist in soil for a period longer than the requisite time for target-pest control are undesirable. Further, the breakdown of pesticide molecule should not result in release of any toxic molecules in the soil. Residual concentration of pesticides in soil depends on the type of soil, quantity of applications and growth stage of plants (Cycon and Piotrowska-Seget 2007). The residual effects of toxic pesticides tend to vary with the initial application dosage. For example insecticides like lindane and unden when applied at elevated concentrations (156 and 125 g ha^{-1}) inhibited the microbial activity as well as crop yields for vegetable crops (Glover-Amengor and Tetteh 2008) while no change in crop yield was observed at lower concentrations. Similarly, key soil processes like nitrification were inhibited at higher dose of hexazinone pesticide i.e. 20 kg ha^{-1} while at lower concentrations of 5 and 10 kg ha^{-1} the same pesticide enhanced the rate of ammonification and decomposition of cellulose in a soddy podzolic soil (Bliev et al. 1985). Further, the residual soil concentration of hexazinone when applied at 5 kg ha^{-1} reached zero level after 450 days however, when applied at 10 kg ha^{-1}; it took 750 days to reach this level. Thus, it may be inferred that when applied at low dosage pesticides tends to be either neutral or less toxic for soil microbes as well as soil functions but same pesticide may tend to become highly toxic through increased application dosage.

8.4.2 Factors Affecting Pesticide Toxicity

The toxicity of a pesticide apart from its chemical composition depends on certain other biotic and abiotic factors of soil. The organism itself is the most critical biotic parameter as various soil organisms respond differently to the same pesticide. This has been discussed in detail in the next part. Next to it, the most influential factor affecting pesticide toxicity is the application dosage. As explained above,

application dosage is one of the most critical parameter that determines the residual pesticide concentration in the soil and hence its long-term toxicity. Low application dosages are either non-toxic or have lesser detrimental effects on the soil microflora as well as other soil properties. However, as the initial application dosage is increased the corresponding residual soil concentrations and the toxicity also tend to increase. On the basis of various culturing techniques, bacterial community-level substrate utilization patterns, community level catabolic profiles (CLCP), PLFA and ARDRA many studies have proved that the changes in the microbial parameters of soil such as microbial biomass, genetic diversity and catabolic activities are more pronounced at higher inputs of pesticides like methamidophos (Wang et al. 2008), herbicide oxadiazon (Rahman et al. 2005), herbicide glyphosate (Sumalan et al. 2010). Soil characteristics also strongly influence the toxic effects of pesticides on microflora. Application of a herbicide glyphosate inhibited the predominant soil bacteria i.e. actinomycetes in humus rich chernozem soils while in case of gleysol type soils where the indigenous microflora is represented by eubacteria, a high growth of these organisms was registered on application of glyphosate (Sumalan et al. 2010).

Other important factors that may affect the toxicity of pesticide include stage of application i.e. pre-seed or in-crop (Lupwayi et al. 2009a); repetition of treatment (Lupwayi et al. 2010), organic amendments in soil (Rahman et al. 2005) and age of crop (Kalyanasundaram and Kavitha 2012). Microbial properties of rhizosphere and bulk soil of canola were analyzed at flowering stage through bacterial community-level substrate utilization patterns and microbial biomass carbon (MBC) (Lupwayi et al. 2009a). The crop was given pre-seed treatment with 2,4-dichlorophenoxyacetic acid (2,4-D), glyphosate and 2,4-D + glyphosate as well as different in- crop treatments including single and double glyphosate application and various combinations of alternative herbicides like ethalfluralin, sethoxydim, ethametsulfuron and clopyralid. It was observed that pre-seed treatments altered the functional structure and reduced the functional diversity of soil bacteria to varied extent and the in-crop applications of various pesticide combinations when applied after pre-seed treatment also reduced the functional diversity of soil bacteria. Similarly, the deleterious effects of herbicide on soil microbiological characteristics of fields cultivated with canola and barley were observed for 3 years (Lupwayi et al. 2010). It was registered that repeated applications of herbicides year after year produced more significant effects on soil biology and biological processes than single applications. In a similar study in Brazil (Araujo et al. 2003) it was observed that in vitro application of glyphosate for a period of 32 days had more pronounced variations in soil that had a long history of repeated glyphosate applications in comparison to the soil sample with no previous exposure to the same chemical herbicide. Soil and microbial parameters such as soil respiration, fluorescein diacetate hydrolysis and most probable number counts responded more strongly in glyphosate long treated soil. An overall increase was observed in the number of actinomycetes and fungi while there was a slight reduction in the total bacterial counts. Thus, it may be established that long term application of a particular chemical agent bring about more noticeable and permanent changes in the structural diversity of soil microbes.

Influence of herbicide oxadiazon on soil microbial activity was compared in soil amended with crop residues as organic input and the unamended soil on the basis of substrate-induced respiration and dehydrogenase activity (Rahman et al. 2005). It was observed that at elevated oxadiazon concentration i.e. tenfold of recommended dose SIR was comparatively higher in the amended soil than the unamended soil. Similarly, in unamended soil oxadiazon application showed no significant influence on DHA while an elevated DHA was recorded in the amended soil. It indicates that organic amendment stimulated the size of microbial population as well as the microbial activity. Thus, the applied herbicide could serve as a substrate for the microbial population and may be easily degraded.

Hence, it may be summarized that pesticide toxicity depends primarily on its chemical composition, application dosage both in terms of size and repetition, soil properties and crop in terms of type and age etc.

8.4.3   Effects of Pesticides on Soil Microflora

In the last two decades many research groups have been actively involved in investigating the changes in soil properties as well as shift in microbial community structure of agricultural soils due to prolonged pesticide inputs.

Soil microorganisms respond differently to various kinds of chemical pesticides applied in agricultural soils depending on a number of factors including the nature of pesticide, soil properties and groups of established microbes in soil. Total number of bacteria, fungi, protozoa and algae may increase or decrease depending primarily on the nature i.e. toxicity and potential of the pesticide as a nutrient or energy source. However, the overall structural and functional diversity of the soil microbial populations definitely get altered due to pesticide applications. For example, the population size of sensitive communities will decrease and at the same time other microbes capable of withstanding the applied concentrations of the chemical pesticide may tend to increase in number as a result of utilization of either the organic compounds released from dead microbial cells or the pesticide itself as an energy or carbon source (Jana et al. 1998; Das and Mukherjee 2000) and also due to reduced competition (Chen et al. 2001). In many cases the overall microbial biomass has been reported to increase following the pesticide application but a corresponding reduction in the functional diversity is observed at the same time (Wang et al. 2008; Lupwayi et al. 2009a). Soil tends to become dominated by only a few functional groups under the effect of applied chemical pesticide thus affecting the overall community structure and hence various biological processes of soil. Even if no significant pesticide effects are manifested on soil microbial biomass or functional microbial diversity the overall functional structures of soil bacteria surely get altered (Lupwayi et al. 2009b). Further, in some cases though in long term no significant changes are observed as a result of continuous pesticide applications still temporary fluctuations in the community structure of soil and rhizospheric microbial populations have been recorded, such as for herbicides like trifluralin and alachlor

(Moorman and Dowler 1991), herbicides atrazine, butylate, ethalfluralin, imazetha-pyr, linuron, metolachlor, metribuzin and trifluralin (Tu 1992) and herbicide buta-chlor (Kalyanasundaram and Kavitha 2012).

High inputs of an organophosphate pesticide methamidophos in soil significantly reduced total microbial biomass carbon and fungal biomass, but improved the cata-bolic activity as well as the biomass of gram- negative bacteria with no significant effects on the gram-positive bacteria under the same conditions. Further, on the basis of ARDRA pattern it was observed that the overall genetic diversity of the bacterial community decreased under this chemical stress (Wang et al. 2008). In a study by Yang et al. (2000) similar RAPD (Random Amplified Polymorphic DNA) fragment richness and Shannon–Weaver index of DNA sequence was observed for pesticide, triadimefon, in treated and untreated soils while a significant decrease in total soil microbial biomass was also observed in case of triadimefon treated soil. Similarly, application of fungicides captan at dose rates of 2.0–10.0 kg ha^{-1} enhanced denitrify-ing and total culturable bacteria while total culturable fungal populations, nitrifying bacteria, aerobic N2-fixing bacteria and nitrogenase activity were significantly decreased at the same concentrations thus establishing that microbes have different tolerance range for various pesticides (Martinez-Toledo et al. 1998). Such studies thus confirm the variable effects of pesticides on different classes of soil microflora.

The toxic effects of pesticides leading to detrimental effects on soil microbial populations have been reported in many studies. For example, application of an insecticide imidacloprid at high concentrations decreased the total bacterial popula-tion of soil and also changed the soil dominate bacteria (Moghaddam et al. 2011). In a similar manner, a decrease in bacterial, fungal and actinomycetes populations as well as soil dehydrogenase activity was observed after application of herbicides atra-zine, primeextra, paraquat and glyphosate for 6 weeks in cassava farms (Sebiomo et al. 2011). In a short-term mesocosm experiment it was found that basal respira-tion, substrate-induced respiration, microbial biomass carbon and enzyme activities were inhibited by the pesticide tebuconazole. On the basis of various functional com-munity profiles at different tebuconazole concentration it was observed that tebuco-nazole application decreased soil microbial biomass and activities (Munoz-Leoz et al. 2011). In a similar study, herbicide herbogil even at low concentrations caused significant decrease in microbial biomass as indicated by reductions in the two bio-mass-related activities i.e. substrate-induced respiration (22 %) and dehydrogenase activity (44 %). Herbogil also demonstrated an inhibiting effect on catabolic poten-tial of microbial population as well as a shift in dynamics of the community (Engelen et al. 1998). Similarly, pesticides like dimethoate, chlorpyrifos and fosthiazate were reported to affect soil microbial parameters like basal respiration, biomass and microorganisms specific respiration but the effects were independent of plant species as well as plant functional group richness (Eisenhauer et al. 2009). It indicates that the detrimental effects of such chemicals are not restricted by crop variety.

Application of three insecticides lindane, unden, karate and a fungicide dithane in vegetable crops like garden egg, okra and tomato in Ghana resulted in reduction of both fungal as well as bacterial populations. However, the effect was more pronounced in case of fungus which was reduced by 50–70 % than on bacterial

population which showed 23.0–38.4 % reduction (Glover-Amengor and Tetteh 2008). Further, it was observed that yields of all the three crops decreased with an increase in pesticides application dose thus confirming adverse effect of these chemicals on soil fertility. A similar observation was reported for fungicide (mancozeb+dimethomorph) which enhanced the number of heterotrophic bacteria with an increase in application dose from 15 mg/kg of soil to 1500 mg/kg of soil while a completely opposite effect was observed in case of fungus (Cycon and Piotrowska-Seget 2007).

Many studies have confirmed that type of pesticide is an important factor that determines the behavior of soil microbial populations. For example, Duah-Yentumi and Johnson (1986) reported that certain pesticides like carbofuran (insecticide), iprodione (fungicide), MCPA and simazine (herbicides) showed either no or very little detrimental effects on soil microbial biomass while in the same soil other pesticides like carbosulfan (insecticide), vinclozolin (fungicide) and paraquat (herbicide) produced a significant biomass reduction. A herbicide zytron, o-2,4-dichlorophenyl o-methyl isopropyl phosphoramidothioate, while itself did not show any adverse effect on molds, actinomycetes and soil bacteria, its degradation product, 2,4- dichlorophenol, was found to be toxic to molds (Fields and Hemphill 1996). At the same time, another degradation product of zytron sodium o-methyl isopropyl phosphoramidothioate, stimulated the growth of a species of *Penicillium*.

Hence, it may be concluded that though variable patterns have been observed in terms of population size and structure with respect to dosage, number of applications and type of pesticide as well as class of microorganisms and soil quality (physical parameters and nutrient content), it has been clearly established that chemical inputs in soil in the form of any class of pesticide do significantly affect the soil microflora and its other biotic properties.

8.4.4 Effects of Pesticides on Soil Fertility

Soil microflora is crucial in maintaining and enhancing the nutrient concentrations of key elements like nitrogen and phosphorus in soil and are also instrumental in many other ecological processes of soil. Thus, shift in microbial community structure of agricultural soil due to any factor is bound to influence the overall soil-fertility. As explained above, pesticide application in most cases significantly affect the microbial properties of soil and the corresponding changes have been observed in soil-fertility as well. In a study, it was found that the population of nitrifying bacteria in soil treated with fungicides mancozeb and dimethomorph was drastically reduced at application dosage of 1500 mg/kg of soil and an exposure time of 28 days. Similar but comparatively less pronounced effect was observed for insecticide diazinon and herbicide linuron as well (Cycon and Piotrowska-Seget 2007). At the same time populations of N2-fixing bacteria were almost equally inhibited by the same three pesticides at this dosage and exposure time. Similar observations were made in another study for nitrifying bacteria, aerobic N2-fixing bacteria and

nitrogenase activity under the effect of fungicide captan at dose rates of 2.0–10.0 kg/ha (Martinez-Toledo et al. 1998). A slight depression of nitrification was reported after continuous treatment of soil with herbicides atrazine, butylate, ethalfluralin, imazethapyr, linuron, metolachlor, metribuzin and trifluralin. At the same time soil dehydrogenase and amylase activities were also inhibited by ethalfluralin treatment (Tu 1992).

Another observation made in some cases is that the breakdown of certain pesticides leads to improved availability of plant nutrients like N in soil thus favorably affecting the crop yield. For example, yield of unden treated vegetable crops was recorded as higher as compared to lindane treated crops in similar conditions and soil as unden degradation led to release of N thus enhancing its concentration in soil (Glover-Amengor and Tetteh 2008).

Organic C and total N has also been find to get reduced under the effect of pesticides and chemical fertilizers like triadimefon and ammonium bicarbonate by considerable amounts of 58.5, 54.8, and 55.0 % as compared to the soil without chemical pollution (Yang et al. 2000).

Thus, it may be said that a change in the population dynamics of microbes due to under the effect of pesticide application disturbs the nutrient balance and availability in soil.

8.4.5   Pesticide Degradation

Pesticides are generally toxic and xenobiotic in nature and a huge number of microbes die in their presence. However, continuous application of these toxic chemicals in the soil generates stress which leads to development of resistance and adaptation among the local microbial populations. Degradation of pesticides is the breaking down of toxic chemicals into non-toxic compounds and, in some cases, back to their original elements. Most commonly found mode of pesticide degradation in soil is through microbial activity particularly that of fungi and bacteria (Vargas 1975). A number of pesticides that may be used as a source of energy or nutrient are transformed or degraded by soil microbes (Tancho et al. 1992; Ishaq et al. 1994; Megadi et al. 2010; Mohamed et al. 2011). At the same time many other pesticides which cannot serve as an energy or nutrient source for soil microflora may also be degraded by microorganisms through the process of cometabolism (Bollag and Liu 1990).

Hence, in many cases where the applied pesticide is utilized as a source of carbon, energy and others nutrient elements by soil microorganisms, higher pesticide dosage tend to increase the bacterial and fungal population when applied for longer duration. For example, insecticide diazinon and herbicide linuron were reported to significantly improve the number of heterotrophic bacteria as well as fungi in soil after 28 days when concentration was gradually increased from 15 mg kg^{-1} of soil to 1500 mg/kg of soil (Cycon and Piotrowska-Seget 2007). A well-known organophosphate pesticide, profenofos, which is extensively used to control lepidopteron

pests of cotton, tobacco and vegetable crops, has been reported to be degraded by many soil bacteria like *Pseudomonas aeruginosa* through hydrolysis mechanism (Malghani et al. 2009). Similarly, a *Pseudomonas putida* strain isolated from agricultural soils, utilized and hence degraded a different organophosphate pesticide cadusafos, used to control nematode and insect pests, at a rapid rate (Abo-Amer 2012). Another organophosphate pesticide, chlorpyrifos was reported to be utilized by a soil bacterium, *Providencia stuartii* up to concentrations as high as 700 mg/l under *in-vitro* conditions (Rani et al. 2008). In a similar study using enrichment culture technique a bacterium, *Acinetobacter johnsonii* MA19 was isolated from malathion- polluted soil samples. Malathion is a wide spectrum organophosphate used in agricultural soils. The isolated strain was found to degrade malathion through cometabolism and the degradation rates were significantly improved by using sodium succinate and sodium acetate as additional carbon sources for the cometabolism (Xie et al. 2009a).

Even the most persistent class of organochlorine pesticides has been registered as biodegdadable by soil microflora. Endosulfan is a toxic and persistent, widely used broad spectrum cyclodiene organochlorine insecticide. A soil bacterium, *Achromobacter xylosoxidans* strain C8B was isolated through selective enrichment technique using sulphur free medium with endosulfan as sole sulphur source. This bacterial strain was reported to degrade 94.12 % α- endosulfan, 84.52 % β-endosulfan and 80.10 % endosulfan sulphate probably through the formation of endosulfan ether (Singh and Singh 2011).

Dichlorodiphenyltrichloroethane (DDT), an organochlorine compound was once most popularly used agricultural pesticide world over. Though currently it has been banned in most of the countries still it is used in many developing countries for agricultural as well other usages such as mosquito control. Thus, high levels of this compound are many times found in soils. A *p,p'*- DDT degrading bacterial strain *Staphylococcus haemolyticus* was isolated from soil that has a DDT residue in the range of 0.17–9.84 ng/g soil. It reduced 37.4 % of *p,p'*-DDT in 10 days (Sonkong et al. 2008).

A popularly used synthetic pyrethroid pesticide, cypermethrin has also been established as sole source of carbon for many soil microbes and thus is degraded by them. A strain of *Micrococcus* species isolated from soil broke down cypermethrin through hydrolysis of ester linkage to yield 3-phenoxybenzoate resulting in the loss of its insecticidal activity (Tallur et al. 2008). The degradation product 3-phenoxybenzoate was further metabolized by diphenyl ether cleavage to yield protocatechuate and phenolwere both of which on oxidation by ortho-cleavage pathway lead to complete mineralization of pyrethroid cypermethrin. Hence, it may be inferred that the isolated strain accomplished complete detoxification of the pesticide. Similarly, Naphade et al. (2012) isolated five different strains of soil bacteria namely *Pseudomonas psychrophila*, *Devosia yakushimensis*, *Paracoccus chinensis*, *Planococcus rifietoensis*, *Pseudomonas aeruginosa* that were found to withstand high concentrations of endosulfan, chlorpyrifos and cypermethrin.

Simazine which is an active substance of 2-chloro-s-triazine herbicides was biodegraded with almost 100 % efficiency within 4 days by a bacterial strain

Arthrobacter urefaciens NC isolated from rhziosphere soil (Błaszak et al. 2011). Bromoxynil octanoate (BOO) is a toxic and common herbicide applied to maize. Cai et al. (2011) reported the degradation of this herbicide by bacterial strain *Acinetobacter* sp. XB2 isolated from contaminated soil. This strain used BOO as its sole carbon source and degraded 100 mg/l BOO to non-detectable levels in 72 h under optimal conditions. Similarly, glyphosate is extensively used as a broad spectrum herbicide used to control both perennial and annual post-emergent weeds. Fan et al. (2012) isolated a bacterial strain *Bacillus cereus* from soil that demonstrated highly effective glyphosate degradation capability. Under optimal conditions, this strain utilized 94.47 % of glyphosate and degraded it to AMPA, glyoxylate, sarcosine, glycine and formaldehyde as products through C-P lyase activity and the glyphosate oxidoreductase activity.

Liu et al. (2010a) isolated a high-efficiency degradation *Arthrobacter* strain T3AB1 that used atrazine as sole carbon and nitrogen source from black soil of maize field suffering atrazine in Nehe, Heilongjiang province. This bacterium was found to degrade more than 99 % of 500 mg/l atrazine (pH 8.0) within 72 h under optimal conditions. Further, this strain was found to use other herbicides such as imazamox, imazethapyr, trifluralinm, clomazone and fomesafen as well as sole carbon and nitrogen source at a degradation rate of 12.66–40.54 % after 168 h.

An organic acid 2, 4-dichlorophenoxyacetic acid (2,4-D) is a popular herbicide used in many parts of the world and Brazil particularly, against crops such as wheat, rice, corn, sorghum and sugar cane. WHO (World Health Organization) has classified this herbicide as a carcinogen agent of level II toxicity. However, some microbial strains like *Acinetobacter* sp., Serratia *marcescens*, *Stenothrophomonas maltophilia*, *Flavobacterium* sp. and *Penicillium* sp. have been reported to quickly adapt to the presence of 2,4-D under with subsequent degradation under *in- vitro* conditions (Silva et al. 2007).

Thus, it may be summarized that a wide range of soil bacteria when continuously exposed to high concentrations of toxic and persistent chemical pesticides in agricultural soils may develop a capacity to not only withstand the presence of these highly toxic substances but may also utilize them as energy and nutrient source. This leads to complete or partial mineralization/transformation of such pesticides in soil to a level that are either non-toxic or significantly less toxic than the parent molecule thereby resulting in bioremediation of such contaminated sites.

8.5 Conclusion

According to a report of FAO, world population is growing at a rate of 160 persons per minute and we need to produce 70 % more food for an additional 2.3 billion people by 2050. Agriculture is the fundamental mode to satisfy the food demands of mankind and soil is the only medium to practice agriculture. Maintenance of soil quality and fertility is thus most critical to satisfy the world food demands. In the last century extensive innovations and improvements have been made with respect

to agricultural practices and productions. A basic approach for this has been the introduction of new and improved crop varieties and use of chemical based agents in order to enhance nutrient availability to crops as well as to protect the crops from all kind of pests. As a result of this, modern agriculture has become capital, chemical and technology intensive. While it has been successful to a large extent in keeping pace with the growing food demands, however this has ended up in a number of economic, environmental and social problems.

One of the most critical outcomes of this chemical and technology intensive agriculture is the environmental degradation. Soil being the most fundamental part of cultivated lands has been severely affected by such agricultural practices. Extensive and unjustified use of chemical fertilizers and pesticides has also led to enormous soil pollution. The biodiversity of soil ecosystems in cultivated lands is not only exposed to high concentrations of a number of toxic, non-toxic and persistent chemical fertilizers and pesticides but is also bound to be affected by any changes in soil properties brought about by such inputs.

Chemical fertilizers and pesticides do affect the soil properties in terms of nutrient content, predominant soil species, structural and functional diversity of microbial populations, activities of soil-enzymes and many others. In both the cases the effects may range from short term and temporary fluctuations to long-lasting and irreversible changes. Though chemical inputs seems to give immediate benefit in the form of enhanced crop-yields through elevated nutrient supply and effective pest-control, yet their continuous and long-term usage result in drastic changes in soil microbial communities. On the other hand, organic fertilizers; manures and biocontrol agents have been established as favorable soil amendments that improve the overall quality and fertility of soil thus contributing towards sustainable agricultural practices. Unlike chemical inputs, organic amendments are cost-effective as well as environment friendly options to move ahead with a sustainable approach.

Since microbial populations constitute an important link in the complex soil ecosystems such minor or major shifts in their structure and composition are bound to affect many soil-functions as well as natural food-webs to a large extent. At the same time, the soil quality and fertility are closely linked with the microbial biodiversity of agricultural lands. Thus, any changes in the composition and properties of soil microflora may in long run pose a threat to global food security. Hence, it may be concluded that excessive and prolonged usage of chemical fertilizers and pesticides has a range of detrimental effects on the soil microflora of agricultural ecosystem.

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Chapter 9
Bambara Groundnut for Food Security in the Changing African Climate

Philip Cleasby, Festo J. Massawe, and Rachael S. Symonds

Abstract   Global food production must respond to the demands of a growing world population, and to the hazards of climate change. Higher temperatures, unpredictable rainfall and weather patterns, changes in growing seasons, increased occurrences of drought and extreme weather events will exert a greater strain on agriculture. These changes are forecasted to have a high impact in Africa. Warming in Africa should be greater than the global average, with decreasing precipitation leading to higher occurrence of drought in many regions. Climate change will cause shifts in food production and yield loss due to more unpredictable weather patterns. Climate change will also affect food prices and increase malnutrition, especially amongst children. Improving crop productivity and nutritional content is therefore vital.

   Here we review the potential of an underutilised crop, Bambara groundnut, to contribute to food security in changing African climates. The major points are: (1) under future climate change scenarios, African rainfall patterns are expected to become more erratic and temperatures will be higher. (2) Climate change predictions in sub-Saharan Africa anticipate cereal yield losses. (3) Actually, with only three plant species accounting for more than 90 % of the world caloric intake, it is clear that an abundance of genetic resources and potentially beneficial crops are being neglected. (4) There is now ample evidence demonstrating Bambara groundnut superior tolerance to drought conditions relative to other legumes. (5) Bambara groundnut has a high nutritive content and can therefore be used in combatting malnutrition. (6) Bambara groundnut can be successfully intercropped with African staple cereals to improve productivity and contribute to soil fertility through nitrogen fixation.

Keywords   Bambara groundnut • Food security • African climate change • Drought • Rainfall

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9.1 Introduction

One of the greatest challenges facing humanity in the twenty-first century is ensuring global food security in a changing global climate. Limited land availability, unprecedented population growth along with urbanisation are issues which agriculture must now confront in the face of changing global climate. In its most recent report, the United Nations-sponsored Intergovernmental Panel on Climate Change (IPCC) predicts with greater confidence that climate change will have adverse effects on food security in many parts of world, particularly the most vulnerable developing countries (IPCC 2013). In particular, climate change poses a threat to one of humanity's most vital natural resources: freshwater. Changing precipitation patterns and other variables are expected to reduce water supply in many areas across the world, resulting in depleting water resources for agriculture and rising water insecurity (Hagemann et al. 2013). These changes will put a significant strain on already fragile food systems and stretch even further the resources needed to ensure food security. While it is expected some regions will benefit in the short term from rising temperatures in terms of crop yields, the majority of the world's poorest and least prepared populations will become increasingly vulnerable to food insecurity (Fig. 9.1).

It is expected that rain-fed agricultural systems in the tropics, which are among the most susceptible regions to climate change, will experience the most severe effects (Calzadilla et al. 2009). Further drying of semi-arid regions is anticipated,

Fig. 9.1 Food production per capita (USD). Despite economic growth in many Africa countries, food production has remained underdeveloped and amongst the lowest in the world (Source: Schaffnit-Chatterjee 2014). SSA: sub-Saharan Africa
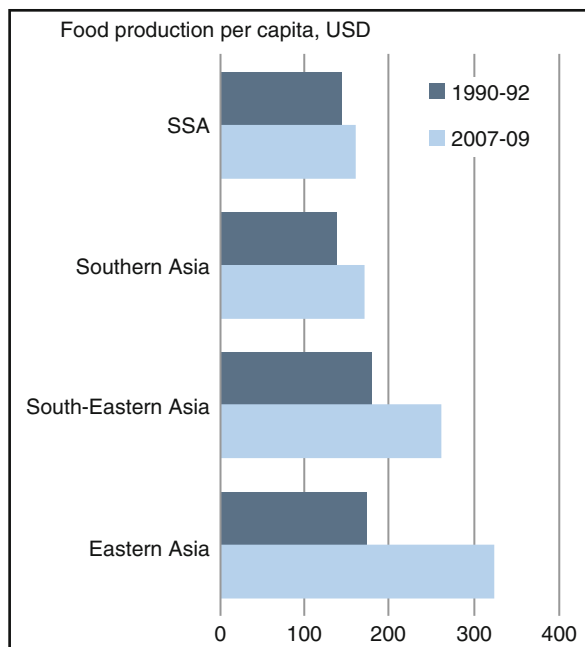
leading to increasing drought conditions which will create challenges for water management. Coincidentally, it is also these regions which from a geographical viewpoint represent some of the world's poorest populations, particularly many countries across semi-arid Africa. With low income potential and adaptive capacity and already widespread poverty, climate change has the ability to impair and even reverse socioeconomic progress made in recent years in sub-tropical Africa (Ford et al. 2014). As rainfall patterns become more unpredictable, drought conditions will become more prevalent and yields of the most important food crops such as maize (*Zea mays*), rice (*Oryza* spp.) will be threatened. In addition, changing weather patterns and increases in extreme weather events will have consequences not only for agricultural productivity but also for the cost and affordability of food (FAO 2013a; Lobell and Field 2007). However, it is an unfortunate paradox that while Africa comprises some of the world's poorest and most vulnerable communities to climate-linked food insecurity, it also represents vast regions of untapped fertile and agricultural land with more than 200 million ha yet uncultivated; almost half of the global availability (Chamberlin et al. 2014). Unlocking the potential of Africa's land and resources could significantly boost productivity, improve incomes and increase agricultural exports (Schaffnit-Chatterjee 2014).

Rising global temperatures and changing weather patterns will alter ecosystems and exert new pressures onto the planet's plant and animal species, including those used for agricultural production. As the climate changes, so do the environmental conditions in which the world's most important food crops grow, potentially making them less resilient and productive. In some cases climatic conditions may change too rapidly for many plant species to adapt (Botero et al. 2015). While these changes will have consequences globally, few countries will be affected more acutely than those in the semi-arid regions of Africa.

9.2 Climate Change in Semi-arid Africa

Africa is often cited as the continent most vulnerable to the negative consequences of climate change as up to 95 % of farmed land is rain fed and agriculture represents the main livelihood for the vast majority of the rural poor (CABI 2009; Boko et al. 2007). Further, of the 32 countries in the world facing food crises and requiring international emergency support, 20 of them are in Africa (FAO 2013). For such countries, agriculture is a significant if not the most significant aspect of the economy. In Malawi for example, 40 % of the country's global domestic product (GDP) comes from agriculture and is a source of employment for three quarters of the population (IFPRI 2008). Similarly agriculture contributes around 35 % towards GDP in Ghana (Jerven and Duncan 2012) whereas in Ethiopia it is as high as 45 % (Tilahun et al. 2011). While the challenges of climate change are global, poor infrastructure and a weak capacity to adapt to new threats and sudden changes pose a significant threat for Africa's poorest nations and its people (UNDP 2012).

The second half of the twentieth century saw a number of food crises throughout Africa which were at least partially the result of extreme weather events. One of the most severe cases in the last 30 years was the 1983–1985 famine in Ethiopia which resulted in more than 400,000 deaths. Although it is believed that government policy and civil war were amongst the underlying causes, drought is regarded as a confounding factor that further exacerbated the crisis (IFPRI 2012a). In 2005 Malawi experienced a more than 60 % drop in maize (*Zea mays*) yields as a direct result of drought, affecting more than five million people and requiring international aid (FAO 2005; UNDP 2006; Devereux 2009). More recently, tens of thousands died from starvation in 2011 throughout the Horn of Africa following severe drought and failed harvests (OCHA 2011). In 2012 severe drought hit the Sahel region, resulting in acute food insecurity with millions today still facing a deteriorating outlook (FAO 2014). Substantial and continuous humanitarian support is needed across the Sahel for the more than 20 million affected, for which the UN has released an urgent international appeal for more than US$2 billion and has developed a regional 3-year strategy to support vulnerable populations (OCHA 2014). As well drought, heat stress events present another major threat to food security in the sub-Saharan region and will become more frequent in tropical regions (Fischer et al. 2005). Battisti and Naylor (2009) predicted that hot spots for heat stress will occur across the Sahel and East Africa, affecting Maize yields in particular. The nature and frequency of such events will be a major factor in determining future climates in sub-tropical Africa.

It is clear that the adverse effects of climate change pose a significant risk to the vulnerable populations in semi-arid Africa. While some progress has been made towards reducing hunger in these countries, large populations still depend on rain fed agriculture for their livelihoods, resulting in high levels of vulnerability to increasing extreme weather events such as drought (Shongwe et al. 2011; Lyon and DeWitt 2012). The nature of the predicted changes will now be explored in more detail.

9.2.1   Rainfall Patterns

As the planet's surface temperature rises, overall there is a twofold effect on global rainfall patterns. On the one hand, the moisture holding capacity of the atmosphere increases as the warmer air can retain more water, while on the other, increased temperatures lead to more evaporation (Houghton et al. 2001). This both adds water to the atmosphere and increases rainfall, but also removes water from oceans, rivers and arable land and increases salinization of soils (Várallyay 2010). In sub-Saharan Africa, the general trend is that rainy seasons are expected to become wetter, while the dry season becomes drier. Rainfall projections by various intermediate climate

change models vary, but at this stage there is some agreement that rainfall will increase in some parts, including East Africa, while Sahelian and Southern Africa are likely to experience decreased rainfall and shorter rainy seasons (James and Washington 2013; Shongwe et al. 2011). Further, reports mention that there has been a continued warming in the Indian-Pacific pacific region over the past 30 years which has contributed to increasing occurrences of droughts in East Africa (Williams et al. 2012). At the same time, heavy precipitation during the rainy season will become more common which can potentially lead to flooding and soil erosion. Hulme et al. (2001) suggest that by 2050 East Africa will be faced with up to 20 % increase in rainfall from December–February and a 5–10 % decrease in rainfall from June to August. Seneviratne et al. (2012) suggested with high certainty that the number of extreme wet days in the same region has and will continue to increase. However, not only is it likely that the increase in rainfall will be more sporadic and unpredictable and therefore difficult to manage, it may well be the case that rainfall comes in the form of intermittent and heavy rainstorms. Heavier rainfall is also expected to contribute to soil erosion and consequently loss of soil fertility due to nutrients lost from the topsoil (Lal et al. 2011; Bates et al. 2008), requiring new management practices, especially at the farm level, to mitigate these effects and ensure soil conservation.

Combined with a decrease in rainfall during the already dry season, countries such as Kenya will be faced with complicated water management issues and the need to adopt sustainable methods for maintaining food production. River systems which are a vital source of water for many populations will be affected by these changes. Bordering Kenya, the basin of the River Pangani of Tanzania is expected to experience a similar increase in rainfall during the wet season (November–March) and a decrease during the dry season (June–October), with increased evapotranspiration expected towards the end of the dry season (IUCN 2011). This is projected to result in a 6–9 % reduction in annual flow, adding further pressure to the demand for water along the river which provides agricultural irrigation for an estimated 55,000 ha of land and is a source of livelihood for over three million people (IUNC 2011). Reduced stream flow will become a more common reality for numerous river systems across Africa, particularly in the southern regions, where most studies suggest reductions of up to 50 % will be seen by 2050 (Kusangaya et al. 2014).

While indications of increasing precipitation suggests a hopeful future, the reality is that water shortages are still occurring and water will become increasingly scarce in the long term with increasing urbanisation and competition for water resources (Druyan 2011). Any increase in rainfall during the rainy season is likely to be more extreme and unpredictable, whilst countries along the Sahel and southern arid countries such as Tanzania, are likely to experience lower rainfall and reduced river flow. Should rainfall follow this pattern of becoming more extreme and erratic, adaptation and mitigation strategies will need to be adopted urgently to optimise water resources for the most susceptible regions.

9.2.2 Rising Temperatures

In addition to changing rainfall patterns, rising temperatures poses a significant threat to rain fed agriculture as a result of increased evaporation, changes in soil moisture and groundwater reserves and increases in the frequency of droughts (Schulze 2011). Warming is expected to be higher than the global mean for all seasons across Africa, with the already rain dependent semi-arid regions facing the most vulnerability (Kusangaya et al. 2014). Mean annual temperature rise across Africa is likely to exceed 2 °C by 2100, with southern Africa, East Africa and the Sahel experiencing the most warming (Kruger and Shongwe 2004). According to Battisti and Naylor (2009), there is a high probability, greater than 90 %, that by the end of the twenty-first century, average growing season temperature in the tropics and subtropics will be higher than the highest seasonal temperatures seen from 1900 to 2006.

This increase in temperature will certainly negate any increase in rainfall, as more water will be lost through evaporation, further exacerbating the dry season (James and Washington 2013). Additional loss of water through evaporation will put an even greater strain on water management, and by 2080 there will be more arid and semi-arid land across Sahelian Africa and an increase in desertification as a result (James and Washington 2013; Nikulin et al. 2012). For those regions receiving less precipitation, a hotter climate will put an even greater strain on the already dry soils and further constrict water availability. East Africa, for example, is expected to continue to experience drought events at 7-year intervals, but will be more extreme and hard hitting as water becomes even scarcer during the dry season (Boko et al. 2007).

9.3 Impact on Food Security

Inevitably these changing climatic conditions will impact crop production across the sub-Saharan region, with cereal yields facing a significant risk. A review by Zinyengere et al. (2013) consolidated the findings of various climate change impact studies in southern Africa from 2001 to 2011. While variation and discrepancies were found to exist across different models, it was concluded that the overall impact of climate change on crops in sub-Saharan Africa will be negative. This correlates with other studies which suggest negative impacts of climate change on crop production (Liu et al. 2013; Müller 2013) (Fig. 9.2).

Maize is one of the dominant crops in Zambia, Tanzania, Kenya, Ethiopia and Nigeria, with wheat (Triticum spp.), rice (Oryza sativa), sorghum (Sorghum bicolor (L.)) and millet (*Pennisetum* glaucum) also having important roles in calorific intake (IFPRI 2012a, b; Romney et al. 2003). While the latter two may display higher yields in some areas due to their higher tolerance of heat and water stress, projections indicate that most cereal crops across semi-arid Africa will face yield losses (Waha et al. 2013). Yield losses will drive food prices higher, resulting in unaffordable food and therefore a greater a risk of malnutrition (FAO 2008; Holden 2009).
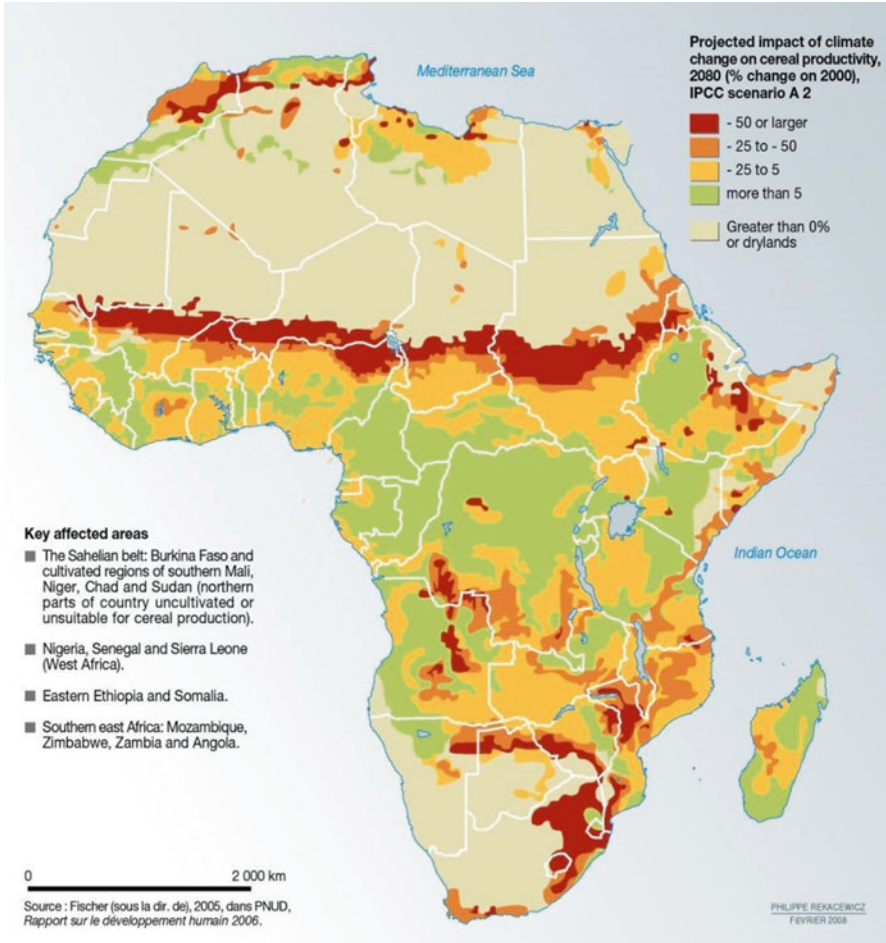
Fig. 9.2 Impact of climate change on African cereal production (Fischer et al. 2005). Changes in cereal production vary greatly, however those regions along the Sahelian belt and the semi-arid countries to the south are at the greatest risk

Knox et al. (2010) incorporated irrigation and water resources into their analysis of how rising temperatures will affect sugarcane (*Saccharum officinarum*) yields in Swaziland, and concluded that in order to only maintain current yields, irrigation would need to increase by more than 20 %. Schlenker and Lobell (2010) proposed that aggregate production changes in maize, sorghum, millet, groundnut and cassava is likely to exceed 7 % and in certain scenarios could reach as high as 27 %, while Berg et al. (2013) projected that even yields of C4 species, such as millet, will decrease by an average 6 %. In fact for the grains alone, Lobell and Gourdji (2012) estimated that for each degree Celsius temperature rise, global yield loss is approximately 5 %. At the regional level, however, the impacts vary. While some regions
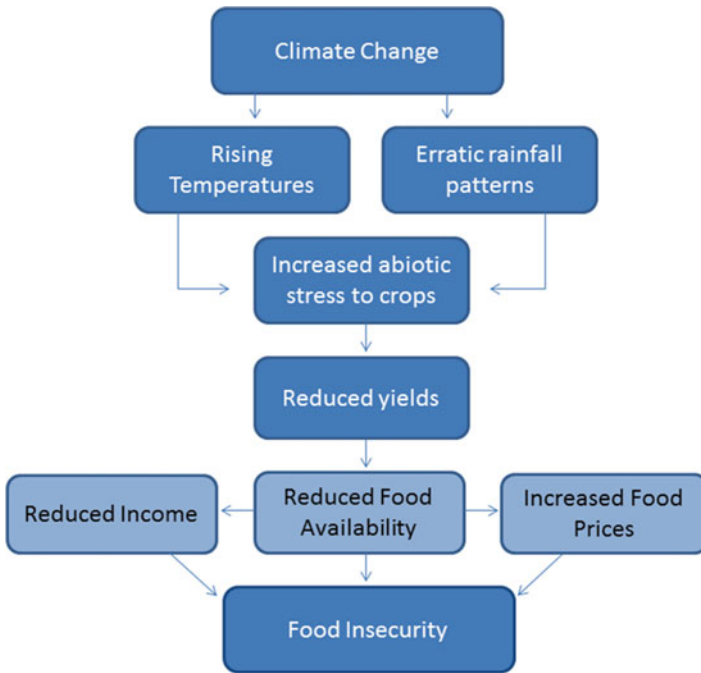
Fig. 9.3 Impacts of drought (Adapted from Gitz and Meybeck 2012). Environmental stresses increase the risk of food security not only through yield losses, but also through reducing income and pushing up food prices. In regions where widespread poverty is prevalent, drought conditions will have increasingly severe social, economic and environmental consequences

will experience an expansion in the areas suitable for growing cereals, this will be negated by the loss of land as a result of increased heat stress in others. For the arid and semi-arid zones, precipitation changes are likely to be the main driver behind yield losses (Berg et al. 2013). In Southern Africa, even moderate temperature increases may lead to significant yield loss. Maize, the staple crop of the region, may face yield reductions of up to 30 % by 2030 if temperature rises along its expected trajectory (Lobell et al. 2008; Easterling et al. 2007).

Global prices of maize, rice and wheat are projected to increase by 4 %, 7 % and 15 % respectively by 2050 as a result of climate change-linked yield loss, in addition to other important crops such as cassava, millet and sorghum which will see price increases of 20 %, 5 % and 4 % respectively (IFPRI 2011). Such an rise in cost will cut the affordability of the most important crops for human consumption and livestock feed, leading to an expected drop of calorie availability of 37 kilocalories per capita per day across SSA, with the central zone being hardest hit (IFPRI 2011). Figure 9.3 below summarises the main impacts of drought that would affect smallholder farming systems across Africa.

9.4 Solutions

It is clear that the primary impact of climate change throughout Africa will relate to water availability, management of water resources and nutritional security, as has been highlighted by the United Nations (UN-Water 2010). Although the precise way in which weather systems will change is not clear for all regions, what is expected is that climate change will cause shifts in cereal growing areas and increase the risk of crop failure due to intensified drought and heat stress. This in turn will increase food prices and therefore lower the affordability of basic staple foods and animal feed for those most vulnerable, further exacerbating malnutrition, especially amongst children (Ringler et al. 2010).

It follows then, that any effort to mitigate the consequences of climate change must explore ways to improve the resilience of farming systems to water and heat stress, particularly those that are rain-fed (Rost et al. 2009). Doing so will help to increase food availability and improve rural incomes, thereby negating the consequences of dropping yields and rising food prices.

One strategy that has been advocated is the development of superior varieties of important cereal crops, enhancing drought resistance or nutritive content through genetic modification or other breeding technologies (Najafi and Lee 2014; Qaim 2011). While such an approach will no doubt be of great importance in improving incomes and ensuring food security in more hostile African climates, it can be argued that what is more pressing is a fundamental restructuring of the way Africa grows and distributes food (UNDP 2012). Poor infrastructure, political instability, limited access to technology and education and financial constraints are amongst the many hurdles that would make difficult the introduction of high yielding varieties of the major crops. The Green Revolution, which was so successful in South and East Asia, has so far not reaped the same benefits in sub-Saharan Africa. This has been the result of a combination of factors including poor infrastructure in many African nations, unfavourable environmental (including irregular rainfall and soil properties) and the high cost of the technologies and inputs involved (Lynd and Woods 2011; Diao et al. 2010). This is perhaps an indication that high input, mechanised agriculture is unlikely to provide the necessary solutions for the resource poor farmers in semi-arid Africa, especially in the face of climate change (Frankema 2014).

Food systems and crops that can withstand the shocks of increasingly hostile climates will be better suited to protecting livelihoods, yet many modern varieties of the major crops are vulnerable to heat and drought stresses (Lobell and Gourdji 2012).

An approach that is likely to prove more relevant and sustainable for the arid regions of Africa, is the exploitation of minor and underutilised crops, especially species native to the continent of Africa. Crops that have evolved and been cultivated in Africa's various climatic conditions are likely to grow more favourably in African climates and will contain characteristics and traits that enable them to withstand abiotic stresses and unfavourable conditions (Sambo 2014). Tapping into these local resources is vital for building resilience in rural communities and their farming systems.

9.4.1   Underutilised Crops

Underutilised crops, also known as 'neglected', 'minor' and 'orphan' crops, are those species which are of little importance globally in terms of production, consumption and economic value but have great potential to contribute to food security, improved income and nutrition or may be of medicinal use (IPGRI 1999). Such species are considered underutilised as they have not been given the necessary support either by research, policies or legislation that would enable them to make such contributions. They are often regarded as traditional crops and are usually found in a specific region or ecological niche and represent an untapped source of agrobiodiversity. Many of these species have been grown locally for centuries and appear to have been chosen due to their ability to tolerate unfavourable environments (Azam-Ali et al. 2001). These crops represent a wide range of species, including cereals: finger millet (*Elusine coracana*) and Teff (*Eragrostis tef*), fruit species: jackfruit (*Artocarpus heterophyllus*), legumes: Bambara groundnut (*Vigna subterranea* (L.) Verdc), the azuki bean (*Vigna angularis*), root crops: African Yam bean (*Sphenostylus stenocarpa*), and many others (FAO 2013b).

In the effort to combat climate-related challenges, issues relating to food distribution, sustainable livelihoods as well as socio-economics are necessary areas of intensified focus and development (Connolly-Boutin and Smit 2015). However, tapping into a wider genetic pool of potentially beneficial agronomic traits and characteristics will increase the resilience and adaptability of agriculture to changing climates. Indeed, José Graziano da Silva, the Director General of the FAO, stated at the International Crops for the twenty-first century Seminar in 2012 that underutilised crop species "play a crucial role in the fight against hunger and are a key resource for agriculture and rural development" (FAO 2012). In recent years, the subject has started to attract more attention from research institutions, organisations and governments as the importance of agro-biodiversity becomes more recognised (Gowda et al. 2007).

This new interest in agro-biodiversity has emerged from an increasing awareness of the risks associated with relying on too few crops and the vast untapped potential of the world's plant resources. Only 30 crop species provide 95 % of the world's food, with maize, wheat and rice providing more than half of the world's calorific needs despite more than 7000 cultivated species being known to exist (FAO 1997). This state in which global agriculture finds itself not only deprives humanity of a vast reservoir of plant resources, but it also gives rise to many risks that are associated with relying on just a handful of species. The increasingly global and interconnected nature of agriculture means that any setbacks in production will have far reaching consequences, for both people and economies.

Diseases and pests pose a particular threat. If a serious outbreak of a particular pathogen occurred amongst any of the world's major crop species, food prices would escalate and food shortages will become widespread, leading to large scale food crises. Such a possibility exists with the fungus *Puccinia graminis tritici* strain known as UG99, which causes stem or black rust disease in wheat. First detected in

Uganda in 1999, several races of the same lineage have already spread to Zimbabwe, South Africa, Sudan and Yemen, and in 2007 the disease was found to have reached Iran (Singh et al. 2011). This particular strain produces devastating yield losses of up to 70–100 % if preventive measures are not taken, and it is feared that spores will continue to travel by wind to India, which is second largest producer of wheat in the world (FAO 2010). While efforts are underway to prevent an outbreak by identifying resistant wheat varieties, the situation highlights the susceptibility of agricultural systems characterised by low crop and genetic diversity.

Similarly, as the future climate across Africa will become increasingly characterised by drought, rising temperatures and erratic rainfall patterns, any susceptibility amongst the staple crops will affect millions of people. Economically, global food prices are delicately linked to crop production and the fluctuating costs of commodities, such as oil. The risk associated with this scenario is perhaps demonstrated most clearly with rice: it is the staple food for more than three billion people and in 2008 when the cost of rice increased threefold, more than 100 million people were pushed into poverty (IRRI 2011).

As climate change intensifies drought conditions, countries affected by political instability are likely to become more vulnerable to poverty, as food and water shortages along with rising prices exacerbate existing economic and socio-political tensions (Smith and Vivekananda 2007). Dependence on rain-fed agriculture and the fragility of political and social infrastructure in many countries makes African agriculture especially vulnerable to further food crises (Knox et al. 2012).

While enhancing the institutional and administrative capacity of nations to adapt to these tensions is vital, ensuring food security for those most vulnerable is no less essential, and the 'cornerstone' of any strategy to achieve food security in the world's poorest countries is, as reiterated by the FAO, the expansion and diversification of food production (FAO 2008).

Many different species around the world have been identified as underutilised and with potential to contribute to food security in their respective regions. The relative success of a Quinoa (*Chenopodium quinoa*) for example, demonstrates the potential for indigenous crops with nutritional benefits to contribute to food production globally (Ruiz et al. 2014). Such crop plants represent a cross section of the planets' plant diversity.

One crop group which is of major agricultural important across sub-Saharan Africa and contains species with potential to contribute to improved incomes, nutritional security and food production are the leguminous crops (family *Facaceae*) (Sprent et al. 2010). Legumes represent almost 18,000 species and include widely grown food crops such as cowpea (*Vigna unguiculata*) pigeonpea (*Cajanus cajan*), common bean (*Phaseolus vulgaris*) and groundnut (Arachis hypogae) (Graham and Vance 2003). Many species are grown in intercropping systems throughout sub-Saharan Africa where they appear to offer protective control for Sorghum and Maize against pests such as *Striga hermonthica* (Khan et al. 2007). The protein-rich food which legumes offer, as well as the ability for many species to contribute to soil fertility through nitrogen fixing symbioses, makes them important components of any future cropping system (Jensen et al. 2011).

As well as improving food security through increased food production, it is also essential to improve the resilience of farming systems to withstand the pressures of climate change. Leguminous species, through their agroecological benefits and many end uses, are examples of untapped plant resources that could be used to improve the productivity and adaptability of food systems in the most resource poor regions of Africa (Ebert 2014).

Amongst this family is Bambara groundnut (*Vigna subterranea* L.); an African grain legume that has received interest in recent years for its tolerance to drought conditions and its potential to contribute to food security, particularly on its home continent. The next sections will now explore the literature on some of the physiological traits of Bambara groundnut that make it a suitable candidate as a food security crop in changing African climates.

9.5 Bambara Groundnut

Since 1988, Bambara groundnut research has steadily increased and has involved international collaboration between various Universities and institutions (Dakora 1998; Amarteifio and Moholo 1998). The result today is a comprehensive body of knowledge about the crop covering genetic diversity, drought resistance and adaptation, nitrogen fixation and intercropping and other physiological and molecular aspects, some of which will be explored in this review. In addition to the gained scientific knowledge, the potential of the crop with regard to market, economy and diet has been considered. This is especially important for many underutilised crops whose acceptance and increased production is limited by poor markets (Mayes et al. 2011). The history of Bambara groundnut research represents a unique case study of how work on underutilised crops can take place (Azam-Ali et al. 2001).

According to Doku and Karikari (1971) bambara groundnut has two botanical forms; the wild type (var. *spontanea*) and the cultivated forms (var. *subterranea*) which originated from the former through gradual changes. The landraces have developed as a result of selections made by farmers based on their traits which make them reliable in various climatic conditions (Doku and Karikari 1971; Massawe et al. 2005) (Image 9.1).

Its centre of origin is believed to stretch from the Yola region of Nigeria down to northern Cameroon and is now cultivated throughout the African continent, Southeast Asia and South and Central America (Azam-Ali et al. 2001; Hepper 1963). Currently regarded as an underutilised crop, it is grown mainly by resource poor women subsistence farmers in sub Saharan Africa. It is regarded as a complete food with many different uses in different regions (Linnemann 1990; Bamshaiye et al. 2011). Its seeds can be eaten fresh, used to make flour, can be pounded or ground into a stew and is often cooked to be consumed with rice and in traditional dishes, while in some countries the seeds and plant are used for livestock (Linnemann and Azam-Ali et al. 1993). In Burkina Faso, for example, the protein and phosphorus-rich leaves are often used for fodder and in Botswana the stems are used for grazing (Bamshaiye et al. 2011). As is characteristic of many underutilised crops, Bambara

Image 9.1 Bambara groundnut seeds and plant morphology. Great diversity of seed colour exists between landraces. As with many legumes, Bambara groundnut leaves are trifoliate and seeds develop in pod fruits, either above or below ground, as seen on the image on the *right* (Images taken from the University of Nottingham (www.nottingham.ac.uk))

groundnut is currently cultivated from landraces as varieties with specific traits are yet to be developed.

9.5.1 Production and Importance

Bambara groundnut is grown throughout the tropical regions of Africa and in 2009 the largest producer was Burkina Faso, with around 44,000 tons harvested, and followed by Cameroon, Democratic Republic of the Congo and Mali (FAO 2011a). Global production in 2009 was just over 100,000 tons (FAO 2011a).

Although compared to major crop species there has been limited research attention, enough has been found to demonstrate that Bambara groundnut has certain advantages over other legumes and indeed other crops. Experimental studies have shown, for example, its superior drought tolerance and nutritive content relative to other legumes (Bamshaiye et al. 2011; Mazahib et al. 2013). Despite its beneficial traits, barriers to wider acceptance exist. Although in some areas there is rising interest in the crop, the popularity and familiarity of other cash crops such as soybean make them a financially safer option (Mkandawire 2007). Its reputation as a snack or food supplement means its status is low and is not considered as a crop with economic value (Azam-Ali et al. 2001) and in some parts has obtained the unfortunate byname of a 'poor man's crop' (Heller et al. 1997). There is no doubt however that this crop deserves more recognition. Through education, support from policy makers, scientific research and initiatives by governments, Bambara groundnut's potential can be more fully realised.

Under ideal conditions and with appropriate management yields up to 4000 kg ha^{-1} have been observed (Heller et al. 1997) and it has been suggested that there is potential for wider cultivation and higher yields than what is currently achieved in sub-Saharan Africa (Azam-Ali et al. 2001). Known in Madagascar as 'a seed that

satisfies', it is highly nutritious with high protein and carbohydrate content (Azam-Ali et al. 2001). Work has shown that Bambara groundnut is a richer source of protein (16–25 %) than cowpea, groundnut and pigeon pea (Brough et al. 1993; Brough and Azam-Ali 1992; Poulter and Caygill 1980). Starch content is also favourable at 43 % and lipid content (7.9 %) exceeds that of cowpea (1.0–1.6 %) and pigeon pea (1.2–1.5 %) but not groundnut (45.3–47.7 %) (Brough and Azam-Ali 1992). In the case of Bambara groundnut, it is a strange paradox that such a nutritionally whole plant that can significantly contribute to alleviating malnutrition should be so neglected and overlooked in its homeland. A more detailed look will now be given to what qualifies Bambara groundnut as a potential food security crop in the sorts of conditions that will increasingly characterise many regions across Africa in the next 50–100 years.

9.5.2   *Bambara Groundnut Tolerance to Water and Heat Stress*

With the changes in weather and rainfall patterns expected to occur across semi-arid Africa, it is essential to grow crops that can ensure reliability during unpredictable climatic conditions. In the tropics soil moisture is the primary limiting factor to yield and crop production and will continue to exert more pressure with increasing temperatures and changing rainfall patterns. For many African countries in the tropics, irrigation and adequate water supply for agriculture presents a long term challenge (UNDP 2012).

There is now strong evidence that Bambara groundnut is more drought tolerant than many of its legume counterparts (Babiker 1989; Collinson et al. 1996; Vurayai et al. 2011; Berchie et al. 2012) Early work indicated the drought tolerance capacity of Bamabra groundnut (Doku and Karikari 1971), however it has only been in the past few decades efforts have been made to understand and quantify how periods of drought are tolerated.

Plants that grow in areas where water is the major limiting factor have evolved various means of ensuring their growth and development in times of water stress. Turner (1979), in a discussion of the physiological aspects of drought tolerance, suggested that there are three main mechanisms by which plants cope with periods of water stress. These three mechanisms are known as 'drought escape', 'drought avoidance' and 'drought tolerance'. The first of these is usually a short-term strategy whereby the life cycle of a plant is completed before periods of drought. 'Drought avoidance' involves physiological changes, such as reducing leaf expansion, promoting root growth or reducing stomatal conductance and can be seen in sorghum and cowpea (Osonubi 1985; Stout and Simpson 1978) as well as Bambara groundnut (Collinson et al. 1997). Finally, 'drought tolerance' is linked with survival strategies which are used in severe drought conditions and is less relevant to agriculture. The lack of quantitative evidence available means that it has not been possible to easily place Bambara groundnut into only one of these categories and instead elements of each have been observed in landraces Jørgensen et al. (2010).

Indeed, great intraspecific diversity exists in terms of how well plants cope with drought. For example, the landrace known as UNISWA red from Swaziland is regarded as having a relatively low tolerance compared to S19-3, a very drought tolerant landrace from Namibia (Jørgensen 2011). In addition, molecular and genetic analyses have confirmed the great diversity that exists between landraces (Amadou et al. 2001; Massawe et al. 2002; Somta et al. 2011; Molosiwa et al. 2013; Mukakalisa et al. 2013; Siise and Massawe 2013). Such diversity demonstrates the vast pool of genetic resources that can be used in future breeding efforts.

Collinson et al. (1997) and Colllinson et al. (1996) and both assessed the response of Bambara groundnut under different irrigation treatments. Collinson et al. (1996) observed that water stress resulted in reduced leaf initiation rates, leaf and pod number per plant, dry matter, leaf area index and the efficiency of conversion of intercepted radiation into dry matter. Interestingly, the treatment which received no further irrigation at all from 35 DAS was still able to produce some pod yield, albeit very low (0.1 t ha^{-1}). This contrasts with previous work carried out on groundnut under similar conditions in which it was unable to produce any pods (Babiker 1989). Importantly, what this study identified were some plant responses that seem to have a role in tolerating drought, namely an economical way of using available water such as by a reduction in leaf area development and an increased partitioning of dry matter to the roots, as well as increasing root density. The later study, Collinson et al. (1997) built on these findings by identifying other apparent adjustments made by the crop. Water potential was maintained during water stress and was associated primarily with a combination of stomatal regulation, osmotic adjustment and reducing leaf area index.

Experimental evidence demonstrates that much potential exists amongst Bambara groundnut for climatic adaptation and that great diversity exists in the ways landraces respond to different environmental conditions. Twin publications by Mwale et al. (2007a, b) looked at the effect of water stress on different aspects of the crops' growth and development. One focused on the impact on dry matter production and yield, while the other explored resource capture and conversion. Both confirmed previous work showing that much variation exists between landraces in terms of responses to drought, as a result of the different environments from which they originated. A plant originating from an environment characterised by low mean annual rainfall and a short growing season is likely to have a relatively short life cycle. On the other hand, a longer life cycle would be permitted in a climate with higher rainfall and a longer growing season. Such differences are reflected in the data. For example, the landrace S19-3, which is from the first of these two climates (Namibia), displayed a better performance under the drought treatment than DipC and Uniswa-red, which are from the latter (Botswana and Swaziland respectively). This is a result of its faster growth and development rate, its early maturity, and also relatively more efficient use of water, as has been confirmed in other studies (Karunaratne 2009; Karunaratne et al. 2010; Jørgensen 2011). Landraces such as S19-3 would be more advantageous where terminal drought is a problem, due to their shorter pod filling periods. Where the growing season is not a limiting factor or where rainfall is more uniformly distributed, Uniswa red would produce higher

yields due to its longer life cycle. Findings such as these are essential as they demonstrate the most suitable conditions for a certain landrace and can help inform crop management in changing weather patterns. Further, such variation can be harnessed for selections in breeding programmes that can develop true varieties of the crop that are adapted to and high yielding in future climates across Africa.

In Mwale et al. (2007a), an average seed yield of 1.65 t ha^{-1} was achieved which is higher than reported yields from cowpea (0.3–0.5 t ha^{-1}) under similar conditions (Leport et al. 1999). Similarly across all landraces was a reduction in the mean fractional intercepted radiation (f) during drought, which was reduced from 0.8 to less than 0.7. Seasonal intercepted radiation was also hindered, which is consistent with the findings of Collinson et al. (1999). An interesting outcome of this work was the observation that those plants in the irrigated treatment extracted water from the top 50 cm of the soil, while the roots of those experiencing drought were found throughout the soil profile, some reaching as far as 90 cm.

An aspect of Bambara groundnut physiology studies that was until recently not fully considered was the relationship between the mechanisms underlying drought tolerance and the final crop yield. This was recently explored by Jørgensen (2011) who imposed drought on four landraces during the early reproductive stage. Stomatal response and transpiration were investigated as with previous studies, however the effects of these on yield components were considered. While there were similarities amongst the landraces in terms of stomatal response, variation could be seen in seed yield reduction and the effect on yield components and cumulative plant water use. Seed yield loss was greater in LunT (from Sierre Leone) and Ramayana (Indonesia) compared to Uniswa-red and S19-3 and the former two also had fewer larger seeds than the latter. LunT and Ramayana are therefore more sensitive to drought than the other two landraces. Again, these responses reflect the adaptations the landraces have developed in response to their local climates. An interesting finding was that the fraction of transpirable soil water (FTSW), while similar among the landraces, was much higher than those identified in other crops (Sadras and Milroy 1996). This may suggest that Bambara groundnut possesses a greater sensitivity to water stress and soil drying than other crops.

As African climates will get hotter as well as generally drier, selection of crops for use in these environments must consider both of these factors. Limited research has been done to identify the response of Bambara groundnut genotypes specifically to temperature stress, but those that have revealed useful initial insights. Berchie et al. (2012) evaluated the response five landraces of Bambara groundnut to temperatures ranging from 34.7 °C to 38.7 °C. As temperatures exceeding 38 °C, pod yield was negatively affected even though irrigation was provided. Nevertheless, the landrace Burkina, from Burkina Faso, proved to be the most drought tolerant landrace and still managed to produce pods at high temperatures whilst other landraces produced none. Likewise, Al Shareef et al. (2013) found that during high temperatures and under moderate drought, the landrace S19-3 produced significantly higher pod yields than Uniswa Red. Further, results suggested that water stress produces a greater response in crops compared to temperature stress; an indication that

plants in the tropics may be better suited to tolerate high temperatures alone. Although both studies demonstrate that traits linked with heat tolerance exist amongst genotypes, more experiment research is needed in this area to produce more valuable insights into the potential of Bambara groundnut in hotter climates.

9.5.3 Potential of Bambara Groundnut as an Intercrop

Any climate smart strategy to ensure food production must include sustainable soil management practices. Further, such an approach will also need to consider the fact that most farmers across semi-arid Africa are not sufficiently equipped with the technological or financial means of applying modern, conventional farm management techniques designed for monocultures (UNDP 2012). Intercropping and agroforestry are common practices throughout Africa and have already proved to be useful means of improving utilisation of plant resources, reducing soil compaction and at the same time improve yields whilst maintaining soil fertility and health (Khan et al. 2014; FAO 2012; Scherr et al. 2011).

Nitrogen fixation makes legumes valuable crops in low-input agriculture where nitrogen fertilizer is rarely used. Bambara groundnut is usually intercropped with cereals and root crops, namely maize, cowpea, sorghum, pearl millet and peanut (*Arachis hypogaea*) and is frequently used in crop rotations (Ncube and Twomlow 2007; Mukurumbira 1985). Studies exploring the nodulation capacity of the crop have found that Bambara groundnut's nitrogen requirement is met by nitrogen fixation and compares favourably against other legumes (Somasegaran et al. 1990; Doku 1969). Investigations into the crop's nitrogen requirements have also shown that while nitrogen fertiliser increases vegetative growth, there is relatively little gain in terms of yield development (Adjetey and Sey 1998). A deeper understanding of this process in landraces of Bambara groundnut is needed as those which are superior in fixing nitrogen may be potential sources of genetic variation for producing improved cultivars.

Kishinevsky et al. (1996) studied the nodulation and nitrogen fixation of 23 Bambara groundnut landraces from Malawi. Two strains of *Bradyrhizobium* (100 M and 280A, from *Macroptilium atropurpureum* and *A hypogea* respectively) were used to inoculate the plants at sowing in fields which were deficient in nitrogen. Much variation was found between the landraces for the attributes measured at maturity, such as nodule number, nodule mass, shoot dry weight, shoot nitrogen and pod yield. While there was no significant change in the number and weight of nodules, overall there was a significant increase in nitrogenase activity and nitrogen content of the inoculated plants which also varied between landraces. On the contrary, non-inoculated plants were free of nodules. The amount of nitrogen fixed accounted for 80 % of the total nitrogen content in the plants, which is in agreement with other similar studies (Kumaga et al. 1994; Dakora et al. 1992). It was also found that nitrogenase activity in most of the landraces increased significantly between 68 and 105 DAS, which represents the pod filling stage, which suggests

that the crop is able to provide nitrogen during pod and seed formation. This is comparable with the conclusions of Kumaga et al. (1994) who found that during reproductive development of Bambara groundnut nitrogen fixation was the primary source of nitrogen. In that same study, one of the landraces investigated fixed almost twice the amount of nitrogen as the other, highlighting the range of nitrogen fixing capacities between landraces. By quantifying such differences it will be possible to identify those landraces which can be used in breeding programmes to develop varieties with higher rates of nitrogen fixation.

In the light of Bambara groundnut's role in intercropping systems, investigating the interactions between the crops is essential. Intercropping provides great benefits in low-input agriculture on low fertility soils, as not only can it increase yields it can also ensure yield stability. The benefits of intercropping systems have thus been demonstrated in many legumes such as soybean (*Glycine max*) (Odhiambo et al. 2011) and groundnut (Langat et al. 2006). In order for intercropping to be of benefit, both crops need to be complimentary in the ecological niches they occupy in such a way that they do not compete for the same resources (e.g. water, nutrients and light) and consequently reduce yield. While uncommon in intensive farming involving monocultures, the method of intercropping is still important for yield stability in low-input systems, especially in Africa (Khan et al. 2014). Studies involving intercropping Bambara groundnut with other crops have demonstrated that for some species, intercropping increases overall yield. In some cases, incorporating Bambara groundnut into a rotation system has been identified as an efficient means of ensuring soil fertility. Work by Mukurumbira (1985) found that Bambara groundnut had a greater residual nitrogen effect than maize and groundnut, and that where rain is not a limiting factor; no nitrogen input is required for maize if it is grown after Bambara groundnut in a rotation. This is in agreement with recent work by Svubure et al. (2010), who assessed the soil fertility effects of different legumes when incorporated into a maize cropping system. Of all the legumes that preceded maize, growing Bambara groundnut lead to the greatest increase in maize yields (5.18 t ha⁻¹) compared to soybean (4.79 t ha⁻¹), groundnut (4.37 t ha⁻¹) and Natal Sugar bean (2.89 t ha⁻¹). In terms of intercropping Bambara groundnut with cereals, studies have generally shown that intercrops are more successful with pearl millet than with maize and sorghum and does better when the crop is quite scattered (fewer plants) (Heller et al. 1997). Karikari (2001) grew Bambara groundnut and sorghum in rows of different combinations of intercrops and as sole crops and determined the productivity of each in terms of a Crop Performance Ratio (CPR). This value is defined as the productivity of the intercrop per unit area of ground compared with the rows consisting of a single crop. The results demonstrated that the two crops are compatible and effective in an intercropping system, and that higher yields were achieved when the Bambara groundnut population in relation to sorghum was high (>50 %) than when it was low (<50 %).

Karikari (2002) furthered this work by intercropping three landraces, Diphiri Cream (DipC), OMotswasele (OM6) and National Tested Seed Red (NTSR) with sorghum. The sorghum variety Segaolane was used and, as in the previous study, was grown in different ratios with Bambara groundnut (3:1, 2:2 and 1:3). Using the

relative crowing coefficient (K) and relative yield total (RYT) to quantify the crop interactions, it was found that neither of the Bambara groundnut landraces inhibited or reduced the yield of sorghum. However, variation between the landraces was observed, with DipC having higher K and RYT values than the other two landraces. This suggests that DipC competes less with sorghum for resources and therefore is a more suitable landrace to use in an intercrop. As the study took place in Botswana, DipC may be a suitable target towards the development of an ideotype that can be used in intercrops with sorghum in that region. An additional benefit of intercropping Bambara groundnut is the protection it can offer as a 'trap' crop. Gworgwor (2002) conducted two trials in which Bambara groundnut and groundnut were grown separately as intercrops with varieties of sorghum that were either susceptible or resistant to *Striga hemonthica* (Del.); a parasitic plant. Groundnut and Bambara groundnut both significantly reduced the number of Striga on sorghum, with as much as a 50 % reduction with groundnut and 56–91 % reduction in Striga shoot counts.

The effect of intercropped millet and Bambara groundnut was studied by Hulugalle (2009) in the Sudan savannah in Burkina Faso. The treatments were grown on both flat land and on ridges and include a millet monocrop, a millet-Bambara groundnut intercrop and a Bambara groundnut monocrop. In the intercrop treatment, light interception and root growth was not significant affected and were lower in the Bambara groundnut monocrop. Soil water content was greater for millet in the intercrop treatment on the ridges and its Relative leaf water content (RLWC) was unaltered, although it was reduced in Bambara groundnut in particular on the flat plots. Further, dry matter yield of Bambara groundnut was increased when intercropped on the tied ridging plot. These findings therefore show that yield increase and the success of a millet-Bambara groundnut intercrop lie with planting being done on ridges.

Considering both sorghum and millet are staple crops in many climate vulnerable regions of Africa, such as the Sudanese and Sahelian savannahs of West Africa (Sultan et al. 2013), there is clearly great potential and a need for greater attention given to these cereal-legume intercrops.

A similar story can be seen with rice. Andika et al. (2010) who investigated the performance of an intercrop involving the new rice for Africa (NERICA) rice and Bambara groundnut paying particular attention to the spatial distribution of roots in the soil. Exploring the interaction below-ground is important as the soil contains the major source of resources for crop growth. It was not until 38 days after sowing (DAS) when changes in Bambara groundnut were first observed and it was not until 52 DAS when significant increases in root length were identified. For NERICA rice, no significant changes in root diameter or length were seen at 24 DAS and 38 DAS, but by 52 DAS there were significant differences in the root diameters and in root length between the landraces. However root length was significantly higher in Bambara groundnut as the root length of rice was actually higher as a sole crop. Conversely, the volume occupied by Bambara groundnut in the soil was higher in the intercrop treatment than as a sole crop. Root densities of both crops increased after 52 DAS in the intercrop although Bambara groundnut roots were still longer.

As for dry matter allocation, NERICA rice had greater allocation to the shoots when intercropped, while Bambara groundnut allocated more to the roots. This is in agreement with previous work which found that wheat (*Triticum aestivum*) increased in total above-ground biomass when intercropped with white clover (*Trifolium repens*) (Thorsted et al. 2006). It was therefore apparent that rice shifted its dry matter allocation depending on whether it was grown as an intercrop or sole crop which itself is an indication of crop competition. Such competition may be beneficial, because as root length and diameter of Bambara groundnut increases, it is able to mobilise nutrients and water from a deeper layers in the soil profile. This is particularly important in times of drought where water is limited. In addition, the nitrogen fixing capacities of Bambara groundnut may lead to less competition for nitrogen when roots are intermingled. This may explain the greater dry matter allocation to the shoots of rice when intercropped. With rice being one of the most important staple crops in many African countries (IFPRI 2012a, b, c), these findings suggest great potential for an intercropping system between the two species which has not yet been fully exploited.

9.6   Conclusion

Meeting the needs of a changing climate and responding to the threats it will bring is without a doubt one of the most urgent issues facing Africa today. These challenges will be further magnified by a rising population, which is expected to reach two billion by 2050 (UNDESA 2011). Indeed, Africa has the highest population growth rates in the world, with a tripling of the population from 230 to 811 million in the second half of the twentieth century (FAO 2011b). With this vast population and an abundance of untapped agriculture land, a more productive Africa would not only improve food security throughout the continent, but would be able to help contribute to the rising demands of food globally.

Sustainable increases in food production and nutritional security are at the heart of ensuring food security and improving livelihoods of families and communities. What is needed however is a new approach to food production that maximises resources, is concordant with the reality and needs of subsistence farmers and is environmentally sustainable. In the context of a drier and hotter Africa, achieving sustainable yields can be helped through utilisation of crop species native to the continent of Africa that can withstand such hostile conditions. Bambara groundnut, a crop native to the semi-arid tropics of Africa, has proved to meet the characteristics required of a potential food security crop. Research has demonstrated Bambara groundnut's considerable resilience as a drought tolerant crop relative to other legumes, its ability to produce yields on marginal lands and its rich nutritional content. Further, work has shown that as an intercrop it can not only contribute to soil nitrogen content but can help boost the yields of some of the continent's most important cereal crops, such as rice, maize, millet and sorghum. Further consideration and efforts from governments, policy makers, organisations and research insti-

tutions is essential for unlocking the potential of Bambara groundnut. While the time frame is small and the task formidable, increased utilisation of Bambara groundnut and other crops indigenous to the continent of Africa, can and should play a central role in adapting to the demands of changing African climates and in ensuring the food security and livelihoods of its people.

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