Progress in Botany

Francisco M. Cánovas
Ulrich Lüttge
Rainer Matyssek *Editors*

Progress in Botany 78

Springer

Progress in Botany

Volume 78

More information about this series at http://www.springer.com/series/124

Francisco M. Cánovas • Ulrich Lüttge •
Rainer Matyssek
Editors

Progress in Botany 78

Springer

Editors
Francisco M. Cánovas
Depto. Biología Molecular y Bioquímica
Universidad de Málaga
Málaga, Spain

Ulrich Lüttge
Department of Biology
TU Darmstadt
Darmstadt, Germany

Rainer Matyssek
Ecophysiology of Plants, School of Life Sciences Weihenstephan
Technische Universität München LS Ökophysiologie der Pflanzen
Freising, Bayern
Germany

ISSN 0340-4773 ISSN 2197-8492 (electronic)
Progress in Botany
ISBN 978-3-319-49489-0 ISBN 978-3-319-49490-6 (eBook)
DOI 10.1007/978-3-319-49490-6

Library of Congress Control Number: 2014949152

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Part IV Ecology

Contributors

Per Ambus Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark

Christian Ammer Silviculture and Forest Ecology of the Temperate Zones and Center for Biodiversity and Sustainable Land Use, Göttingen University, Göttingen, Germany

Lucía Arenas-Alfonseca Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Ángeles Aroca Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Daniel J. Ballhorn Department of Biology, Portland State University, Portland, OR, USA

Erwin H. Beck Department of Plant Physiology, University of Bayreuth, Bayreuth, Germany

Vagner A. Benedito Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA

Dan Bruhn Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark

Jürgen Burkhardt Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany

Marco Stefano Caccianiga Department of Life Sciences, Università degli Studi di Milano, Milan, Italy

Nathalie Ceusters Faculty of Engineering Technology, Department of Microbial and Molecular Systems, Bioengineering Technology, TC KU Leuven, Technology Campus Geel, Geel, Belgium

Johan Ceusters Faculty of Engineering Technology, Department of Microbial and Molecular Systems, Bioengineering Technology, TC, KU Leuven, Technology Campus Geel, Geel, Belgium

Samson Chimpango Department of Biology, Faculty of Science, University of Cape Town, Rondebosch, South Africa

Francisco J. Corpas Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Granada, Spain

Joanna Dames Department of Microbiology and Biochemistry, Faculty of Science, Rhodes University, Grahamstown, South Africa

Rafael Durán-García Centro de Investigación Científica de Yucatán A.C. (CICY), Unidad de Recursos Naturales, Mérida, Yucatán, Mexico

Wim Van den Ende Faculty of Sciences, Department of Biology, Laboratory of Molecular Plant Biology, KU Leuven, Leuven, Belgium

Brigitta Erschbamer Institute of Botany, University of Innsbruck, Innsbruck, Austria

Irene García Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Cecilia Gotor Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

David A. Grantz Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany

University of California at Riverside, Kearney Agricultural Center, Parlier, CA, USA

Sabine Hilt Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

Martyn Kelly Bowburn Consultancy, Bowburn, Durham, UK

Aleysia Kleinert Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Matieland, South Africa

Alfonso Larqué-Saavedra Centro de Investigación Científica de Yucatán A.C. (CICY), Unidad de Recursos Naturales, Mérida, Yucatán, Mexico

Ana M. Laureano-Marín Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Ting Liu College of Life Sciences, Hebei Normal University, Shijiazhuang, Hebei, P. R. China

Martha Méndez-González Centro de Investigación Científica de Yucatán A.C. (CICY), Unidad de Recursos Naturales, Mérida, Yucatán, Mexico

Teis Nørgaard Mikkelsen Department of Environmental Engineering, Technical University of Denmark, Lyngby, Denmark

Inmaculada Moreno Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Bruno Peixoto Faculty of Sciences, Department of Biology, BioISI – Biosystems & Integrative Sciences Institute, University of Porto, Porto, Portugal

Susana Pereira Faculty of Sciences, Department of Biology, BioISI – Biosystems & Integrative Sciences Institute, University of Porto, Porto, Portugal

José Pissarra Faculty of Sciences, Department of Biology, BioISI – Biosystems & Integrative Sciences Institute, University of Porto, Porto, Portugal

Luis C. Romero Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas, Universidad de Sevilla, Sevilla, Spain

Martin Schädler Department Community Ecology, Helmholtz-Centre for Environmental Research (UFZ), Halle, Germany

iDiv – German Centre for Integrative Biodiversity Research, Leipzig, Germany

Susanne C. Schneider Norwegian Institute for Water Research, Oslo, Norway

Zhonglin Shang College of Life Sciences, Hebei Normal University, Shijiazhuang, Hebei, P. R. China

Rochelle Thuynsma Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Matieland, South Africa

Alex J. Valentine Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Matieland, South Africa

Jan E. Vermaat Institute of Environmental Sciences, Norwegian University of Life Sciences, Ås, Norway

Torn Between Nature and Lab: A Dying Breed of Plant Scientists?

E.H. Beck

Contents

E.H. Beck
Department of Plant Physiology, University of Bayreuth, Bayreuth 95447, Germany
e-mail: erwin.beck@uni-bayreuth.de

Progress in Botany (2017) 78: 3–42, DOI 10.1007/124_2016_2,
Published online: 8 June 2016

Abstract In his life as a scientist, E. Beck addressed questions, concerning a very broad spectrum of disciplines in plant science. He likes research in the laboratory as well as in the field. Admitting that specialization is inevitable given the complexity of the systems we deal with, he is not flagging to encourage his students and colleagues to go back and consider the entire system once in a while for verification of the reasonability of their research questions and approaches.

1 Introduction: Studying Biology, Chemistry, and Geography

I grew up in an atmosphere created for generations by teachers. No wonder that I, too, aimed at becoming a teacher with a special interest in biology kindled by my mother. Due to the chaos of world-war II and the post-war period, biology was taught in school either not at all or as a minor subject not every year to the classes. Apart from the Mendelian laws I never received a lesson in botany during my entire school days. Starting from the scratch, lectures in botany at the University of Munich opened a new world for me when I learned that algae, herbs, shrubs, and trees, which I already knew by name, belong to the same kingdom, unified by a common type of metabolism, termed photosynthesis. In 1956 when I started my teacher's studies in biology, chemistry, and geoscience, vitaminized by educational

Preamble Having been asked to report on 55 years of my life as plant scientist is a great honor for which I am very grateful to Ulrich Lüttge, and which I would like to pass on to the numerous friends, colleagues, and students to whom I feel deeply indebted for their contributions to my projects and ideas, and *vice versa* for sharing their projects with me. It is a pity that space limitation constrains my presentation to the more complex subjects and to leave out many projects and experiences which, while challenging my skills, enriched my life as human being and as scientist as well. Unforgettable are, e.g., the three expeditions with the group of soil scientists led by my friend Wolfgang Zech to the Himalaya where we tried to reconstruct landscape history around the Annapurna massif from soil cores and pollen diagrams (Zech et al. 2001a, b). Thinking of Nepal and the Tribhuvan University in Kathmandu is also not possible without mentioning the fruitful cooperation in plant molecular biology with my friend Tribikram Battarai (Beck et al. 2007) and his doctoral fellow Deepak Pant. I apologize for not expanding to the fruitful and long-standing collaboration with Prof. J. C. Onyango PhD (e.g., Netondo et al. 2004a, b), and his students from Maseno University (Kenya) and to the fantastic revival of my experiences on Mt. Kilimanjaro with my friends Andi and Claudia Hemp. These names stand for many others whom I could not mention for shortage of space and I hope that they would forgive me. To illustrate the environments of my professional life and its relationship with the time spirit and with academic and social events, I include a few snapshots, termed "interludes" in the text. As this contribution is dedicated to research, I will abstain from expanding my experience in academic teaching which in the retrospective was inherently positive, whenever I had to do with students. Special highlights were the botanical fieldtrips to many exciting regions in Europe, Africa, and Australia. Representatively for such fieldwork, I refer to the outstanding Summer Schools of the German Studienstiftung in the Alps where teaming up with my friend Ulrich Lüttge, we could introduce highly interested students to the exciting life of plants in the stressful alpine environment.

theory, philosophy and psychology, the path of carbon in photosynthesis had just been published with many open questions and the term "Calvin Cycle" had not yet been coined. The required sources of reducing equivalents and ATP were still unclear, as neither the pathway of the photosynthetic electron transport, nor the chemi-osmotic ATP-synthesis were yet known. Later, while doing advanced practical in botany, dedicated to details of plant structure, our supervisor, Meinhart Zenk, who just had returned from Purdue University fascinated us with the latest news in plant biochemistry, such as the Krebs cycle and Daniel Arnon's idea of the *zig-zag*-photosynthetic electron transport. In the late 1950s, neither microbiology nor genetics was represented in the Biological Faculty by a professorship in Munich and therefore also not included in the curricula. Microbiology was an excursus of the lecture "General Botany" and genetics likewise of "General Zoology". Studying also chemistry promoted my interest in biochemistry and I was happy to attend a lecture by the later Nobel Prize laureate Feodor Lynen.

2 Chemotaxonomy

At that time, a few groups, in particular Hans Reznik in Muenster had introduced secondary plant constituents as characters in plant taxonomy, especially the recently discovered "nitrogen-containing anthocyanins" (now known as betacyanins) as a unique character of the entire group of the Centrospermae. Interested equally in plant taxonomy and plant biochemistry I asked the plant systematist,[1] Prof. Herrmann Merxmüller for a problem that could be solved by chemotaxonomy. This was pioneer work in Munich, and since there was no lab in the Institute of Systematic Botany I got a working place in the new Institute of Pharmacy of the University of Munich where paper chromatography had just been introduced as a method of analysis. My question was, whether the Centrospermae form a consistent group with only one exception, the carnations (Silenoideae) or whether there are more families in this group which share the chemical marker "true anthocyanins." With a thesis in which I could show that also the Molluginaceae, Plumbaginaceae, and the Alsinoideae contain anthocyanins instead of betacyanins and thus together with the Silenoideae form a separate branch (Caryophyllinae) within the Centrospermae (Beck et al. 1962), I finished my teacher's study in 1961 with the state examination. As a trainee teacher in a secondary high school in Munich I had the opportunity to continue my studies in plant chemotaxonomy for a doctoral thesis on more Centrospermae as well as on the Primulaceae for which a separation of the genera *Lysimachia*, *Anagallis*, and *Cyclamen* from the Primulaceae *sensu stricto* had been proposed (Lys 1956). In addition to confirming my earlier results on the Caryophyllinae I could show that a separation of the

[1] At that time plant taxonomy (without emphasis in phylogenetic relationship) was differentiated from plant systematics, aiming at elucidating phylogenetic relations.

Primulaceae into the proposed branches could not be supported by chemical characters (Beck 1963).

Interlude 1: Attaining Dr. Rer. Nat. in the Sixties *It might be of interest to our junior staff that at that time the realization of a doctoral study was quite different from to-day's mode: The subject was usually proposed by the student after a thorough discussion with the supervisor and an approximate date of submission of the thesis in 2 or 3 years was agreed upon; regular reports about the progress of the work were not expected and the costs for equipment and consumables had to be defrayed by the candidate, as well as the costs for using lab facilities. Doctoral students were not payed, except a small compensation, if supervising students in a practical. Of course the subjects of the doctoral theses were less comprehensive and the volumes of the scripts were smaller, usually not exceeding 100 pages. There was no pressure on publishing the work, as a doctoral thesis was acknowledged as a publication providing more details than a publication in a journal. External funding of projects was an exception, as doctoral students did not participate in the departments' budget. Also the final exam, termed "Rigorosum" was more comprehensive: Mine consisted of two orals (1 h each) in botany (systematics and physiology), one in zoology and one in inorganic chemistry. The system clearly promoted a broad knowledge in natural sciences including biology. With that knowledge and skills postdocs could enter a university career or could join a research team, mostly in the chemical/pharmaceutical industry. This system changed, triggered by the students' riots in the late 1960s and the advancing specialization that was promoted by the upcoming technical revolution in laboratory equipment and methodology, concomitant with a dramatic rise in the costs of research. As a consequence external project funding by the government, the German Research Foundation or the industry became more important including salaries for the doctoral candidates.*

My training in plant taxonomy was and still is a fruitful basis of my research, as well as of my teaching botany in lectures, practicals, and fieldtrips. Even when investigating chemical markers, I had to know about all other types of characters and their importance for taxonomy, had to know about the phylogeny of my groups in particular and of the plant kingdom in general, had to know details of their way to propagate, about their ecology and geographical distribution. Having the entire plant in mind when following a particular research question helps to place this problem into a wider context, to comprehend the experimental plants as individuals with a certain life history and in a given environment. This has now been termed "Systems Biology."

Interlude 2: A Plea for Systems Biology *To illustrate the value of that kind of systems biology, let me briefly tell a story which is only partly publishable (nevertheless, we can learn a lot from experiments which do not meet our expectations): A doctoral student tried to improve the yield of a wheat cultivar from his country Argentina by enhancing the cytokinin content especially of the flag leaf which is the main carbohydrate provider for the grains. The idea was to extend the lifetime of this*

leaf by a higher cytokinin content. From his study we learned three lessons. First, it is possible to increase the cytokinin content and also lifetime of the flag leaf a little by introducing an additional gene for cytokinin synthesis, the isopentenyltransferase gene from Agrobacterium tumefaciens under the control of the senescence-specific promotor HvS40. But expression of the gene was not consistent and increase of the grain yield was either negligible or sporadic (Souza-Canada 2012). Second, not only a prolonged carbohydrate input is crucial for an enhanced yield, but also the supply of the seeds with other nutrients. Grain development and filling is a multi-faceted complex process, encompassing the entire plant. It cannot be over-run by fortifying only one single reaction, even if this controls one of the major processes. Third, and this tells us about the importance of the life history of our experimental plant material: Bread–wheat as a hexaploid species comprises the genomes of 3 other grass species, one of which is Aegilops tauschii (genome DD). When growing the T2 generation of our transformed wheat, several plants emerged which clearly showed the characters of Aegilops; we interpreted this as an effect of the changed hormonal pattern; however the effect was not reproducible and finally it turned out that the overly expression of the Aegilops genome must have resulted from an infection of the plants by the barley midge known as Hessian fly.

3 Plant Biochemistry

Soon after receiving my doctoral degree in 1963, Prof. Dr. Otto Kandler offered me a fellowship (later a staff position) in the Department of Applied Botany at the Technical University of Munich. So I left school teaching and started for a career as plant scientist. Kandler is a plant physiologist and a microbiologist as well. I decided to stay with plants moving from taxonomy to plant biochemistry. But at the same time I learned the basics of working with bacteria of which I could make good use in my later work. During his postdoc period in the USA Kandler had worked with Melvin Calvin and Martin Gibbs joining their research in photosynthetic carbon metabolism. In the context of the Calvin cycle were a lot of open questions and Kandler was not absolutely convinced of the proposed mechanism of CO_2 fixation resulting in two molecules of 3-phosphoglycerate. He favored the idea that the CO_2-fixation product could be immediately reduced to yield a branched-chain hexose. The respective monosaccharide D-hamamelose was known as glycosidic moiety of a secondary constituent of the bark of witch hazel (*Hamamelis virginiana*). He suggested to investigate the biosynthesis of this sugar. To that end he offered me a working place in his isotope lab in the dairy institute of the Technical University in Freising which was the only laboratory for studies with radioactive tracers in plant science in Munich at that time.

3.1 Plant Branched-Chain Monosaccharides

To achieve the lecturer's degree (Habilitation) I had to deliver a thesis to the faculty, and I decided to address the biosynthesis of the two branched-chain monosaccharides of higher plants: the pentose D-apiose and the hexose D-hamamelose. D-apiose was well known as the glycosidic component of the flavone apiin, but its occurrence also in cell walls of a few plant species had been suggested. I succeeded in isolating a D-apiose containing pectin form the cell wall of duckweed and could establish its structure (Beck and Kandler 1965; Beck 1967). With sterile cultures of another duckweed species (*Lemna gibba*) I could elucidate the entire biosynthetic route from glucose via D-glucuronic acid, decarboxylation of carbon-6 to yield 4-keto-D-xylose from which D-xylose results upon reduction, and in a parallel step, D-apiose by intramolecular rearrangement and, again, reduction (Fig. 1). Since glucuronic acid, xylose and apiose are incorporated into cell wall polysaccharides we concluded that the entire pathway takes place in the nucleotide-activated state of the sugars (Beck and Kandler 1966).

3.2 The Hamamelose Story, Part I

Because of Otto Kandler's interest I further concentrated on the biosynthesis of D-hamamelose. A doctoral student of Kandler, Josef Sellmair at the same time investigated the occurrence of hamamelose in the plant kingdom establishing a kind of "chemical herbarium." To that end some 600 plant species were exposed to air containing 1% $^{14}CO_2$ under high light and the labeled compounds were separated by 2-dim paper chromatography and identified to the extent possible. This "^{14}C-herbarium" was the basis for many theses in plant carbohydrate metabolism in the Kandler group. Surprisingly, D-hamamelose could be demonstrated in almost all investigated species except for the Leguminosae. Highest concentrations were in the primrose family, where also the corresponding polyol hamamelitol (Sellmair et al. 1968) and a hamamelitol-containing disaccharide clusianose (Beck 1969) could be identified. While also participating in these studies my group focused on the biosynthesis of hamamelose as a potential early product of photosynthetic CO_2

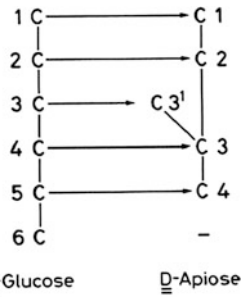

Fig. 1 Biogenetic relations between the carbon atoms of D-glucose (respective D-glucuronic acid) and of D-apiose (from Beck 1982)

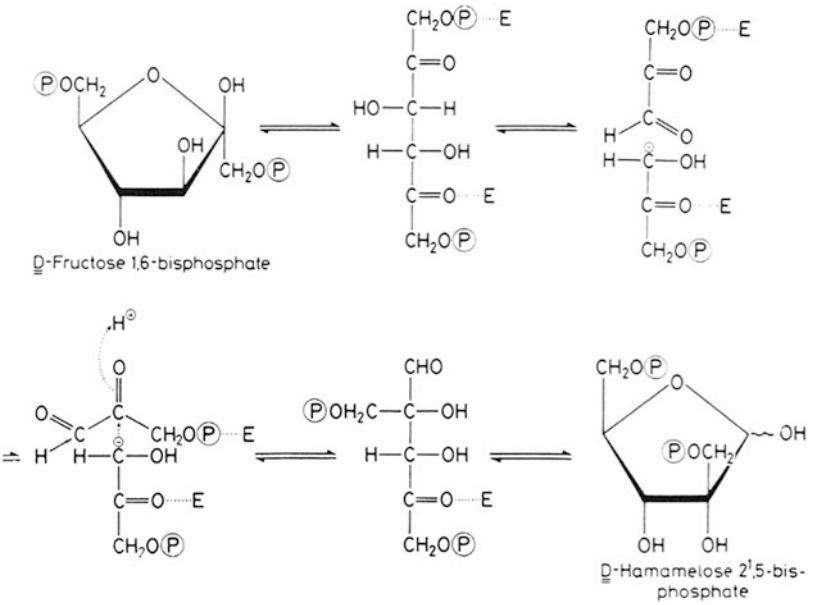

Fig. 2 Interconversion of D-fructose 1,6-bisphosphate and D-hamamelose 2^1,5-bisphosphate in chloroplasts. E... suggests fixation to the enzyme (from Beck 1982)

fixation. Therefore we incubated photosynthetically active intact spinach chloroplasts in bright light with ^{14}C-bicarbonate for a short-time span and investigated the labeled products. In place of free hamamelose in the chloroplast extract we could identify labeled D-hamamelose2^1-5-bisphosphate (Beck et al. 1971). The urgent question now was, as to whether carbon2^1 which could have derived directly from CO_2 was the "hottest" carbon after short-time photosynthesis in $H^{14}CO_3^-$.

With primrose leaves (*Primula clusiana*) we had already elucidated the biosynthetic relations between the carbon atoms of glucose and hamamelose and proposed an intramolecular rearrangement by which all carbons of glucose are incorporated into hamamelose (Beck et al. 1968). To that end I developed a procedure of chemical degradation of hamamelose and isolation of the individual carbons. Similarly the hydrogens of hamamelose could be traced to those of ^{3}H-position-labeled glucoses. Using these methods we could show that the immediate precursor of hamamelose2^1,5-bisphosphate is fructose 1,6-bisphosphate and that the carbon2^1 is not the carbon immediately derived from CO_2 (Gilck and Beck 1973, 1974). We could further show that free hamamelose results from a stepwise and presumably unspecific dephosphorylation (Beck and Knaupp 1974) in the cytosol where free hamamelose accumulates. However we could not show any kind of metabolism apart from reduction to hamamelitol, mainly in the primroses (Fig. 2). Hamamelose-bisphosphate in contrast is metabolized, at least by reconversion into fructose-bisphosphate. The enzyme that catalyzes the interconversion appears to follow an aldolase mechanism. Unfortunately we were not able to isolate and investigate this enzyme in detail, we only found it with the thylakoid fraction. At that state we finished our work on hamamelose.

3.3 Out of Hibernation

The issue slept for 15 years when a potent inhibitor of Ribulose-bisphosphate carboxylase/oxygenase (RUBISCO) was found (Gutteridge et al. 1986). It was described as 2-carboxyarabinitol-1-P. By several methods we could show that this compound is D-hamamelonic acid2[1]-P (Beck et al. 1989b). By infiltration of bean leaves with ^{14}C-labeled hamamelose in the dark, Andralojc et al. (1996) showed the formation of ^{14}C-labeled 2-carboxy-D-arabinitol (CA, D-hamamelonic acid) and 2-carboxy-D-arabinitol 1-phosphate (CA1P, hamamelonic acid2[1]-P). The crucial step filling the gap between our studies and those on the inhibitor is the oxidation of hamamelose to hamamelonic acid (CA). It escaped our attention because it is formed in the dark or under very low light, whereas our experiments were performed in highlight. Andraloic et al. (2002) showed the entire pathway to CA1P: Dephosphorylation of HDP to free H, NADP-dependent oxidation to CA, and rephosphorylation to CAP1, which as an analogue to the transition product of CO_2 fixation binds to RUBISCO. Removal of the inhibitor is either by a specific phosphatase producing free CA or by the light-activated RUBISCO activase. The additional regulation of the RUBISCO activity is neither ubiquitous in the plant kingdom, nor a feature of special plant families.

3.4 The Hamamelose Story, Part II

In the leaves of *P. clusiana,* D-hamamelose and in particular hamamelitol (and to a lower extent clusianose) accumulate to considerable amounts especially under low temperatures. Even prolonged cultivation of the plants did not result in a measurable decrease and senescent leaves still contained substantial amounts of these carbohydrates. We therefore tried to find microorganisms that grow on D-hamamelose and used soil from underneath rosettes of the primrose for our search. We found a strain of *Pseudomonas* which could use hamamelose as a hydrogen donor, producing hamamelonic acid (Thanbichler et al. 1971), however, no degradation of the carbon skeleton could be observed. Therefore we collected bacteria from the surface of the leaves and isolated a strain identified as *Kluyvera citrophila*, from the Enterobacteriaceae. It differs from the American Type-Culture Collection strains only by the capability to grow on hamamelose as the only carbon source and was given the strain number 627. Using uniformly ^{14}C-labeled D-hamamelose in a time kinetics experiment we could show its fermentative dissimilation and compare it to the dissimilation of uniformly and position-labeled ^{14}C-glucose (Thanbichler and Beck 1974). The sequence also comprises an intramolecular rearrangement from the branched-chain sugar to a straight-chain hexose which takes place at the stage of monophosphates:

$$\text{Hamamelose} \rightarrow \text{hamamelose } 2^1\text{-P} \rightarrow \text{fructose 1-P} \rightarrow \text{fructose 1,6-bisP} \rightarrow \text{glycolysis}$$

The first enzyme, ATP: hamamelose2^1 phosphotransferase, is specific for hamamelose and different from hexokinase or glucokinase from *Kluyvera* (Beck et al. 1980). The second enzyme requires free hamamelose for activation and catalyzes the intramolecular rearrangement without liberation of triose or triose-P (Wieczorek 1976). The third enzyme is complementary to phosphofructokinase. Under aerobic conditions, only $^{14}CO_2$ was found as degradation product of hamamelose by *K. citrophila 627*. So, at least a microbial pathway for degradation of D-hamamelose could be shown. However, the question of a physiological or ecological function of the high concentrations of hamamelose and its derivatives in the primrose leaves still remained open. As mentioned above, these sugars accumulate especially in the cold and their content in leaves of same age fluctuates during the course of the year, being highest in winter. Polyols are known as compatible solutes improving drought and cold resistance. Compounds which resist metabolic degradation are in particular suitable for that function and the members of the hamamelose family appear to be striking examples.

Interlude 3: Experimental Skills and Efforts *At this point I would like to briefly mention how we measured radioactivity (^{14}C, ^{3}H) in the 1960s. In 1968 Otto Kandler's group bought the first liquid scintillation counter in Munich. The technicians of the company and we were similarly pioneering with the new method which all of us had to explore from the scratches. Before we got this advanced tool, ^{14}C and ^{3}H had to be measured in the gas phase. The crystalline and dry compounds were combusted in a special oven and $^{14}CO_2$ and ^{3}H (as water) were separated as gases by their different freezing points in high vacuum, purified and frozen at −196°C into the so-called counter tubes which were backfilled with an inert gas (mostly helium) before attaching to a counter. The character line of the filled tube had to be determined for each sample to find the "plateau," where an increase of the voltage did not further enhance the count rate. The plateau represented the amount of radioactivity as "counts per unit time." Measuring a plateau took between two and several hours depending on the amount of radioactivity. At that time I was not yet married and had an air mattress in my office and an alarm, working day and night for counting my radioactive samples in due time.*

3.5 From Postdoc to Lecturer

In October 1967, Otto Kandler encouraged me to combine the pieces of my work on branched-chain carbohydrates in a thesis ("Isotopenstudien zur Biosynthese der verzweigt-kettigen Monosaccharide der Höheren Pflanzen") to be submitted to the Faculty of Natural Sciences of the Technical University Munich for achieving the lecturer's degree (Habilitation). The following examination was in January 1968.

At that time, the candidates in addition to delivering a lecture on a subject selected by the faculty (without connection to the research of the applicant) had to undergo an interview in a faculty assembly where members could ask any kind of questions. Preparation for that event gave me a tough time due to the breadth of the faculty's disciplines, including mathematics, geometry, physics, chemistry, biology, earth sciences, ergonomics, and photography – in total there were 32 members who might ask questions. It was a check of the candidate's general scientific standing.

As a lecturer I could supervise diploma and doctoral students in my group that grew since 1968. I had so many ideas – but now I had to raise the funds. In the 1970s the competition for funds was not as strong as today, and I received funds from the Bavarian Ministry of Research, from the Federal Ministry of Education and Research, from the VW-Foundation, and in particular from the German Research Foundation. In 1969 I accepted an associated professorship at the Botany Department of the University of Munich with a small budget, ample laboratory space, and a considerable teaching load.

My particular task, the establishment of an advanced practical course and a lecture on plant ecology, was the trigger of another shift in my interests in plant science. The exciting field work in the scope of the practical course urged me to also enter basics of geobotany, phytosociology, and soil science which partly complied with my training as plant taxonomist. While still working in the lab, I got interested in ecophysiological problems, such as frost resistance of perennial plants and the association between nitrogen metabolism and the competitive strength of weeds.

4 Plant Physiology: Isolated Chloroplasts as a Powerful Tool

Between 1969 and 1975 (when I followed a call to a full professorship for plant physiology at the University of Bayreuth), we worked mainly with photosynthetically competent isolated chloroplasts. After the move to Bayreuth, we continued these studies but started additional projects, which I will report on later.

With isolated chloroplasts we worked on five major topics: The formation of glycolate during photosynthesis (J. Eickenbusch), the role of oxygen in photosynthesis (H.M. Steiger, D. Groden), formation and degradation of assimilatory starch (P. Pongratz), light activation and inactivation of chloroplastic enzymes (R. Scheibe), and frost hardening and dehardening of spruce and its chloroplasts (M. Senser). The available space does not allow me to report many details about this fascinating work with intact and "broken" chloroplasts (thylakoids) striving for the "world record" in chloroplastic photosynthetic CO_2 assimilation; unfortunately, we were consistently second to David Walker (Sheffield) who used pea chloroplasts.

Interlude 4: Safety Inspectors Not Allowed *Preparing such chloroplasts was an art by itself, starting from the optimal growth of the spinach, the time of leaf harvesting and then, most important the speed of the preparation. Less than 10 minutes were allowed for processing from the leaves to the purified intact*

chloroplasts, which was only possible by braking the centrifuges by hand. Richard Jensen (Phoenix Arizona), Erwin Latzko (TU Munich), and Ulrich Heber (at that time University of Düsseldorf) were my taskmasters in chloroplast preparation and David Walker introduced us in using his clark electrode for oxygen measurements.

4.1 The Chloroplast's Handling of Oxygen

With our chloroplasts we could show that a minor amount of the early photosynthetic product glycolate resulted from the oxidation of "activated," i.e., transketolase-bound glycolaldehyde by an unknown oxidant, whereas the bulk of glycolate originates in an oxygen dependent reaction from Ribulose-bisphosphate (Eickenbusch and Beck 1973; Eickenbusch et al. 1975; Beck et al. 1974). This coincided with Tolbert's group (Lorimer et al. 1973) publishing the oxygenase reaction of RUBISCO. During the course of our studies on glycolate formation, we became interested in the role of oxygen in the chloroplast metabolism. When searching for the unknown oxidant of activated glycolaldehyde we came across H_2O_2 and its formation and biochemical destruction by the chloroplast. In the scope of these studies we measured the oxygen concentration in photosynthesizing intact chloroplasts (Steiger et al. 1977) and found that H_2O_2 is formed especially if the natural electron acceptor NADP falls short. Continuous removal of oxygen from the chloroplasts inhibits photosynthesis by over-reduction of compounds of the linear electron flow (Steiger and Beck 1981). Although initially considered without special function we investigated the role of ascorbate in photosynthetic CO_2 assimilation. In these studies we found the membrane-bound ascorbate peroxidase, showed its substrate H_2O_2, and characterized its properties (Groden and Beck 1979). Now, we could put together the reactions of the so-called pseudocyclic photosynthetic electron transport, now known also as the "water-water cycle" and explained the function of oxygen for the poising of photosynthetic NADP reduction and ATP-formation (Steiger and Beck 1981). In that context we could also demonstrate the presence of a specific ascorbate transporter in the chloroplast envelope which facilitates equilibrium between the stromal and the cytosolic ascorbate concentrations or the exchange of didehydroascorbate for ascorbate (Beck et al. 1983).

Interlude 5: A High-Ranked Guest *Let me, for a moment jump ten years ahead. Having been competitors in the early days of glycolate research, I was surprised to receive a telephone call from Ed Tolbert, in which he asked me, whether he (and his wife) could come to my department for half a year with money from a Humboldt award. I agreed and we had a very pleasant and interesting time together, and the former competitors became friends. Our idea was to check, whether in analogy to the photosynthetic CO_2 compensation point, C_3-plants would also show an oxygen compensation point (Γ_{O2}), i.e., an atmospheric oxygen level with a given CO_2 level and temperature, at which net oxygen exchange is zero. For tobacco at 220 ppm CO_2, Γ_{O2} was at 23% O_2, increasing to 27% at 350 ppm CO_2 and 35% at 700 ppm*

CO_2 (Tolbert et al. 1995). Unfortunately, Ed died 10 months after his return to East Lansing, and since I was his last "coworker," I was invited for an opening lecture of a Gordon Conference that was dedicated to his memory. Later, we continued that research growing tobacco plants at different oxygen concentrations. Expectedly plants showed reduced growth at an atmosphere with a higher oxygen concentration, however, the data are still sitting in my desk, waiting for publication.

4.2 Poising in Photosynthesis

It opened another branch of our studies with intact photosynthesizing chloroplasts. The Calvin cycle alone consumes more ATP as the linear electron transport can produce. Support by the cyclic electron transport could take up the slack but obviously cannot completely prevent over-reduction of the NADP and associated pools of electron acceptors, leading to H_2O_2 formation. In the mid-1970s the question of **light activation of chloroplastic enzymes** in particular those of the Calvin cycle came up. Interestingly, a non-photosynthetic enzyme, NADP-dependent malate dehydrogenase showed strong activation by light (Scheibe and Beck 1979). It could prevent over-reduction of NADP by consuming reduction equivalents in the presence of oxaloacetate and was therefore termed "malate valve" (see also Scheibe and Beck 1994). An activator protein, whose nature was unknown (now known as the thioredoxin system, see Schürmann and Buchanan 2008), was concluded to effect light activation of chloroplastic enzymes. In 1979 Renate Scheibe went for a postdoc stay to Louise Anderson (University of Illinois, Chicago) to work on that light effector mediator (LEM) which controls the activity of the enzymes catalyzing the irreversible reactions of the Calvin cycle (as well as the inactivation of glucose-6-P-dehydrogenase in the light). It was shown that the LEM was identical with the thioredoxin system.

Interlude 6: The Origin of the Wallenfels Meetings *With respect to the light activator, Louise Anderson and Bob Buchanan were strong opponents. For the 6th International Congress in Photosynthesis in Brussels (1984), Renate Scheibe, after her return to Bayreuth, had proposed a satellite meeting on light activation of enzymes. We organized this meeting in the ecological field station of the University of Bayreuth in the little city of Wallenfels north of Bayreuth, and hired a bus to bring the 33 participants in 2 days from Brussels to Wallenfels. As this meeting was not only very successful but also highly delightful, e.g., with a ride on a raft, it was decided, that such a meeting on photosynthesis should be held every spring in Wallenfels. This was the beginning of the almost legendary Wallenfels meetings, meanwhile 32 by number, which are always overbooked, although they are not externally funded. Illustrious names garnish the list of the organizers: Hans Heldt, Ulrich Heber, Erwin Latzko, Renate Scheibe, Stephan Clemens, and Ekkehard Neuhaus. In the meanwhile the topic of the meetings has been widened from photosynthesis to plant biochemistry. Many professors of plant physiology and*

biochemistry had entered the Wallenfels community as junior staff, selected by their professors to present their work.

Two more topics merit mentioning when reflecting on our work with chloroplasts.

4.3 Starch Metabolism and Frost Hardening

Preparation of photosynthetically competent chloroplasts depends on the integrity of the thylakoids as well as the envelope. Therefore it is necessary to harvest the leaves at the end of the night when the granules of assimilatory starch have disappeared and would not disrupt the chloroplast upon centrifugation. On this background we became interested in the starch metabolism of chloroplasts (Beck and Ziegler 1989), especially in the degradation of the starch granules, their chemical composition and changing appearance. We found an oscillating amylolytic activity (endo-, exo-amylase, and a debranching enzyme) in isolated chloroplasts which is partly due to the diurnal change of the stromal pH that strongly curtails the activity of the system during the light phase, while inhibiting it only slightly in the dark (Pongratz and Beck 1978; Beck et al. 1981). We could show that the major end-product of the nocturnal starch degradation is maltose, which is exported by a maltose-specific transporter in the chloroplast envelope (Rost et al. 1996). It took a while, until this contribution to the chloroplastic starch metabolism was recognized and acknowledged, but meanwhile the transporter MEX1 (maltose export 1) has been studied in detail (Niittylä et al. 2004). We further concentrated on the debranching enzyme as the pacemaker of the degradation of the starch granules. The enzyme which consists of a single polypeptide chain showed up to seven interconvertible forms upon gel electrophoresis and isoelectric focusing which all exhibited debranching activity (Ludwig et al. 1984; Henker et al. 1998). Since this was a strange behavior of a protein, we isolated and sequenced its DNA and expressed it in *Escherichia coli* (Renz et al. 1998). The heterologous protein showed the same interconvertible forms which had different specific activities. Interconversion and change of the overall specific activity is triggered by a pH–shift, by addition of a substrate (e.g., amylopectin), or by reduction/oxidation. A shift in this microheterogeneity could serve the regulation of the enzyme activity (Schindler et al. 2001) in a way that could be considered a primitive form of allosteric regulation.

4.4 Frost Hardening of Plants, Part I

Reversible deposition of starch in chloroplasts associated with their change in function was the starting point of our investigations on frost hardening and

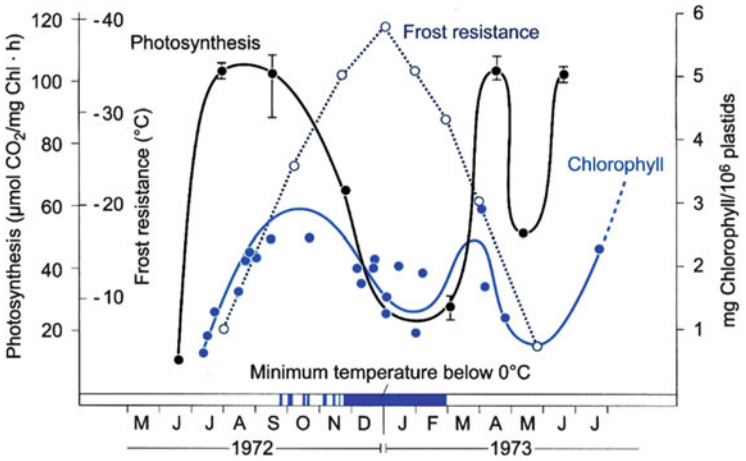

Fig. 3 Photosynthetic capacity (1% CO_2), chlorophyll content, and frost-resistance of one generation of spruce (*Picea abies*) needles measured under identical conditions in the course of the year (from Senser and Beck 1979)

dehardening of spruce needles which we later extended also to Scotch pine needles. We started with an EM study of the shape, thylakoid system, and starch deposition in spruce chloroplasts during the annual course. Evergreen conifers do not need to accumulate storage material in autumn for sprouting in spring, as, e.g., apple trees, because they can produce it by photosynthesis when needed. Shortly before sprouting of the new needle generation the chloroplasts of the older needles accumulate large amounts of starch, mostly from ongoing photosynthesis and thus become amyloplasts with only a few thylakoids remaining. After consumption of the starch for the formation of the new needles, the chloroplasts rebuild their thylakoid system and act as normal chloroplasts accumulating starch during daytime and degrading it during night. Upon frost hardening in autumn the chloroplasts swell, become amoeboid, and partly disintegrate the thylakoid system which recovers at the end of the winter to photosynthesize and deposit the big amount of starch for sprouting. Degradation of the thylakoid system, either during frost hardening or upon the functional change into amyloplasts is mimicked by the chlorophyll content of the plastids (Senser et al. 1975). Division of chloroplasts in the overwintering needles was observed very rarely and thus the entire annual change of form and function of the plastids is understood to take place in the extant organelles. Subsequently, we focused on the annual fluctuations of frost hardiness and sensitivity, measuring the photosynthetic capacity of needles and chloroplasts. Frost resistance is lowest in June and July when minus 10°C kills the needles while in winter temperatures as low as minus 40°C and even lower can be survived. Photosynthetic capacity in winter is about 25% of that achieved in summer. In the amyloplast stage of the plastids, a residual photosynthetic activity of around 40% remains (Senser and Beck 1977, 1979). Frost hardiness and photosynthetic capacity of the chloroplasts are countercurrent (Fig. 3; for physiological interpretation see

Sect. 4.6). Changes of frost hardiness in spruce chloroplasts are accompanied by changes of the lipid: protein ratio in favor of the lipids and by a desaturation of the lipid fatty acids resulting in a lower viscosity of the membrane system and the envelope (Senser and Beck 1982a). With small spruce trees which we cultivated for at least 3 years under different combinations of day length and temperatures we showed that the changes in membrane lipids could never be separated from the degree of frost resistance and that short-day conditions as well as subzero temperatures induced these changes (Senser and Beck 1982b). The changes in lipid composition could also be related to the photosynthetic capacity, as indicated by an Arrhenius plot of the Hill reaction.

Interlude 7: Gaining Momentum in Bayreuth *This might be the right place to change the topic for a while and report about my move to the University of Bayreuth in 1975, where the first building was just brought up and the offices of the few professors were put together in a wooden barrack, while their labs were still at their home universities. As ecology has been put up as one main focus of this university, each professor should dedicate part of his research to an ecological problem. One of my ecological interests was frost resistance of plants. While still working on that topic in Munich (see above), I also tried to broaden my view having come across another type of frost resistance which lasts all year round: The frost tolerance of tropical alpine plants. After his retirement from the University of Tübingen my former academic teacher in cryptogam botany in Munich, Karl Mägdefrau, inspired me with his reports about the afroalpine vegetation and the giant rosette Senecios. After a student's fieldtrip to Mt. Kenya in 1974 I burnt for research on these plants. At the same time, our proposal for a botanical garden of the University of Bayreuth was accepted by the Government of Bavaria under the condition, to create a novel type of garden which at the same time serves research, academic teaching, and the public as an area of education and recreation. The plan was an ecological botanical garden with several stations for demonstration purposes and ecological experimentation, also showing typical vegetation of selected areas. Instead of an alpine garden which is of the common jewelry of most botanical gardens we proposed a greenhouse for tropical alpine vegetation which would be unique worldwide in cultivating the giant rosette plants.*

4.5 Facing Mt. Kenya[2]

For collecting these plants and to investigate and document their environmental conditions, I organized an expedition with my entire group to Mt. Kenya in 1979. At that time export of living plants and seeds was easy, as the Convention on Biological Diversity was not yet on the horizon. That expedition was supported by

[2] Title of a book by Yomo Kenyatta, the first President of Kenya after its liberation.

the Bavarian Ministry of Research and Arts and we worked for 6 weeks in an absolutely fantastic landscape between 4,000 and 5,000 m. With the produced data, we could not only feed the engineers who worked on the construction of the above-mentioned special greenhouse, but together with my colleague Detlef Schulze could also prepare a concept for ecophysiological research on the tropical alpine plants, to be submitted to the DFG for funding. We succeeded and went several times to the East African high mountains including also vegetation analysis initiated by my former colleague Helmut Rehder from the Technical University in Munich. The scientific return of these expeditions was remarkable. Among others we now understand the type of frost hardiness which is required by plants living in a climate of "everyday summer, every night winter" (Hedberg 1964), which follows several mechanisms: Extracellular freezing of the cell water creating an extremely negative water potential at which water vapor bubbles form in the vacuole. This contrasts with supercooling, where crystallization of cellular and tissue water is avoided. Other mechanisms are delaying of freezing by accumulation of large volumes of liquid, or insulation, either permanent or temporarily during the cold night by nyctinastic leaf movement, forming a closed "night bud" which protects the central leaf bud from radiation emission and freezing (Beck 1982, 1984). We included also photosynthesis in our program, showing that the leaves are photosynthetically fully competent immediately after thawing, but that too high intensities of radiation result in photoinhibition. The plants avoid this stress by an almost vertical position of the leaves during hours of bright sunshine (Schulze et al. 1985; Bodner and Beck 1987). Within the scope of the project several other studies could be placed, the most important of which is the result, that fire might be more selective for the composition of the afroalpine vegetation than the nocturnal frost all year round (Beck et al. 1986a; Hemp and Beck 2001). Together with my Kenyan friend Prof. O. Kokwaro (Nairobi) we produced a vegetation map of the alpine zone of Mt. Kenya and described the afroalpine plant communities (Rehder et al. 1988a, b, 1989). It was a special honor for me, to publish a paper on the biology of the unique globular mosses together with my former academic teacher Karl Mägdefrau (Beck et al. 1986a, b). And we described a new species of the giant rosette groundsels *Senecio x saundersii* Sauer & Beck and investigated the population biology of this hybrid and of its parents (Beck et al. 1992). Two doctoral students did their geobotanical theses on the vegetation of the East African high mountains (Klaus Schmitt on the Ruwenzori: Schmitt and Beck 1992, and on the Aberdare Mountains, Schmitt 1991), and Rainer Bussmann on the forests and forest turnover on Mt. Kenya (Bussmann and Beck 1995a, 1995b, 1995c; Lange et al. 1998).

4.6 Frost Hardening of Plants, Part II

After this short excursion to the tropics I will return to our further work on cold hardening and frost resistance of higher plants. Related to the freezing behavior of

the afroalpine plants we started to investigate the freezing process and metabolism in our local overwintering plants. With respect to water relations we worked with ivy, winter barley, and the ornamental plant *Pachysandra terminalis* (Buxaceae). At subzero temperature the leaves of these plants freeze, adopting a glassy appearance. The question was how much of the cellular water crystallized in the intracellular spaces and which kind of damage may still occur in frost-hardened plant organs. The idea was that cellular water moves out of the cell and crystallizes until a physicochemical equilibrium (i.e., of the water potentials) between the concentrating cellular solutions and the extracellular ice is reached. This process is termed equilibrium freezing and the portion of the cellular water that freezes depends on the subzero temperature and the original osmolality of the cellular solutions. We elaborated on the theory of extracellular freezing (Hansen and Beck 1988) and showed equilibrium freezing for ivy leaves down to about −10°C where some 80% of the leaf water has solidified as ice. However, in the rosette leaves of winter-barley less water froze than calculated from the water potentials and we termed this non-ideal equilibrium freezing. A doctoral student from China who traveled 6 weeks by train from Shanghai to Bayreuth, arriving on Christmas Eve 1987, investigated the phenomenon of non-equilibrium freezing with leaves of *Pachysandra* in detail. Inspecting the leaves at −20°C by light microscopy he could for the first time produce photographs of extracellular ice and show that upon dehydration of cells during extracellular freezing a negative turgor develops that counteracts further freeze dehydration (Zhu et al. 1989; Zhu and Beck 1991). The negative turgor results from the fact that air in contrast to water cannot penetrate imbibed cell walls which are therefore sucked inwards. This negative turgor saved the cell about 10% liquid water giving rise to the phenomenon of non-equilibrium freezing. Nevertheless, damage by frost of frost-hardened leaves could still occur, mainly by a too high concentration of ionic solutes upon cell dehydration and a too low concentration of osmolytes (cryoprotectants) or membrane protecting proteins (dehydrins). Such a situation could result in a breakdown of the membrane potential or disintegration of membranes. Within the scope of the EUROSILVA-EUREKA program established under the debates about the Central European forest dieback in the 1980s and 1990s and needs for clarification, we investigated the frost hardening process in *Pinus sylvestris* as affecting chloroplast ultrastructure and photosynthetic activity. Like in spruce the amounts of the light harvesting complexes as well as the photosynthetic activity decreased significantly during frost hardening. Reduction of light harvesting complexes was interpreted as a protective measure to mitigate overexcitation of the photochemical machinery and its destruction by radicals when the biochemical reactions of the chloroplast are blocked by the low temperatures (Vogg et al. 1998a). Incorporating a spin label in the thylakoid membranes, we could show that the reduction of the protein content of the thylakoid membranes maintains the fluidity of the membranes at subzero temperatures (Vogg et al. 1998b). By applying artificial light regimes to young trees of Scots pine we could demonstrate that day length measured via the phytochrome system triggers the onset of frost hardening whereby a low level of phytochrome$_{fr}$ promotes hardening and a high level dehardening. The highest rate of frost hardening

achievable with short-day conditions was −0.7°C per day. The first incidence of a subzero temperature triggers a second hardening process with a daily rate of −0.9°C. Both processes are additive resulting in maximum hardening of −1.5°C per day (Beck et al. 2004).

5 Nitrogen, Cytokinins, and Plant Growth: A New Era and New Friends

Already 5 years after the opening of the University of Bayreuth, biologists and soil scientists succeeded in implementing a Collaborative Research Center (CRC, Sonderforschungsbereich 137) on "Regulation and Flux Control in Ecological Systems" which was funded for 12 years (1981–1993) by the German Research Foundation (DFG). The contribution of my coworker Inge Rosnitschek to that program aimed at understanding the mechanisms of growth regulation of plants by the nitrogen supply. Already in Munich, I had started a pot experiment with stinging nettles (*Urtica dioica* and *U. urens*) to investigate the dependence of their competitive strength on the nitrogen supply to the soil. In Bayreuth the first greenhouses of the University were finished in the early 1980s which allowed to continue the experiments on the nettles under controlled conditions. We grew the plants from the seeds in washed quartz sand which was continuously percolated with nutrient solution of different nitrogen content. Biometric data showed the influence of the nitrogen supply on the total biomass, on the root to shoot ratio of biomass and on the leaf area (Rosnitschek-Schimmel 1982). Other traits were determined as well, such as the light response curve of the net CO_2 uptake rates of the individual leaves and nocturnal losses of carbon by respiration. Combining an infrared gas analyzer with a setup for $^{14}CO_2$ application allowed assessment of the photosynthetic performance of the individual leaves on the nodes of the experimental plants and the carbon export and distribution over the entire plants. In that way the leaves could be characterized as source or sink leaves and the plastic response of the plant to the nitrogen supply could be traced to their ratios of carbon import: carbon export. Especially under low nitrogen supply we could also show sink limitation of photosynthetic net CO_2 uptake, by removing all except one source leaves what tripled the rate of CO_2 uptake by the remaining source leaf (Fetene et al. 1993). At that time, the cytokinin concentration in the plant, especially in the root has been proposed to mediate the growth response of *Plantago* to the nutrient supply (Kuiper 1988). With our experimental setup and a split-root system allowing application of cytokinin (zeatin-riboside) to part of the root system, we could show a change of the carbon export from the source leaves in favor of the shoot apex, enhanced CO_2 net uptake and growth of the shoot, and concomitant reduction of carbon import into the root (Fetene and Beck 1993). Supervised by my coworker Anton Fußeder my doctoral student Bernd Wagner elaborated a method for comprehensive analysis of cytokinins in plant tissues allowing the quantification of

15 species of cytokinins and their derivatives, by column chromatography, HPLC, deglycosylation, and ELISA. Using this method they could address the missing link between the nitrogen supply to the plant and the cytokinin-mediated modulation of growth (Fußeder et al. 1988b; Wagner and Beck 1993; Beck and Wagner 1994) showing that the cytokinin content of the root and the export to the shoot are proportional to the nitrogen content of the root (Beck 1994). This work brought us in contact with Miroslav Strnad and his coworker Karel Doležal from the Czech Academy and University of Olomouc, who spent several months in our department. They had specific antibodies for cytokinins and auxins which were very welcome in order to broaden our spectrum of quantifiable phytohormones. We had a successful Conference in Liblice (1991) on all aspects of plant hormones known at that time, from which we could produce a well-received book (Strnad et al. 1999). The connections to this very successful group in Olomouc remained vivid (e.g., Strnad et al. 1992, 1994; Doležal et al. 2002) and I was lucky to meet the grand-seigneur of cytokinin research in the Czech Republic Miroslav Kaminek several times.

Interlude 8: Ethiopian Skills and Consequences *Although the program and the experimental setup appear straight forward and not exceptionally complicated, one of the major difficulties was the appropriate growth of the nettles: Stinging by the nettles, pests (pathogenic fungi, aphids), controlling and adjusting of the continuous flow of the nutrient solution simultaneously through many pots, the necessity to change the position of the pots for equal illumination gave us a lot of problems and several students gave up finally. At that moment I received a message by my friend Ulrich Lüttge from the TU Darmstadt, that a student from Ethiopia, who had just finished the doctoral exam was interested to spend the rest of his DAAD stipend in another group to widen his physiological scope. This was Masresha Fetene from Addis Ababa University (AAU). We combined Masresha's remaining and our money and could employ him as a postdoc for more than a year. He was keen to work in our CRC, meeting other physiologists, ecologists, and soil scientists, and agreed to work in the nettle project. Thanks to his skills and the dedicated assistance by his wife Selome Bekele, we finally succeeded in the proper growth of the nettles. He performed the above-described experiments and participated in a study unveiling the pathway architecture of the vascular system of the nettles by autoradiography of the carbon flow from given source into particular sink leaves (Fetene et al. 1997). With a series of well-placed publications and continuously increasing his international connections Masresha made a steep career as professor of plant ecology and physiology of AAU, which he finally served as Deputy Vice President and Head of the publication office for years, before being appointed the Executive Secretary of the Ethiopian Academy of Science. While associate professor in the AAU, Masresha later received a fellowship of the Georg Forster Program of the Alexander von Humboldt foundation and is a regular visitor of Germany in official functions. There was never any doubt that he would bring back the knowledge achieved in Germany into his country and would encourage his students, who came to Germany or to other European countries and to the USA to follow his example. Since 1994, at the end of the last civil war in Ethiopia I have regularly*

visited Ethiopia and several times served as co-supervisor or external examiner of his masters and doctoral students. Together with his doctoral supervisor Ulrich Lüttge (TU Darmstadt) we succeeded in raising funds for an Ethiopian-German student's fieldwork and later achieved funding by the Volkswagen-Foundation and for several years also by the DFG. More than ten further papers on afroalpine plants of Ethiopia, especially on the giant Lobelias,[3] *but more recently on the trees in the remnants of the montane forests have meanwhile been jointly published. Our families have become close friends and it is a great honor for me having been elected associated member of the Ethiopian Academy of Science.*

After this excursus to Ethiopia, let me finish the nitrogen story. The investigations with the nettles were complemented by studies on the carbon sinks in the growing leaves. We investigated the invertases as potential mediators of phloem unloading, but could rule out a function at least of the apoplastic invertases in that process (Fahrendorf and Beck 1990; Möller and Beck 1992). Therefore symplastic unloading and splitting of sucrose by non-cell wall invertases appears likely. The entire story, from the nutrient supply to the cytokinins and to the growth response of the whole plant, was summarized by Beck (1999).

6 Photoautotrophic and Other Cell Suspension Cultures

Nitrogen supply to or the C/N ratio of a plant tissue reveals still an undifferentiated picture of the fate of nitrate or ammonium in a photosynthesizing cell. Therefore we started to work with a suspension culture of photoautotrophic *Chenopodium rubrum* cells (new name: *Oxybasis rubra*). Special growth conditions allowed the use of this cell culture as a model for the regulation of the intracellular metabolism, being aware of the fact of neglecting the exchange of metabolites and signals with the natural neighborhood in a plant tissue. Nevertheless this cell suspension culture turned out as a very potent tool and was used for various investigations. One topic was nitrogen metabolism, e.g., nitrate and ammonium uptake, regulation of nitrate reductase (NR) by nitrate, light and cytokinins, and determination of intracellular fluxes of nitrogenous compounds (Renner and Beck 1988; Beck and Renner 1989, 1990). I will not go into detail because several of our questions, e.g., the regulation of NR by 14-3-3 proteins, have been solved in the meantime in great detail by others (e.g., Lambeck et al. 2012). However, the cell culture brought us back to the cytokinins in the context of ageing and senescence of photosynthesizing cells. From a not successful endeavor to implement a collaborative research center on the cellular processes associated with ageing of plants, bacteria, humans, and animals, a few promising projects remained which we combined in a research

[3] The up to 6 m tall *Lobelia rhynchpetalum* from Ethiopia was the first of the giant Lobelias which could be brought to blossom in our tropical alpine greenhouse of the University of Bayreuth. Now, it flowers every year, mostly in winter and serves as object for further ecophysiological studies.

unit, "Biology of Ageing" (Beck and Scheibe 2003), funded by the DFG for 6 years. My group focused on the ageing of the photoautotrophic *Oxybasis* cells and in particular on the role of cytokinins and auxin. To characterize the individual developmental stages of that cell culture, we investigated the respective patterns and metabolic interconversions of these phytohormones and their derivatives (Fußeder et al. 1988b). Bearing in mind the "stay-green" mutants (e.g., Hauck et al. 1997) and the green islets in pathogen-attacked leaves (Faiss et al. 1997) we identified the point of no return from ageing in our cell cultures as that state at which addition of phytohormones could not reverse the process of ageing (Peters et al. 2000). One of the genes whose expression is under the control of auxin turned out as the gene of a glycan-exohydrolase *OrGEH* that is essential for growth of the cell wall and therefore also for the growth of the cell culture (Dominguez et al. 2014). Overexpression of *OrGEH* in *Arabidopsis* resulted in dramatic stimulation of growth (size and number of leaves) associated with a lower tolerance of desiccation stress (unpublished results).

7 The Plant Cell Cycle

Resumption of cell division in resting cells by supplying cytokinin and auxin brought us to another field, which in plant science had just come up: Constituents and regulation of the plant cell cycle. Our main questions were the regulation of the cyclins and the cyclin dependent protein kinases (CDKs) as the drivers of the cell cycle, and the response of their genes and transcription factors to cytokinins and auxins. Due to cancer research, knowledge of the cell cycle of animal and human cells had a considerable lead compared to that of plant systems. Identification and sequencing of new compounds, especially cyclins, inhibitors, a CDK, a retinoblastoma protein, and transcription factors and their expression in the course of the cell suspension culture and as response to phytohormones could be achieved (e.g., Fountain and Beck 2003; Fountain et al. 2003). Joining a Priority Program of the German Research Foundation on "Molecular Analysis of Phytohormone Action" (SPP 322, chaired by my friend Ralf Reski Freiburg), we included also a heterotrophic plant cell culture, the well-studied tobacco BY2 culture, in which cell division could be synchronized at least for two rounds. This is important for identifying the stages of the cycle at which regulatory steps are taking place. Using this culture we could show that cytokinin synthesis and degradation, resulting in oscillations of cytokinin concentrations and patterns, in crosstalk with auxin and carbohydrate resources regulate the expression of cell cycle control genes and thus the stages of the tobacco By2 cycle (Hartig and Beck 2005, 2006).

8 An Open Door May Not Only Tempt a Saint, . . . Projects Induced by Calls

In contrast to many countries, Germany's funding agencies state that the subjects of topical research arise from the scientific community and are not introduced top-down following political decisions. This is mostly true; however, e.g., collaborative programs in forest decline or in biodiversity research had their origin in political discussions. Nevertheless, at least for the German Research Foundation, calls for a collaborative priority program require previous application for the program by experts of the respective scientific community. If such an application is successful, calls are sent out. Fortunately, our hesitance to join such programs was low, in particular as I had the pleasure to participate in the expert groups for 2 collaborative research programs, the already mentioned program on the phytohormones and a program on **growth of plants under elevated CO_2**. The speaker of the latter program was Mark Stitt who had spent several years as an associated professor in my department. It was a fruitful joint research, in which my group followed the question of a potentially unlimited growth of a (tobacco) plant. High CO_2 concentration (700 ppm), saturating light, unlimited nutrient supply, and a non-limiting pot volume resulted in vast tobacco plants which produced a multiple biomass compared to plants under ambient CO_2. However, their growth was not unlimited because it was terminated by the onset of flowering. In that project we could trace growth limitation by a cramped root bed volume to the cytokinins in the root and the exudation from the root into the shoot (Schaz et al. 2014). An earlier priority program which met our interest in root physiology was that on **matter transport in the rhizosphere** (speaker Horst Marschner, Stuttgart-Hohenheim). Our interest was the phosphate uptake by the maize root, the development of a phosphate depletion zone around the root, and its replenishment from the surrounding soil which we could show in ^{33}P-labeled soil cores. To that end we sliced the soil after deep freezing with a special saw and exposed the frozen sections on X-ray films (Kraus et al. 1987a, b). Labeling neighboring maize plants with different isotopes (^{33}P and ^{35}S) we could demonstrate that the roots of individual maize plants avoid intergrowth and this is also true for different plant species (Fußeder et al. 1988a; Beck et al. 1989a, b; Hübel and Beck 1993). Extending the study to phytate, the major form of organic phosphate in a fertilized crop field, we could describe and localize a phytase in the maize root and assess its contribution to the phosphate uptake by the root which was much less than expected (Hübel and Beck 1996).

Interlude 9: Not a Lapse: Fascinated by Inconspicuous Insects *Commonly plant physiologists consider insects as pest which interfere with the culture of their plant material. Nevertheless collaboration with entomologists in 2 postgraduate programs ("Plant-Herbivore-Interactions" 1987–1993, Speaker Erwin Beck, and "Active and Signal Compounds of Insects – from Structure to Function in Ecosystems" 2001–2007, speaker Klaus Hoffmann) was an outstanding experience.*

Several doctoral theses which were supervised together with my colleague Helmut Zwölfer, centered around galls on the stems of the Canada thistle (Cirsium arvense), induced by the gall-fly Urophora cardui by oviposition into the young stem just below the developing flower head. Such galls are micro-ecosystems as parasitoids of the gall fly can establish higher trophic levels (Zwölfer et al. 2007, 2015). The 3 major questions were: How is gall growth initiated, which cues lead the insects to the thistle, which signals lead the female parasitoids (Eurytoma serratulae, Eu. robusta) to the egg of Urophora which is buried in the stem tissue of the thistle. Growth initiation is by cytokinins which are already present in the larvae before they start feeding on the plant tissue (Sakuth 1996). Visual and olfactory cues are used for the detection of the host plant (Daniels 2004) and most probably physical/acoustic responses indicate the position of the Urophora egg to the female parasitoid. The experimental challenge was to establish the plant-parasite system under laboratory conditions for becoming independent of the short natural window in June/July. Theoretically the gall fly could be used to control invasions by the Canada thistle which propagates with an enormous number of wind-dispersed seeds and the production of many rhizomes. However, not least because of the hyperparasitism, the gall fly Urophora is too weak for an effective control of the weed.

9 Condensing Experiences: Tree Ecophysiology

9.1 Water and Carbon Relations

Upscaling from the subcellular to the whole-plant level as described in the previous chapters confronts us with a plethora of new questions, e.g., of resource production, transportation and storage, of growth rates and patterns, of the functionality of structures, longevity of organs, pollination, propagation, tolerance of a stressful environment, and adaptation to seasons. Apart from the mechanisms and strategies which cause the enormous competitive strength of weeds, I was fascinated by trees as that plant life-form which under natural conditions outlives their human contemporaries. Calls from several German and European programs prompted us to join a priority program on tree physiology and another one on the forest dieback in which we investigated the fate of photosynthates in damaged and healthy spruce needles (Kuhn and Beck 1987) and the uptake of sulfate, simulating acid rain (Kuhn and Beck 1989) using adult trees in their natural environment. With several years old pine trees in a separated quarter of our Ecological Botanical Garden we could unravel the seasonality of the trees carbohydrate metabolism by applying a pulse of $^{14}CO_2$ to a number of trees which were then harvested over the year. The fate of ^{14}C was analyzed after separating roots, stem, twigs, and needles, by determination of their carbon budgets and analyzing the labeled compounds. Simultaneously the flow of ^{14}C-labeled compounds in the needle and through the stem to the roots was

investigated by radioautography (Hansen and Beck 1990, 1994; Hansen et al. 1996, 1997). To put it in a nutshell, we showed that in pine rather than in deciduous trees starch plays several physiological roles in adaptation to the developmental stages of the tree in the annual growth cycle. Bud sprouting and the development of the new needles and shoots are fed from starch that had been accumulated by photosynthesis in the chloroplasts of the older needles just before bud break (see Sect. 4.4) whereas contribution of reserve material from the last year is negligible. In summer starch is used as transitory carbohydrate deposit in the mesophyll chloroplasts warranting a continuous supply of carbohydrates to the axes for secondary growth. During this time hardly any newly fixed carbon reaches the roots. In spite of the partial degradation of the photosynthetic apparatus during frost hardening, photosynthesis is going on even at temperatures around the freezing point and the carbohydrates, mainly sucrose and its galactosides raffinose and stachyose (cryoprotectats) are transported to the root where they are finally deposited as starch. Root growth takes place during the cold season with maxima in autumn and spring, when the deposited starch is used. The path of carbon from a branch to the root uses only a small sector of the phloem, indicating sectorial connection between branches and roots. Within the scope of research collaboration with my Ethiopian colleague Masresha Fetene we investigated water and carbon relations of tropical evergreen or deciduous trees in an upland forest subjected to a climate with pronounced dry and wet seasons. Since a considerable part of that forest (Munessa) is no more natural forest but managed as plantations of eucalypt, pine, and cypress we started a collaborative program to compare the respective forest types as ecosystems, combining vegetation studies with investigations in soil science, on mycorrhizae, tree ecophysiology, and forest management. Receiving funding for a research station in the forest by the German Research Foundation and the Addis Ababa University, we could intensify the studies by involving more Ethiopian colleagues and students. Several doctoral theses of German and Ethiopian students originated from these studies contributing much to our understanding of niche utilization and coexistence of the adult trees and the rejuvenation potential of indigenous tree species which together result in the current tree composition of that forest (Lüttge et al. 2003; Fetene and Beck 2004; Fritzsche et al. 2006; Tesfaye et al. 2010, 2011; Strobl et al. 2011; Seyoum et al. 2012, 2014). From a multitude of interesting details of tree ecophysiology, the presentation of which would go too far, I only will pick up two: The claim that *Eucalyptus* is a bulk water consumer, withdrawing water from competitors in mixed stands must be put in a fair perspective: Although the daily water consumption of *Eucalyptus* trees was remarkable, it was still significantly less than of the indigenous tree species *Podocarpus falcatus*. The second point concerns the importance of facilitation of the regeneration of the natural tropical forest through a shelter effect even by exotic trees (Feyera et al. 2002; Strobl et al. 2011).

9.2 Defense by Alkaloids

Another type of studies in tropical tree ecophysiology examined the hypothesis of chemical pest defense by alkaloids in two members of the Apocynaceae, *Rauvolfia mombasiana* and *Tabernaemontana pachysiphon* which grow in the coastal forests of Kenya and are considered as medicinal plants by the local people. The patterns of alkaloids and their dependence on abiotic environmental factors were investigated in collaboration with the alkaloid specialist Robert Verpoorte from the Netherlands. In contrast to the expectation a high content of alkaloids at least in the leaves was an attractant rather than a repellent for moths and their caterpillars which completely defoliated the trees (Höft et al. 1996, 1998a, 1998b).

Interlude 10: Traditional Plant Knowledge in Kenya *When talking to indigenous healers and witchdoctors about our studies I became interested in the botanical knowledge of these people who got and passed on traditional knowledge from generation to generation. So I proposed a project on the indigenous botanical knowledge of one of the Kenyan coastal tribes, the Digo, together with two colleagues from the Faculty of Languages and Literature, Franz Rottland and Gudrun Miehe. By personal relations of Franz, we came across an MSc holder in Botany, Mohamed Pakia from that tribe, who was very interested in the subject. The combination of academic (and some practical) knowledge in plant science from my side together with the knowledge of colleagues whose interest was reaching far beyond the structural and historical traits of a language kin to Kisuaheli combined with the input of the local knowledge by the student can be considered an outstanding privilege on my side. In spite of an obvious unconsciousness of many botanical facts in the Digo community, and obscurantism in their medical treatments, we learned to appreciate the wealth of accumulated inherited wisdom especially in the local types of agri- and silviculture. Mohamed wrote a high-profile PhD-thesis: "African Traditional Plant Knowledge Today," which can be considered a showcase of an ethnobotanical study.*

10 Catching a Flying Bird: The Final Turn

In 2006 I was pleased to hand over the chair of the Department of Plant Physiology to my successor Stephan Clemens who, using modern analytical and molecular methods (genomics to metabolomics), continues research in plant stress physiology, focusing on heavy metals and drought.

Several years before retirement I was thinking of a project which would fit my ecophysiological interests, preferentially in the tropics, but could be done without much lab facilities.

10.1 The History of a Great Project

A Priority Program of the DFG on the "Mechanisms of maintaining tropical diversity" had just come to an end, in the frame of which Rainer Bussmann's work on the forests of East African High Mountains had been financed. In spite of several endeavors, the former PIs could not compound on a new subject for an extensive collaboration in the tropics. While still in the phase of brainstorming for a new program, Rainer Bussmann reported of a business gentleman, Ivan Gayler by name from California who had bought some territory in the South Ecuadorian Andes and was willing to build a research station for ecological studies if its further use could be ensured. What an opportunity! However, prior to moving in that direction, the German Research Foundations wanted to see the research station. In an iterative process Rainer and I could convince both sides, sending monthly photographs of the construction progress to the DFG and reports on the development of research projects to Ivan Gayler, who in the meantime had successfully implemented a foundation "Naturaleza y Cultura Internacional" (NCI) with offices in Seaside (Cal.) and Loja. In October 1997 six projects focusing on climate (Michael Richter, Erlangen), soils (Wolfgang Zech, Bayreuth), vegetation analysis (E. Beck), geobotany (Klaus Müller-Hohenstein, Bayreuth), plant–animal interactions (Gerhard Gottsberger, Ulm), bats, and crickets (Otto von Helversen, Erlangen) could celebrate research kick-off in the new Estación Científica San Francisco (ECSF) in the narrow valley of the Rio San Francisco about 1,800 m above sea level. With only one interruption of about 9 months, biodiversity and ecosystem research was continuously running there for 18 years and most of the projects were funded by the German Research Foundation within the scope of 2 Research Units and 3 the so-called bundles of research projects. The sparsely populated area in the eastern Cordillera of the Andes was largely unexplored and we had to begin from the very scratches as several of our original hypotheses and ideas turned out as wrong and irrelevant for that ecosystem. Gradually, we realized that we are investigating the second hottest hotspot of biodiversity worldwide which prompted a plethora of new but also basic questions. Since the beginning considerable efforts were undertaken to amalgamate the temporarily 30 individual projects from up to 18 universities under a common topic but due to the extremely multifaceted ecosystem, the mutual streamlining was not easy. Likewise problematic was the development of trustful relations to our local counterparts, to the above-mentioned US-American NGO and the two Universities of the area in Loja. Nevertheless, after 10 years of chairmanship and 1 year after my retirement, I could handover an effective "Ecuador group" to Jörg Bendix from the Faculty of Geography of the University of Marburg, who successfully further strengthened the scientific ties between biology, earth-science, forestry, economics, and sociology, on the one hand, and between the Germans and their Ecuadorian partners, on the other. Assessing all the achievements in science and capacity building would widely overstress the volume of this chapter (Pohle et al. 2013). Before briefly touching on my own projects, I would like to quote a publication by Pitman et al. (2011) on ecological research in the tropical Andes and the Amazon: "The most productive

Fig. 4 The two faces of the San Francisco valley in South Ecuador. (**a**) The southern slope with tropical mountain rain forest (Pico de Antennas 3,200 m); (**b**) the northern slope with active (*bright green*) and abandoned (*grayish green*) pastures. Fotos: Joerg Bendix, Marburg (with kind permission)

field station in the tropical Andes, Ecuador's San Francisco Scientific Station, accounted for 14.3 percent of all Andean studies" (three books: Beck et al. 2008; Bendix et al. 2013; Liede-Schumann and Breckle 2008 and more than 600 publications, most of them in peer-reviewed journals).

10.2 Ecophysiology Between Field and Lab

The contributions of my groups were and still are channeled by the fundamental contrast of the two faces of the W-E-oriented valley of the Rio San Francisco (Fig. 4): While on the southern slopes the natural tropical mountain rain forest has been preserved as part of the Podocarpus National Park, the forest has been cleared on the northern slopes and converted into pastures except a few remnants in ravines (Richter et al. 2013). Initially we started with a botanical inventory of the natural forest (Bussmann 2002), compiling the herbarium of the ECSF which has meanwhile become a useful complement of the recognized herbarium Reinaldo Espinosa at the Universidad Nacional de Loja. Admittedly, not just a few specimens have been misnamed, due to our ignorance of the neotropical flora, the frequent lack of flowers or fruits or simply because the species had not yet been described. Limiting ourselves to patches of the even more biodiverse secondary forest, my well-trained Ecuadorian PhD-student Alfredo Martinez Jerves collected 779 vascular plant species of which 90% could be assigned to a family, 50% to a genus, but only

20% could be identified to the species level, while 8% could not be identified at all (Martinez Jervez 2007). Interestingly the mode and approximate time of disturbance could be assessed from the tree diversity patterns (Mahecha et al. 2007, 2009; Martinez et al. 2008). Another series of studies dealt with the propagation of ecologically important tree species from seeds or cuttings for reforestation purposes. Studies on phenology, seed production, seed storage, and germination were mainly done by my Ecuadorian coworker Eduardo Cueva-Ortiz (Cueva-Ortiz et al. 2006; Stimm et al. 2008), while vegetative propagation by cuttings contrary to all expectations failed. In her PhD-Thesis Sina Heppner (2010) showed that the high content of cytokinins in the twigs from which the cuttings were collected inhibits formation of adventitious roots.

Currently my group works in a program that strives for recognizing early effects of a climate change on the water and carbon relations of the natural forest, lining measurements of the above-canopy atmospheric water vapor and CO_2 content with a modification of the Eddie covariance technique. On the northern valley slopes, where the forest had been cleared we investigate the pasture management of the local framers. The main problem there is the use of fire for further clearing the forest and for rejuvenation of the pastures. Any use of fire paves the way for the extremely invasive bracken fern (*Pteridium arachnoideum* and *Pt. caudatum*), which in a few years outcompetes the pasture grasses, rendering the pastures unusable (Hartig and Beck 2003). We studied the fire-triggered invasiveness of bracken ecophysiologically (Roos et al. 2010) and in the field after burning with a time series of aerial photographs taken with a Helium balloon (Silva et al. 2014). We also modeled competitive growth of bracken and the dominant pasture grass *Setaria sphacelata*, driven by the microclimate over the year showing different preferences of both competitors for wet and dry weather (Silva et al. 2012). Over 10 years we finally developed procedures for repasturization and sustainable pasture management of areas which had been abandoned because of complete infestation with bracken (Roos et al. 2011). In a synoptical study, using data deposited in our data warehouse in the University of Marburg, we opposed the ecological services of different options of land management (repasturization, re-afforestation, and leaving the land abandoned) with the economic and sociological services to the indigenous people (Knoke et al. 2014). Having caught a flying bird that was looking for a place to settle down in Ecuador we have finally arrived at comprehensive ecosystem studies and the transfer of our results to the people concerned and their authorities. Hundreds of scientists and students, as well as people from the management side, have contributed over the years to this endeavor, which recently has been commended by a reviewer as a “showcase project for the German Research Foundation.” Thanking them all individually is not possible; therefore I cordially acknowledge their services and inputs by saying “muchissimas gracias al todos.”

11 A Special Postscript

11.1 Serving the Scientific Community

I allocated a considerable share of my time to matters of the scientific community, be it the academics, knowledge transfer to practitioners, the scientific societies and unions, and last but not least German and European funding agencies. This is not the place to extend much on that issue. Whether my acting as a President of the German Biologists Association, of the German Botanical Society, of the International Working Group on Comparative High Mountain Research, as treasurer of the International Union of Biological Sciences, in several responsible functions of the University of Bayreuth, for the Humboldt University in Berlin, or in the fields of research funding and publication was successful with a sustainable outreach, is a matter of judgement by the people concerned. For 10 years I accompanied the BIOLOG/BIOTA program of the German Federal Ministry for Education and Research as chairman of the reviewers board, and an inspection of the achievements of this program 5 years after its termination merits the overall predicate "successful," what cannot be taken for granted in a politically highly diverse continent like Africa. Since almost 20 years I serve the German Research Foundation in various functions and panels and from 2008 to 2014 as chairman of the new Commission on Biodiversity Research. This commission works on a wide range of tasks on the national and the international biodiversity scene. One of the major issues was and still is the picking up of a paradigm shift of the conditions for biodiversity research challenged by the UN Convention on Biological Diversity and the subsequent "Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization" which, together with the respective regulations by the European Union and the German Law for the Implementation of the Nagoya Protocol opens a new political dimension of biological research. To my experience, the new system is not just promoting the realization of the projects as many administrative loose cannons are still in the pipe. On the national side, implementation of the national center for biodiversity research (iDiv) and of the German biodiversity data center GFBIO are major achievements of the Commission's work.

Engaging for the scientific community is not always pure pleasure, less so because of the difficulties and problems to be solved, but more so due to the unavailability, not to say unwillingness of colleagues to participate in the tasks. As a communicating editor of the Springer Journal TREES, recruitment of one reviewer from a list of 10 proposed is already a success. The same holds for the reviewing of grant applications and work in commissions and boards. Spending time for others without an immediate pay back holds little attraction in our days. Nevertheless a sustainable success of our scientific endeavors depends on the willingness of the entire community, in particular of the leading scientists to invest an adequate part of their time for community issues. Although knowing about the efficiency of such plea, I cannot abstain from encouraging my colleagues to participate voluntarily in the tasks of our scientific community. The claim that the

payback of such activities surpasses the expenditures is as true as any generalizing statement, since we deal with humans and not with an inanimate matter. However, there is always some payback! Looking into the listings of my grants and publications, I feel deeply indebted to those colleagues who spent their time and experience to promote my work.

11.2 A Contingent Legacy

Since the beginning of my career the Zeitgeist and the working conditions have greatly changed and commercialism has found its way into the various fields of academic research. We all know that competition for financial resources has become extremely stressful, pushing scientists more and more into specialization. Specialization is inevitable given the complexity of the systems we deal with, however, going back to the entire system once in a while is not any less important for verification of the reasonability of the research questions and approaches. When working biochemically with cell suspension cultures or isolated chloroplasts, I urged my coworkers to use also the microscope from time to time or include even electron microscopy. Visual inspection proved in no case needless and finally saved time. Today, data-driven research is en vogue and clouds of data are used to extract correlations, demanding global validity. Admittedly, research questions are raised in many of these papers which could not have been approached with classical methods. But reading such articles I often get the impression of an uncritical belief in statistics and in the weight of a high number of data which as to my own experience may be used by the authors in spite of inconsistency of their production. The temptation of a high-ranked publication is immense. To say it in a provocative way: What our research must provide is an explanatory view of nature rather than a wealth of correlations proofing factitious hypotheses. Being aware of presumably annoying colleagues when presenting any example, I will illustrate my statement by confessing that to my understanding a global map of (potential) maximal CO_2 assimilation calculated from the specific leaf area and as a derivative of it, from the "N-concentration" is not only unrealistic but also dangerous as it suggest knowledge which is neither meaningful nor does it exist, but may be used in an uncritical way by the public. Around the turn of the millennium several endeavors were made to revive "systems biology" and to implement it as a general basis for academic teaching and research. Since then it has gained momentum but still struggles against specialization. In current collaborative research projects specialists are sought for "synthesis" of a general outcome of the research using the data generated by the various special subprojects. To this end a broad as well as fundamental experience is needed and therefore the common "synthesis postdocs" may be frequently overcharged. Synthesis is the supreme art of systems biology using data as well as epistemology; it should be elaborated and taught by the most experienced scientists. Unfortunately, these are forced by to-day's fund-raising mechanisms to become more and more specialists. A fatal vicious circle with no silver-bullet for

breaking through! The Millennium Ecosystem Assessment (2005) emphasized in addition to others also the cultural services of Nature; especially scientists should use Nature not only for edification and recreation but also to critically reflect their intuition and research questions against real evidence. My academic teachers' power of observation, sharpened by knowledge, was something that I wholehearted admire while striving to develop my own skills. There is no better approach for a biologist to a scientific problem.

Acknowledgements Finally, I am anxious to cordially thank my coworkers and colleagues, with many of them I became friends. I had an exciting time in Munich and especially in Bayreuth, where as a professor of the first hour, I could realize several of my ideas, e.g., the establishment of an ecological botanical garden at the University of Bayreuth, or the first biological graduate college in Germany. In most cases I found a sympathetic ear in the boards and administration of the University. I am deeply grateful to the funding agencies, in particular to the German Research Foundation (DFG), which provided the financial means to work on my ideas and at the same time supporting the training and qualification of students and coworkers. It was (and still is) a give and a take and even in the rare cases, when an application failed, I am convinced of a fair treatment. Particular thanks go also to my long-standing coworker Christiane Reinbothe, who joined my department in 1998 and finally was the scientific coordinator of the DFG-commission on biodiversity research until 2015. Last but not least I would like to cordially thank all those sympathetic colleagues to whom I owe my highly acknowledged honors. This is not the place to expand on the support of my work by my family, for which I am particular grateful and whose backup I hardly can overstate.

References

Andraloic PJ, Keys AJ, Kossmann J, Parry MAJ (2002) Elucidating the biosynthesis of 2-carboxyarabinito 1-phopshate through reduced expression of chloroplastic fructose 1,6-bisphosphate phosphatase and radiotracer studies with $^{14}CO_2$. Proc Natl Acad Sci U S A 99:4742–4747. doi:10.1073/pnas.072137099

Andralojc PJ, Keys AJ, Martindale W, Dawson GW, Parry MAJ (1996) Conversion of D-hamamelose into 2-carboxy-D-arabinitol and 2-carboxy-D-arabinitol 1-phosphate in leaves of Phaseolus vulgaris L. J Biol Chem 271:26803–26809

Beck E (1963) Beiträge zur Chemosystematik einiger Centrospermen, Plumbaginaceen und Primulaceen. PhD thesis, University of Munich, 82 pp

Beck E (1967) Isolierung und Charakterisierung eines Apiogalacturonans aus der Zellwand von Lemna minor. Z Pflanzenphysiol 57:444–461

Beck E (1969) Isolierung und Identifizierung von Clusianose, einem 1-0-α-D-Galactopyranosyl-Hamamelit. Z Pflanzenphysiol 61:360–366

Beck E (1994) The morphogenic response of plants to soil nitrogen: adaptive regulation of biomass distribution and nitrogen metabolism by phytohormones. In: Schulze E-D (ed) Flux control in biological systems. Academic, Orlando, pp 119–151

Beck E (1999) Towards an understanding of plant growth regulation: cytokinins as major signals for biomass distribution. In: Strnad M, Péc P, Beck E (eds) Advances in regulation of plant growth and development. Peres Publishers, Prague, pp 97–110

Beck E, Kandler O (1965) Apiose als Bestandteil der Zellwand höherer Pflanzen. Z Naturforsch 20b:62–67

Beck E, Kandler O (1966) Isotopenstudien zur Biosynthese von Apiose in Lemna. Z Pflanzenphysiol 55:71–84

Beck E, Knaupp I (1974) Biosynthese der Hamamelose. III. Identifizierung von Hamamelose-2[1]-diphosphat, Hamamelose-5-phosphat und Hamamelose-2[1]-phosphat. Z PflanzenphysioL 72:141–147

Beck E, Renner U (1989) Ammonium triggers uptake of NO_3^- by Chenopodium rubrum suspension culture cells and remobilization of their vacuolar nitrate pool. Plant Cell Physiol 30:487–495

Beck E, Renner U (1990) Net fluxes and pools of nitrogenous compounds during suspension culture of photoautotrophic *Chenopodium rubrum* cells. Plant Cell Environ 13:111–122

Beck E, Scheibe R (2003) Senescence and aging in plants and cyanobacteria. Physiol Plant 119:1–4

Beck E, Wagner BM (1994) Quantification of the daily cytokinin transport from the root to the shoot of *Urtica dioica* L. Bot Acta 107:342–348

Beck E, Ziegler P (1989) Biosynthesis and degradation of starch in higher plants. Annu Rev Plant Physiol Plant Mol Biol 40:95–117

Beck E, Merxmüller H, Wagner H (1962) Über die Art der Anthocyane bei Plumbaginaceen, Alsinoideen und Molluginaceen. Planta 58:220–224

Beck E, Sellmair J, Kandler O (1968) Biosynthese der Hamamelose. I. Die intramolekulare ^{14}C-Verteilung in Hamamelose nach Assimilation von $^{14}CO_2$ und ^{14}C-positionsmarkierter Glucose durch Blätter von Primula clusiana Tausch. Z Pflanzenphysiol 58:434–451

Beck E, Stransky H, Fürbringer M (1971) Synthesis of hamamelose-diphosphate by isolated spinach chloroplasts. FEBS Lett 13:229–234

Beck E, Eickenbusch JD, Steiger HM (1974) Characterization of glycolate producing reactions in isolated spinach chloroplasts. In: Proceedings of the 3rd International Congress on Photosynthesis, Elsevier Science Publishing Company, pp 1329–1334

Beck E, Wieczorek J, Reinecke W (1980) Purification and properties of hamamelosekinase. Eur J Biochem 107:485–489

Beck E, Pongratz P, Reuter I (1981) The amylolytic system of isolated spinach chloroplasts and its role in the breakdown of assimilatory starch. In: Proceedings of the 5th International Congress on Photosynthesis Halkidiki, Balaban International Science Services, Philadelphia, pp 529–538

Beck E (1982) Branched-chain sugars. Encyclopedia of plant physiology, New Ser, Vol 13A, pp 124–157

Beck E, Burkert A, Hofmann M (1983) Uptake of L-ascorbate by intact spinach chloroplasts. Plant Physiol 73:41–45

Beck E, Schulze E-D, Senser M, Scheibe R (1984) Equilibrium freezing of leaf water and extracellular ice formation in Afroalpine 'giant rosette' plants. Planta 162:276–282

Beck E, Mägdefrau K, Senser M (1986a) Globular mosses. Flora 178:73–83

Beck E, Scheibe R, Schulze E-D (1986b) Recovery from fire: observations in the alpine vegetation of western Mt. Kilimanjaro (Tanzania). Phytocoenologia 14:55–77

Beck E, Fußeder A, Kraus M (1989a) The maize root system in situ: evaluation of structure and capability of utilization of phytate and inorganic soil phosphates. Z Pflanzenernähr Bodenk 152:159–167

Beck E, Scheibe R, Reiner J (1989b) An assessment of the Rubisco inhibitor. 2-Carboxyarabinitol-1-phosphate and D-hamamelose acid 2[1]-phosphate are two identical compounds. Plant Physiol 90:13–16

Beck E, Scheibe R, Schlütter I, Sauer W (1992) *Senecio x saundersii* SAUER & BECK (Asteraceae), an intermediate hybrid between *S. keniodendron* and *S. keniensis* of Mt. Kenya. Phyton (Horn, Austria) 32:9–37

Beck E, Heim R, Hansen J (2004) Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. J Biosci 29:449–459

Beck E, Fettig S, Knake C, Hartig K, Bhattarai T (2007) Specific and unspecific responses of plants to cold and drought stress. J Biosci 32:501–510

Bodner M, Beck E (1987) Effect of supercooling and freezing on photosynthesis in freezing tolerant leaves of Afroalpine 'giant rosette' plants. Oecologia 72:366–371

Bussmann RW (2002) Estudio fitosociológico de la vegetation en la Reserva Biológica San Francisco (ECSF) Zamora Chinchipe. Herbario Loja N° 8, 1–106
Bussmann RW, Beck E (1995a) Regeneration- and cyclic processes in the Ocotea-forests (*Ocotea usambarensis* Engl.) of Mount Kenya. Verh Ges Ökol 24:35–38
Bussmann RW, Beck E (1995b) Regeneration and succession processes in the cedar forests (*Juniperion procerae*) of Mount Kenya. Ecotropica 1:79–84
Bussmann RW, Beck E (1995c) The forests of Mt. Kenya (Kenya), a phytosociological synopsis. Phytocoenologia 25:467–560
Cueva-Ortiz E, Homeier J, Breckle S-W, Bendix J, Emck P, Richter M, Beck E (2006) Seasonality in an evergreen tropical mountain rainforest in southern Ecuador. Ecotropica 12:69–85
Daniels W (2004) The complex foraging strategy of the specialised gallfly Urophora cardui (Diptera: Tephritidae) for host plants (Cirsium arvense, Asteraceae). PhD thesis, University of Bayreuth, 177 pp
Doležal K, Astot C, Hanuš J, Holub J, Peters W, Beck E, Strnad M, Sandberg G (2002) Identification of aromatic cytokinins in suspension cultured photoautotrophic cells of Chenopodium rubrum by capillary liquid chromatography/frit – fast atom bombardment mass spectrometry. Plant Growth Regul 36:181–189
Dominguez OV, Peters W, Reinbothe C, Schwarz W, Zverlov V, Beck E (2014) Characterization of a glycan exo-hydrolase that shows a biphasic expression in the course of an in vitro culture of photoautotrophic oxybasis rubra cells. J Plant Biochem Physiol 2:137–146. doi:10.4172/2329-9029.1000137
Eickenbusch JD, Beck E (1973) Evidence for involvement of 2 types of reaction in glycolate formation during photosynthesis in isolated spinach chloroplasts. FEBS Lett 31:225–228
Eickenbusch JD, Scheibe R, Beck E (1975) Active glycolaldehyde and ribulose diphosphate as carbon sources for oxidative glycolate formation. Z Pflanzenphysiol 75:375–380
Fahrendorf T, Beck E (1990) Cytosolic and cell-wall bound acid invertase from leaves of Urtica dioica L.: a comparison. Planta 180:237–244
Faiss M, Zalubilová J, Strnad M, Schmülling T (1997) Conditional transgenic expression of the *ipt* gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. Plant J 12:401–415
Fetene M, Beck E (1993) Reversal of the direction of photosynthate allocation in Urtica dioica L. plants by increasing cytokinin import into the shoot. Bot Acta 106:235–240
Fetene M, Beck E (2004) Water relations of indigenous versus exotic tree species, growing at the same site in a tropical montane forest in southern Ethiopia. Trees 18:428–435
Fetene M, Möller I, Beck E (1993) The effect of nitrogen supply to Urtica dioica L. plants on the distribution of assimilate between shoot and roots. Bot Acta 106:228–234
Fetene M, Benker C, Beck E (1997) The pathway of assimilate flow from source to sink in Urtica dioica L., studied with 14C under ambient atmospheric conditions. Ann Bot 79:585–591
Feyera S, Beck E, Lüttge U (2002) Exotic trees as nurse-trees for the regeneration of natural tropical forests. Trees 16:245–249
Fountain MD, Beck E (2003) Isolation of full length cDNAs of two cell cycle control factors from proliferating *Chenopodium rubum* cells: a CDKB; 1 and a transcription factor E2F. Physiol Plant 119:40–43
Fountain MD, Valdes O, Fettig S, Beck E (2003) Expression of cell cycle control factors in non-dividing and ageing photoautotrophic plant cells. Physiol Plant 119:30–39
Fritzsche F, Abate A, Fetene M, Beck E, Weise S, Guggenberger G (2006) Soil-plant hydrology of indigenous and exotic trees in an Ethiopian montane forest. Tree Physiol 26:1043–1054
Fußeder A, Kraus M, Beck E (1988a) Reassessment of root competition for P of field-grown maize in pure and mixed cropping. Plant Soil 106:299–301
Fußeder A, Wagner B, Beck E (1988b) Quantification by ELISA of cytokinins in root-pressure exudates of *Urtica dioica* plants grown under different nitrogen levels. Bot Acta 101:220–228
Fußeder A, Ziegler P, Peters W, Beck E (1989) Turnover of O-glucosides of dehydrozeatin and dihydrozeatin-9-riboside during the cell growth cycle of photoautotrophic cell suspension cultures of *Chenopodium rubrum*. Bot Acta 102:335–340

Gilck H, Beck E (1973) Über den Mechanismus der Hamamelosebiosynthese. Hoppe-Seyler's Z Physiol Chem 354:1194
Gilck H, Beck E (1974) Biosynthese der Hamamelose. IV. Nachweis der Biosynthesesequenz: Fructosediphosphat Hamamelose-diphosphat Hama-melosemonophosphat Hamamelose. Z Pflanzenphysiol 72:395–409
Groden D, Beck E (1979) H_2O_2 destruction by ascorbate-dependent systems from chloroplasts. Biochim Biophys Acta 546:426–435
Gutteridge S, Parry MAJ, Burton S, Keys AJ, Mudd A, Feeney J, Servaites JC, Pierce J (1986) A nocturnal inhibitor of carboxylation in leaves. Nature 324:274–276. doi:10.1038/324274a0
Hansen J, Beck E (1988) Evidence for ideal and non-ideal equilibrium freezing of leaf water in Frosthardy Ivy (*Hedera helix*) and Winter Barley (*Hordeum vulgare*). Bot Acta 101:76–82
Hansen J, Beck E (1990) The fate and path of assimilation products in the stem of 8-year-old Scotch pine (*Pinus sylvestris* L.) trees. Trees 4:16–21
Hansen J, Beck E (1994) Seasonal changes in the utilization and turnover of assimilation products in 8-year-old Scots pine (*Pinus sylvestris* L.) trees. Trees 8:172–182
Hansen J, Vogg G, Beck E (1996) Assimilation, allocation and utilization of carbon by 3-year-old Scots pine (*Pinus sylvestris* L.) trees during winter and early spring. Trees 11:83–90
Hansen J, Türk R, Vogg G, Heim R, Beck E (1997) Conifer carbohydrate physiology: updating classical views. In: Rennenberg H, Eschrich W, Ziegler H (eds) Trees – contributions to modern tree physiology. SPB Academic, The Hague, pp 97–108
Hartig K, Beck E (2003) The bracken fern (*Pteridium arachnoideum* (Kaulf.) *Maxon*) dilemma in the Andes of Southern Ecuador. Ecotropica 9:3–13
Hartig K, Beck E (2005) Endogenous cytokinin oscillations control cell cycle progression of tobacco BY-2 cells. Plant Biol 7:33–40
Hartig K, Beck E (2006) Crosstalk between auxin, cytokinins, and sugars in the plant cell cycle. Plant Biol 8:389–396
Hauck B, Gay AP, Macduff J, Griffiths M, Thomas H (1997) Leaf senescence in a non-yellowing mutant of Festuca pratensis: implications of the stay-green mutation for photosynthesis, growth and nitrogen nutrition. Plant Cell Environ 20:1007–1018
Hedberg O (1964) Features of afro-alpine plant ecology. Acta Phytogeogr Suecica 48:1–144
Hemp A, Beck E (2001) Erica excelsa as a fire-tolerating component of Mt. Kilimanjaro's forests. Phytocoenologia 31:449–475
Henker A, Schindler I, Renz A, Beck E (1998) Protein heterogeneity of spinach pullulanase results from the coexistence of interconvertible isomeric forms of the monomeric enzyme. Biochem J 331:929–935
Heppner S (2010) Vegetative Vermehrung einheimischer Baumarten in Südecuador: Physiologische Grundlagen und deren Umsetzung. Südwestdeutscher Verlag für Hochschulschriften, pp 1–144, ISBN: 978-3-8381-1703-4
Höft M, Verpoorte R, Beck E (1996) Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Stapf (Apocynaceae) as influenced by light intensity, water and nutrient supply. Oecologia 107:160–169
Höft M, Verpoorte R, Beck E (1998a) Leaf alkaloid contents of *Tabernaemontana pachysiphon* as influenced by endogenous and environmental factors in the natural habitat. Planta Med 64:148–152
Höft M, Verpoorte R, Beck E (1998b) Growth and alkaloid patterns of roots of *Tabernaemontana pachysiphon* and *Rauvolfia mombasiana* as influenced by environmental factors. Bot Acta 111:222–230
Hübel F, Beck E (1993) In-situ determination of the P-relations around the primary root of maize with respect to inorganic and phytate P. Plant Soil 158:1–9
Hübel F, Beck E (1996) Maize root phytase. Purification, characterization, and localization of enzyme activity and its putative substrate. Plant Physiol 112:1429–1436
Knoke T, Bendix J, Pohle P, Hamer U, Hildebrandt P, Roos K, Gerique A, Sandoval ML, Breuer L, Tischer A, Brenner S, Calvas B, Aguirre N, Castro LM, Windhorst D, Weber M, Stimm B,

Günter S, Palomeque X, Mora J, Mosandl R, Beck E (2014) Afforestation or intense pasturing improve the ecological and economic value of abandoned tropical farmlands. Nat Commun 5, Article number 5612; doi:10.1038/ncomms6612

Kraus M, Fusseder A, Beck E (1987a) In situ-determination of the phosphate-gradient around a root by radioautography of frozen soil sections. Plant Soil 97:407–418

Kraus M, Fusseder A, Beck E (1987b) Development and replenishment of the P-depletion zone around the primary root of maize during the vegetation period. Plant Soil 101:247–255

Kuhn U, Beck E (1987) Conductance of needle and twig axis phloem of damaged and intact Norway spruce (Picea abies (L.) Karst.) as investigated by application of ^{14}C in situ. Trees 1:207–214

Kuhn U, Beck E (1989) Short-term studies of sulfate uptake by Norway spruce needles under outdoor conditions. In: Schulze E-D, Lange OL, Oren R (eds) Acid rain and forest decline of Picea abies, a case study, vol 77, Ecological studies. Springer, Berlin, pp 215–218

Kuiper D (1988) Growth responses of Plantago major L. ssp. pleiosperma (Pilger) to changes in mineral supply. Evidence for regulation by cytokinins. Plant Physiol 87:555–557

Lambeck IC, Fischer-Schrader K, Niks D, Roeper J, Chi J-C, Hille R, Schwarz G (2012) Molecular mechanism of 14-3-3 protein-mediated inhibition of plant nitrate reductase. J Biol Chem 287:4562–4571

Lange S, Bussmann RW, Beck E (1998) Stand structure and regeneration of the subalpine *Hagenia abyssinica* forests of Mt. Kenya. Bot Acta 110:473–480

Liede-Schumann S, Breckle S-W (2008) Provisional checklist of flora and fauna of the San Francisco valley and its surroundings. Ecotropical Monogr 4, 978-3-9807780-3-9, 256 pp

Lorimer GH, Andrews TJ, Tolbert NE (1973) Ribulose diphosphate oxygenase II. Further proof of reaction products and mechanism of action. Biochemistry 12:18–23

Ludwig I, Ziegler P, Beck E (1984) Purification and properties of spinach leaf debranching enzyme. Plant Physiol 74:856–861

Lüttge U, Berg A, Fetene M, Nauke P, Peter D, Beck E (2003) Comparative characterization of photosynthetic performance and water relations of native trees and exotic plantation trees in an Ethiopian forest. Trees 17:40–50

Lys MJ (1956) Les Glucides de quelques Primulacées. Rev Gén Bot 63:95–100

Mahecha MD, Martinez A, Lischeid G, Beck E (2007) Nonlinear dimensionality reduction: alternative ordination approaches for extracting and visualizing biodiversity patterns in tropical montane forest vegetation data. Ecol Inform 2:138–149

Mahecha MD, Martinez A, Lange H, Reichstein M, Beck E (2009) Identification of characteristic plant co-occurrences in neotropical secondary montane forests. J Plant Ecol 2:31–41

Martinez Jervez JA (2007) Sekundäre Bergregenwälder in Südecuador: Der Einfluss der Art der Störung auf das Spektrum der Pflanzenarten und die Waldstruktur, eine vegetationskundliche Analyse. PhD thesis, University of Bayreuth

Martinez A, Mahecha MD, Lischeid G, Beck E (2008) Succession stages of vegetation regeneration: secondary tropical mountain forests. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador, vol 198, Ecological studies. Springer, Heidelberg, pp 409–415. ISBN 978-3-540-73525-0

Möller I, Beck E (1992) The fate of apoplastic sucrose in sink and source leaves of Urtica dioica. Physiol Plant 85:618–624

Netondo GW, Onyango JC, Beck E (2004a) Sorghum and salinity: I. Response of growth, water relations, and ion accumulation to NACl salinity. Crop Sci 44:797–805

Netondo GW, Onyango JC, Beck E (2004b) Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of Sorghum under salt stress. Crop Sci 44:806–811

Niittylä T, Messerli G, Trevisan M, Chen J, Smith AM, Zeeman SC (2004) A previously unknown maltose transporter essential for starch degradation in leaves. Science 303:87–89

Pakia M (2006) African traditional plant knowledge today: an ethnobotanical study of the Digo at the Kenya Coast. LIT Verlag, Berlin, 217 pp. ISBN 3-8258-9056-2

Peters W, Ritter J, Tiller H, Valdes O, Renner U, Fountain M, Beck E (2000) Growth, ageing and death of a photoautotrophic plant cell culture. Planta 210:478–487

Pitman NCA, Widmer J, Jenkins CN, Stocks G, Seales L, Paniagua F, Bruna E (2011) Volume and geographical distribution of ecological research in the Andes and the Amazon, 1995–2008. Trop Conserv Sci 4:64–81

Pohle P, López MF, Beck E, Bendix J (2013) The role of biodiversity research for the local scientific community. In: Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W (eds) Ecosystem services, biodiversity and environmental change in a tropical mountain ecosystem of South Ecuador. Ecological studies, vol 221. Springer, Heidelberg, pp 411–428

Pongratz P, Beck E (1978) Diurnal oscillation of amylotic activity in spinach chloroplasts. Plant Physiol 62:687–689

Rehder H, Beck E, Kokwaro JO (1988a) The afroalpine plant communities of Mt. Kenya (Kenya). Phytocoenologia 16:433–463

Rehder H, Beck E, Kokwaro JO (1988b) Vegetation map of Mt. Kenya. Survey of Kenya, Nairobi

Rehder H, Beck E, Kokwaro JO (1989) The afroalpine plant communities of Mt. Kenya (Kenya): comment of the vegetation map of Mt. Kenya. Phytocoenologia 17:287–288

Renner U, Beck E (1988) Nitrate reductase activity of photoautotrophic suspension culture cells of Chenopodium rubrum is under the hierarchical regime of NO_3^-, NH_4^+ and light. Plant Cell Physiol 29:1123–1131

Renz A, Schikora S, Schmid R, Kossmann J, Beck E (1998) cDNA sequence and heterologous expression of monomeric spinach pullulanase: multiple isomeric forms arise from the same polypeptide. Biochem J 331:937–945

Richter M, Beck E, Rollenbeck R, Endix J (2013) The study area. In: Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W (eds) Ecosystem services, biodiversity and environmental change in a tropical mountain ecosystem of South Ecuador. Ecological studies, vol 221. Springer, Heidelberg, pp 3–18

Roos K, Rollenbeck R, Peters T, Bendix J, Beck E (2010) Growth of tropical bracken (Pteridium arachnoideum): response to weather variations and burning. Invasive Plant Sci Manag 3:402–411

Roos K, Rödel HG, Beck E (2011) Short- and long-term effects of weed control on pastures infested with *Pteridium arachnoideum* and an attempt to regenerate abandoned pastures in South Ecuador. Weed Res 51:165–176; doi:10.1111/j.1365-3189.2010.00833.x

Rosnitschek-Schimmel I (1982) Effect of ammonium and nitrate supply on dry matter production and nitrogen distribution in Urtica dioica. Z Pflanzenphysiol 108:329–341

Rost S, Frank C, Beck E (1996) The chloroplast envelope is permeable for maltose but not for maltodextrins. Biochim Biophys Acta 1291:221–227

Sakuth T (1996) Die Bedeutung der Cytokinine für Entstehung und Wachstum von Urophora cardui-Gallen im Stengel der Ackerdistel (Cirsium arvense). PhD thesis, University of Bayreuth, 90 pp

Schaz U, Düll B, Reinbothe C, Beck E (2014) Influence of root-bed size on the response of tobacco to elevated CO_2 as mediated by cytokinins. AoB Plants 6, pii: plu010; doi:10.1093/aobpla/plu010

Scheibe R, Beck E (1979) On the mechanism of activation by light of the NADP-dependent malate dehydrogenase in spinach chloroplast. Plant Physiol 64:744–748

Scheibe R, Beck E (1994) The malate valve: flux control at the enzymatic level. In: Schulze E-D (ed) Flux control in biological systems. Academic, Orlando, pp 3–11

Schindler I, Renz A, Schmid FX, Beck E (2001) Activation of spinach pullulanase by reduction results in a decrease in the number of isomeric forms. Biochim Biophys Acta 1548:175–186

Schmitt K (1991) The vegetation of the Aberdare National Park Kenya. Hochgebirgsforschung-High Mountain Research 8:259 pp, Universitätsverlag Wagner Innsbruck

Schmitt K, Beck E (1992) On the afroalpine vegetation of the Ruwenzori Mountains, Uganda. Phytocoenologia 21:313–332

Schulze E-D, Beck E, Scheibe R, Ziegler P (1985) Carbon dioxide assimilation and stomatal response of afroalpine giant rosette plants. Oecologia 65:207–213

Schürmann P, Buchanan BB (2008) The ferredoxin/thioredoxin system of oxygenic photosynthesis. Antioxid Redox Signal 10:1235–1274. doi:10.1089/ars.2007.1931

Sellmair J, Beck E, Kandler O (1968) Isolierung und Identifizierung von Hamamelit aus Primula clusiana. Tausch. Z Pflanzenphysiol 59:70–79

Senser M, Beck E (1977) On the mechanism of frost injury and frost hardening of spruce chloroplasts. Planta 137:195–201

Senser M, Beck E (1979) Kälteresistenz der Fichte. Ber Deutsch Bot Ges 92:243–259

Senser M, Beck E (1982a) Frost resistance in spruce (Picea abies (L.) Karst.): IV. The lipid composition of frost resistant and frost sensitive spruce chloroplasts. Z Pflanzenphysiol 105:241–253

Senser M, Beck E (1982b) Frost resistance in spruce (Picea abies (L. (Karst.): V. Influence of photoperiod and temperature on the membrane lipids of the needles. Z Pflanzenphysiol 108:71–85

Senser M, Schötz F, Beck E (1975) Seasonal changes in structure and function of spruce chloroplasts. Planta 126:1–10

Seyoum Y, Fetene M, Strobl S, Beck E (2012) Foliage dynamics, leaf traits, and growth of coexisting evergreen and deciduous trees in a tropical montane forest in Ethiopia. Trees 26:1495–1512. doi:10.1007/s00468-012-0723-6

Seyoum Y, Fetene M, Strobl S, Beck E (2014) Daily and seasonal courses of gas exchange and niche partitioning among coexisting tree species in a tropical montane forest. Flora 209:191–200

Silva B, Roos K, Voss I, König N, Rollenbeck R, Scheibe R, Beck E, Bendix J (2012) Simulating canopy photosynthesis for two competing species of an anthropogenic grassland community in the Andes of South Ecuador. Ecol Model 239:14–26. doi:10.1016/j.ecolmodel.2012.01.016

Silva B, Lehnert L, Roos K, Fries A, Rollenbeck R, Beck E, Bendix J (2014) Mapping two competing grassland species from a low-altitude helium balloon. IEEE J Sel Top Appl Earth Observations Remote Sensing 7:3038–3049

Souza-Canada ED (2012) Transformation einer argentinischen Sommerweizenvarietaet (Triticum aestivum L.) durch Transfer eines Seneszenz-verzoegernden Gens zur Verbesserung der Ertragsleistung. PhD Thesis, Bayreuth

Steiger HM, Beck E (1981) Formation of hydrogen peroxide and oxygen dependence of photosynthetic CO_2 assimilation by intact chloroplasts. Plant Cell Physiol 22:561–576

Steiger HM, Beck E, Beck R (1977) Oxygen concentration in isolated chloroplasts during photosynthesis. Plant Physiol 60:903–906

Stimm B, Beck E, Günter S, Aguirre N, Cueva-Ortiz E, Mosandl R, Weber M (2008) Reforestation of abandoned pastures: seed ecology of native species and production of indigenous plant material. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador, vol 198, Ecological studies. Springer, Heidelberg, pp 417–429. ISBN 978-3-540-73525-0

Strnad M, Peters W, Beck E, Kaminek M (1992) Immunodetection and identification of N^6-(o-Hydroxybenzylamino)purine as a naturally occurring cytokinin in Populus x candensis Moench cv Robusta leaves. Plant Physiol 99:74–80

Strnad M, Peters W, Beck E (1994) Ortho-topolin-9-glucoside, a new aromatic cytokinin from Populus x canadensis Moench cv Robusta leaves. Phytochemistry 37:1059–1062

Strobl S, Fetene M, Beck EH (2011) Analysis of the "shelter tree-effect" of natural and exotic forestcanopies on the growth of young Podocarpus falcatus trees in southern Ethiopia. Trees 25:769–783

Tesfaye G, Teketay D, Fetene M, Beck E (2010) Regeneration of seven indigeneous species in a dry Afromontane forest southern Ethiopia. Flora 205:135–143

Tesfaye G, Teketay D, Fetene M, Beck E (2011) Phenology of seven indigenous tree species in a dry Afromontane forest, southern Ethiopia. Trop Ecol 52:229–241

Thanbichler A, Beck E (1974) Catabolism of hamamelose. The anaerobic dissimilation of D-hamamelose by *Kluyvera citrophila* 627. Eur J Biochem 50:191–196

Thanbichler A, Gilck H, Beck E (1971) Über den Katabolismus von Hamamelose (2-C-(Hydroxymethyl)-D Ribose; I. Die Oxidation von Hamamelose durch Pseudomonas. Z Naturforsch 26b:912–915

Tolbert NE, Benker C, Beck E (1995) The oxygen and carbon dioxide compensation points of C_3 plants: possible role in regulating atmospheric oxygen. Proc Natl Acad Sci U S A 92:11230–11233

Vogg G, Heim R, Hansen J, Schäfer C, Beck E (1998a) Frost hardening and photosynthetic performance of Scots pine (*Pinus sylvestris* L.) needles. I. Seasonal changes in the photosynthetic apparatus and its function. Planta 204:193–200

Vogg G, Heim R, Gotschy B, Beck E, Hansen J (1998b) Frost hardening and photosynthetic performance of Scots pine (*Pinus sylvestris* L.). II. Seasonal changes in the fluidity of thylakoid membranes. Planta 204:201–206

Wagner BM, Beck E (1993) Cytokinins in the perennial herb *Urtica dioica* L. as influenced by its nitrogen status. Planta 190:511–518

Wieczorek J (1976) Anreicherung und Charakterisierung der Enzyme des Hamamelosemetabolismus. PhD thesis, University of Munich, 84 pp

Zech W, Bäumler R, Madhikarmi D, Gerl T, Beck E (2001a) Zur pleistozänen und holozänen Landschaftsgeschichte des Modi Khola-Tals (Annapurna, Nepal). Z Gletscherkunde Glazialgeol 37:159–190

Zech W, Madhikarmi D, Gerl T, Beck E (2001b) Rekonstruktion der spätglazialen und holozänen Vergletscherung des Annapurna III-Nordgletschers unter besonderer Berücksichtigung der Böden als Indikatoren für Klimaschwankungen. Z Gletscherkunde Glazialgeologie 37:141–158

Zhu JJ, Beck E (1991) Water relations of Pachysandra leaves during freezing and thawing: evidence for a negative pressure potential alleviating freeze-dehydration stress. Plant Physiol 97:1146–1153

Zhu JJ, Steudle E, Beck E (1989) Negative pressure produced in an artificial osmotic cell by extracellular freezing. Plant Physiol 91:1454–1459

Zwölfer H, Böheim M, Beck E (2007) Eurytoma robusta (Hym. Eurytomidae), a local key factor in the population dynamics of *Urophora cardui* (Dipt. Tephritidae) – a comparative analysis. Eur J Entomol 104:217–224

Zwölfer H, Böheim M, Beck E (2015) Eurytoma serratulae and E. robusta (Hymenoptera, Eurytomidae): complementary host exploitation strategies of coexisting parasitoids and their impact on the host Urophora cardui. J Hymenoptera Res 42:47–62

Selected Books

Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) (2008) Gradients in a tropical mountain ecosystem of Ecuador. Ecological studies, vol 198. Springer, Heidelberg, 525 pp. ISBN: 978-3-540-73525-0

Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W (eds) (2013) Ecosystem services, biodiversity and environmental change in a tropical mountain ecosystem of South Ecuador. Ecological studies, vol 221. Springer, Heidelberg, 480 pp

Schulze E-D, Beck E, Müller-Hohenstein K (2005) Textbook: plant ecology. Springer, Heidelberg, 702 pp. ISBN: 3-540-20833-X

Strnad M, Pěc P, Beck E (eds) (1999) Advances in regulation of plant growth and development. Peres Publishers, Prague, 258 pp

CV Prof. em. Dr. Dr. h.c. Erwin Beck, Bayreuth Erwin H. Beck, 02 November 1937, studied Biology, Chemistry and Earth Sciences at the University of Munich and the Technical University of the same city. In 1963, he got his Dr. rer. nat. with a dissertation in plant taxonomy on the Chemotaxonomy of Primulaceae (with H. Merxmueller) at the University of Munich. After 5 years as postdoctoral fellow at the Department of Applied Botany of the Technical University of Munich, he was promoted lecturer for "Botany". In 1970, he was appointed "Associate Professor" at the Botanical Institute of the University of Munich. More than 30 years, from 1975 to 2006, Erwin Beck was Full Prof. and Head of the Department of Plant Physiology at the University of Bayreuth. Since 2007, he is Prof. *emeritus* at the same university.

His scientific interests are plant (eco)physiology and molecular biology, plant hormone physiology, biodiversity research, vegetation ecology and ecosystem theory. In 1979, he started ecophysiological studies in the tropics, first in Kenya, Tanzania and Ethiopia (from 1996 until recent) and later on also in South America (Ecuador 1997 –). His projects which were all performed in close collaboration with partners from the host countries were funded by the German Academic Exchange Service (DAAD), the German Research Foundation (DFG), the Volkswagen Foundation, the Bavarian Ministry for Education and Research, and via high ranked counterparts by the Alexander von Humboldt Foundation.

His publication record lists more than 230 papers mostly in peer-reviewed journals, as well as the edition and co-edition of nine scientific books. He was Speaker of several German Research Units and of a Graduate college at the University of Bayreuth. He supervised around 50 successful doctoral theses.

Erwin Beck served the Academic as Dean of the Faculty (1979–1981), Vice-president for Academic affairs and Research of the University Bayreuth (1985–1988), and External Examiner of the University of Nairobi (Kenya), of Addis Ababa University (Ethiopia) and Tribhuvan University, Kathmandu (Nepal).

He was elected President of the German Biologists Association, President of the German Botanical Society (1995–2002), President of the German National Committee of the IUBS, Treasurer of the IUBS (2004–2009) and Chairman of the Senate Commission for Biodiversity Research of the German Research Foundation (2008–2014). He served as examiner in many evaluation panels and boards, e.g. of the Alexander Humboldt Foundation, the Federal Ministry for Education and Research, the German Research Foundation and was and still is a member of the Editorial Boards of scientific journals.

His honours are: Order of Merit of the German Federal Republic, Professor Honoraria of the Universidad Técnica Particular de Loja (Ecuador), Dr. rer. nat. h.c. from the Technical University of Kaiserslautern (Germany), Honorary Member of the German Botanical Society, the Treviranus-Medal of the German Biologists Association and most recently Associate Membership of the Ethiopian Academy of Sciences.

Advances in Plant Sulfur Metabolism and Signaling

Cecilia Gotor, Ana M. Laureano-Marín, Lucía Arenas-Alfonseca, Inmaculada Moreno, Ángeles Aroca, Irene García, and Luis C. Romero

Contents

Abstract Sulfur is an essential element for life, and the entry point of organic sulfur into the metabolism and into the human food chain is through cysteine, initially synthesized in plants. Cysteine is synthesized in the last stage of the plant photosynthetic assimilation of sulfate and is a very important metabolite because it is crucial for the structure, function and regulation of proteins and also because it is the precursor of essential biomolecules and defense compounds. Recent research focused on determining the specific roles of the enzymes involved in the biosynthesis of cysteine and the metabolites they produce has provided new perspectives on their functions. Thus, the cases of the less-abundant cytosolic DES1 with L-cysteine desulfhydrase activity and the major mitochondrial enzyme CAS-C1 with β-cyanoalanine synthase activity highlight the importance of sulfide and cyanide as signaling molecules involved in regulating essential processes for plant performance. The research has provided insight into the role of the sulfide specifically generated from cysteine in the cytosol as a signaling molecule regulating the processes of autophagy and of abscisic acid-dependent stomatal movement. One of the proposed mechanisms of action of sulfide involving the post-translational modification of cysteine residues in proteins by *S*-sulfhydration has been

C. Gotor (✉) • A.M. Laureano-Marín • L. Arenas-Alfonseca • I. Moreno • Á. Aroca • I. García • L.C. Romero
Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicias, Universidad de Sevilla, Avenida Américo Vespucio, 49, 41092 Sevilla, Spain
e-mail: gotor@ibvf.csic.es

Progress in Botany (2017) 78: 45–66, DOI 10.1007/124_2016_5,
Published online: 3 June 2016

demonstrated recently in plants. In addition, in the mitochondria, cysteine plays a central role in maintaining discrete concentrations of cyanide with signaling purposes for proper root hair development to occur and for the modulation of the plant immune system. Therefore, a fundamental conceptual change regarding cysteine and related molecules performing signaling roles is proposed.

1 Introduction

Sulfur is an essential element for all living beings because it is involved in numerous biochemical processes. The element sulfur exists in nature in multiple chemical structures and in a wide variety of sulfur-containing compounds due to the different oxidation states of sulfur. In organic form, sulfur is a component of all proteins in the form of the amino acids cysteine and methionine, and the thiol groups of cysteines are determinants of protein structures and functions (Trivedi et al. 2009). Many enzymes require the involvement of cysteines in the active sites for reactions (Richau et al. 2012), and in other cases, the regulation of enzymes occurs through the reduction/oxidation of thiol groups (Buchanan and Balmer 2005). Another aspect of the reactivity of the thiol group of cysteines is its high affinity for metallic ions, thus originating metal-binding proteins involved in electron transfer reactions such as in the respiratory and photosynthetic pathways (Balk and Schaedler 2014). Sulfur is also present in vitamins such as biotin and thiamine (Miret and Munne-Bosch 2014) and in antioxidant molecules such as glutathione (Noctor et al. 2012).

Sulfur is considered an element with beneficial properties associated with health in animals and is derived from the sulfur amino acids of proteins in the diet. Other sources of sulfur are plant secondary metabolites derived from cysteine, such as glucosinolates and alliins, which have been evaluated regarding human health. Glucosinolates constitute a group of thiosaccharidic compounds found in plants of the *Brassicaceae* family, such as broccoli or cauliflower, and have been suggested to have anti-carcinogenic activity (Dinkova-Kostova and Kostov 2012). Alliin is a naturally occurring cysteine sulfoxide in the *Alliaceae* family, which includes garlic and onion and shows a variety of healthful properties (Borlinghaus et al. 2014). Because animals are unable to synthesize sulfur amino acids, the entry point of reduced sulfur into the metabolism, and also into the human food chain, is through cysteine synthesized first in plants.

Cysteine is the first organic compound with reduced sulfur produced in plants from the inorganic molecule sulfate by the photosynthetic assimilation pathway (Takahashi et al. 2011; García et al. 2015). As in all living beings, cysteine is essential in plants for protein structure, function and regulation, and as a precursor

molecule of a high variety of sulfur-containing metabolites necessary for life. In these metabolites, the sulfur moieties derive from cysteine and are the functional groups responsible for their bioactivities. An example is the antioxidant glutathione, considered the major determinant of cellular redox homeostasis (Foyer and Noctor 2011; Noctor et al. 2012). Similarly, the high reactivity of its thiol is also the cause of other functions, such as heavy metal and xenobiotic detoxifications (Rea 2012; Dixon et al. 2002) or plant defense against pathogens (Rausch and Wachter 2005). Other defense compounds formed in response to biotic stresses also derive from cysteine, such as the above-mentioned glucosinolates and phytoalexins such as camalexin (Rausch and Wachter 2005). Another very important metabolite synthesized from methionine via *S*-adenosyl-L-methionine (SAM) is ethylene. This hormone is essential for adequate plant performance, as it is involved in many aspects of plant development and in plant responses to various stresses (Bleecker and Kende 2000). Sulfur is also present in the sulfolipids of the thylakoid membranes, and sulfation is important for the activity of plant hormones and peptide hormones. Many other cellular components and metabolites also contain sulfur (Ravilious and Jez 2012).

Due to the importance of sulfur-containing compounds for the development of life, knowledge of the biochemical and metabolic processes related to sulfur in photosynthetic organisms has been progressively deepened since the late 1980s. Before 2000, the photosynthetic pathway of sulfate assimilation was completely established in plants; however, many open questions related to the last step of the biosynthetic pathway of cysteine remained unanswered. After the completion of the genome sequencing of the model plant *Arabidopsis thaliana*, the possibility of functional genomics and the explosion of *omics* technologies have produced a dramatic breakthrough in knowledge of the plant sulfur metabolism. Currently, the most novel aspect of plant research on sulfur is focused on plant signaling. A fundamental change in concept has occurred, with a new view of sulfur compounds and related molecules as performing signaling roles instead of metabolic functions.

In this chapter, we provide a brief overview of the established photosynthetic sulfate reduction pathway, as recent reviews have nicely covered all the available information (Takahashi et al. 2011; Ravilious and Jez 2012; Rennenberg and Herschbach 2014; Koprivova and Kopriva 2014; García et al. 2015). We focus in more detail on the biosynthesis and metabolism of cysteine, which have undergone intense investigation in recent years, mainly in *A. thaliana*, and significant knowledge has emerged. We finish with the novel view of two molecules related to the metabolism of cysteine, sulfide and cyanide, which have been very recently found to be involved in the signaling and control of essential plant processes.

2 Photosynthetic Assimilation of Sulfate in Plants

Plants perform a crucial function in obtaining the element sulfur, which is essential for the development of life. As mentioned above, plants are able to transform inorganic sulfur compounds, unused for metabolism, to cysteine, an organic reduced sulfur compound. The primary and most abundant source for plants of the various inorganic forms of sulfur present in nature is sulfate, which is reduced and assimilated to cysteine through the photosynthetic sulfate assimilation pathway. This pathway has four stages, designated as transport, activation, reduction, and incorporation (Fig. 1).

In the first step, the sulfate enters actively from the soil into the roots and is subsequently distributed to the rest of plant through the xylem vessels by the action of proton/sulfate co-transporters. In *Arabidopsis*, a family of 12 genes for sulfate transporters has been identified and classified into different groups depending on affinity, localization, and function (Takahashi 2010). The uptake of sulfate is the most important point of control of the pathway, and the high-affinity sulfate transporters in root cells are induced under sulfur limitation conditions (Davidian and Kopriva 2010; Yoshimoto et al. 2007). In the cells, the sulfate transporters located in the tonoplast can release sulfate into the vacuoles for storage, or the sulfate can be transported to chloroplasts or plastids where the reduction of sulfate occurs.

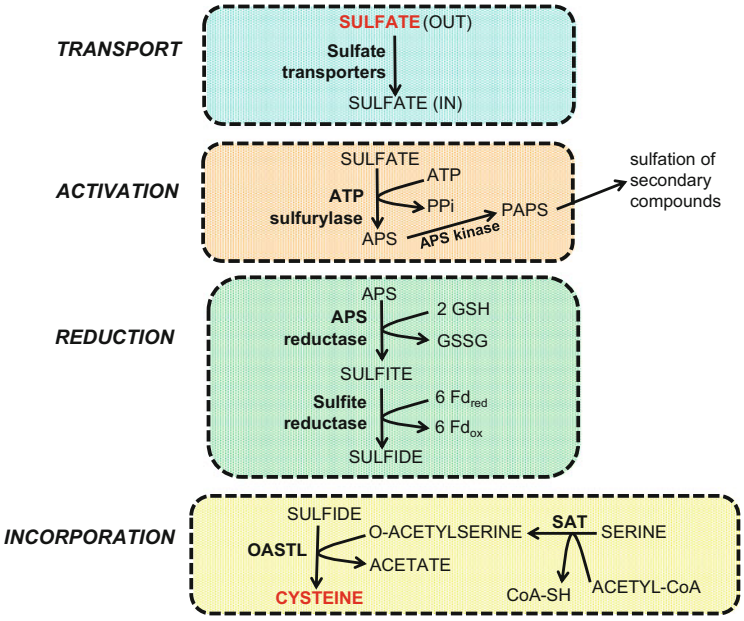

Fig. 1 Photosynthetic assimilation of sulfate. *APS* adenosine 5′-phosphosulfate, *Fd* ferredoxin, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *OASTL O*-acetylserine(thiol)lyase, *PAPS* 3′-phosphoadenosine 5′-phosphosulfate, *PPi* pyrophosphate, *SAT* serine acetyltransferase

Prior to sulfate reduction, it is necessary to lower the redox potential of sulfate to make it reducible by physiological reducing molecules, which is the activation step of the pathway. In this step, the sulfate is adenylated to produce adenosine 5′-phosphosulfate (APS), catalyzed by the ATP-sulfurylase enzyme (Fig. 1). Although the posterior reduction of APS occurs in the chloroplast, the activation step is performed in both the cytosol and the chloroplast, and different ATP-sulfurylase enzymes are present in the two cell compartments. The cytosolic APS constitutes a deviation of the primary sulfate assimilation, with the fate of sulfation of secondary compounds such as glucosinolates, flavonoids, hormones, and peptide hormones. The APS kinase enzyme phosphorylates APS to form 3′-phosphoadenosine 5′-phosphosulfate (PAPS), which is the donor of activated sulfate for the sulfation reactions (Rotte and Leustek 2000; Herrmann et al. 2014).

The sulfate reduction stage comprises two enzymatic steps strictly located in the chloroplasts, and the final product is sulfide (Fig. 1). APS is first reduced by APS reductase to produce sulfite, using reduced glutathione as the reductant molecule, and sulfite is subsequently reduced to sulfide by the action of sulfite reductase using reduced ferredoxin. In *Arabidopsis,* three chloroplastic APS reductases exist and contain two protein domains, the N-terminal with the reductase activity and an Fe-S center as a prosthetic group, and the C-terminal, which is a thioredoxin/glutaredoxin-like domain (Gutierrez-Marcos et al. 1996; Setya et al. 1996; Kopriva and Koprivova 2004). APS reductase has been established as a key regulatory point in the sulfate assimilation pathway, being regulated by sulfur availability (Martin et al. 2005). The second reduction involves a six-electron reaction catalyzed by the sulfite reductase enzyme, which contains siroheme and Fe-S centers as prosthetic groups. This enzyme is encoded in *Arabidopsis* by a single gene, in contrast to other enzymes of the pathway that are encoded by gene families (Khan et al. 2010). Sulfide is finally incorporated into a carbon/nitrogen skeleton to form cysteine, and this last step constitutes the final stage of the assimilation pathway (Fig. 1), which is described in more detail in the next section.

As mentioned above, the main control points of sulfate assimilation are sulfate uptake and APS reduction, with transcription regulated by sulfate availability. The transcription factor SLIM1, belonging to the ethylene-insensitive 3-family, has been shown to be a key transcriptional regulator of sulfate uptake but not of APS reductase in response to sulfur limitation (Maruyama-Nakashita et al. 2006). In addition, microRNA-395 operates in the same circuit of regulation because SLIM1 induces the accumulation of the microRNA, which has ATP-sulfurylase isoforms and the sulfate transporter SULTR2;1, as targets (Kawashima et al. 2009). Other transcription factors such as the ones in the MYB family have been found to be involved in the activation of the sulfate assimilation pathway and glucosinolate biosynthesis (Hirai et al. 2007). Moreover, two sulfur-responsive cis elements have been identified: one is the SURE element in the promoter of the sulfate transporter SULTR1;1, which contains an auxin response factor binding sequence (Maruyama-Nakashita et al. 2005), and the other is the UPE-box identified in the promoter of the tobacco UP9 gene, which is homologous to the Low Sulfur Upregulated (LSU) genes from *Arabidopsis*, where the UPE-box has also been found in APS reductase

genes together with the LSU promoters (Wawrzynska et al. 2010). The central regulator of photomorphogenesis, the HY5 transcription factor, also binds to the promoters of APS reductase isoforms and the sulfate transporter SULTR1;2 (Lee et al. 2011).

3 Biosynthesis and Metabolism of Cysteine: An Update

Cysteine is synthesized in plants in the last stage of the photosynthetic sulfate assimilation pathway, consisting of the sequential reaction of two enzymes (Fig. 1). Serine acetyltransferase (SAT, also known as SERAT) synthesizes the intermediary product *O*-acetylserine (OAS) from acetyl-CoA and serine, and *O*-acetylserine (thiol)lyase (OASTL) incorporates the sulfide into OAS to produce cysteine, with the participation of pyridoxal-5′-phosphate (PLP) as cofactor. These two enzymes physically interact to form a hetero-oligomeric complex named cysteine synthase, which has been extensively studied in plants (Droux et al. 1998; Wirtz and Hell 2006, 2007; Francois et al. 2006; Yi et al. 2010; Jez and Dey 2013; Heeg et al. 2008). It is well known that the domain in the enzyme SAT that is involved in the OASTL interaction, namely the β-sheet-containing C-terminal domain, is also responsible for catalysis. The protein interactions inside the complex modify the catalytic properties of the two components, allowing the activation of SAT for the synthesis of OAS and, by contrast, the inactivation of OASTL for the synthesis of cysteine. Thus, the bound SAT synthesizes OAS, and the unbound OASTL is the responsible for the synthesis of cysteine. The relative activities of SAT and OASTL in different cell compartments have demonstrated that all of the SAT enzyme binds in the complex, whereas the majority of OASTL is free. In this way, the formation of this complex is considered a regulatory circuit to modulate the production of cysteine depending on the sulfur status of the plant, and therefore, it is another point of control of the sulfate assimilation pathway. The formation of the complex is modulated by the relative amounts of sulfide and OAS, such that under sulfur limitation, the amount of sulfide is low, consequently producing an increase in OAS concentration, provoking the dissociation of the complex to prevent more OAS from being synthesized by the bound SAT. In contrast, sulfide produced under sulfur-replete conditions stabilizes the complex to promote the formation of OAS and therefore the synthesis of cysteine (Fig. 2).

The plant cells contain different SAT and OASTL enzymes localized in the cytosol, chloroplasts, and mitochondria, resulting in a complex variety of isoforms and different subcellular cysteine pools. In general, there is a higher proportion of OASTL isoforms than SATs in all the different photosynthetic organisms studied, as observed in the available databases (www. phytozome.org). This information raises many open questions, such as why there are so many isoforms and whether they are redundant or have different functions. Other questions that arose were why cysteine biosynthesis occurs in different cell compartments, whether each pool of cysteine has a different fate, and whether there is any kind of regulation between the

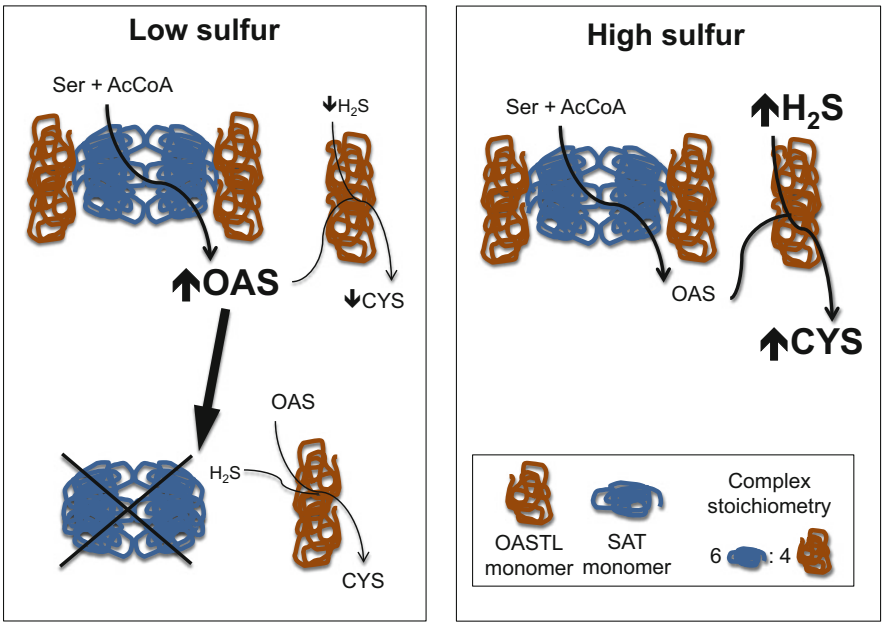

Fig. 2 Mode of action of the cysteine synthase complex. Details are described in the text

different pools. From the beginning of the new century, intense investigation has been performed to try to answer all the open questions, mainly driven by the completion of the genome sequence of the model plant *Arabidopsis thaliana*, the availability of *Arabidopsis* T-DNA insertion mutants in each isoform and the application of *omics* technology.

In the *A. thaliana* genome, five different *SAT* and nine *OASTL* genes have been identified (García et al. 2015; Gotor et al. 2015) (Fig. 3). The SAT family includes the three most abundant isoforms located in different compartments that can interact with their corresponding OASTL partners to form the cysteine synthase complex, namely the chloroplastic SAT1/SERAT2;1 (At1g55920); the mitochondrial SAT3/SERAT2;2 (At3g13110); and the cytosolic SAT5/SERAT1;1 (At5g56760). The other minor isoforms with cytosolic localization that cannot interact with OASTL are SAT2/SERAT3;1 (At2g17640) and SAT4/SERAT3;2 (At4g35640) (Howarth et al. 2003; Bonner et al. 2005; Kawashima et al. 2005; Heeg et al. 2008). With respect to the OASTL family, the most abundant are the cytosolic OAS-A1 (At4g14880), the chloroplastic OAS-B (At2g43750), and the mitochondrial OAS-C (At3g59760), which are considered authentic OASTLs due to their ability to interact with their SAT partners (Bonner et al. 2005; Heeg et al. 2008). The *OAS-A2* gene does not code for a functional protein due to an in-frame stop codon in its sequence. Another highly expressed enzyme belonging to the family based on very high sequence homology is CAS-C1 (former CYS-C1, At3g61440) located in the mitochondria, which is a β-cyanoalanine synthase and

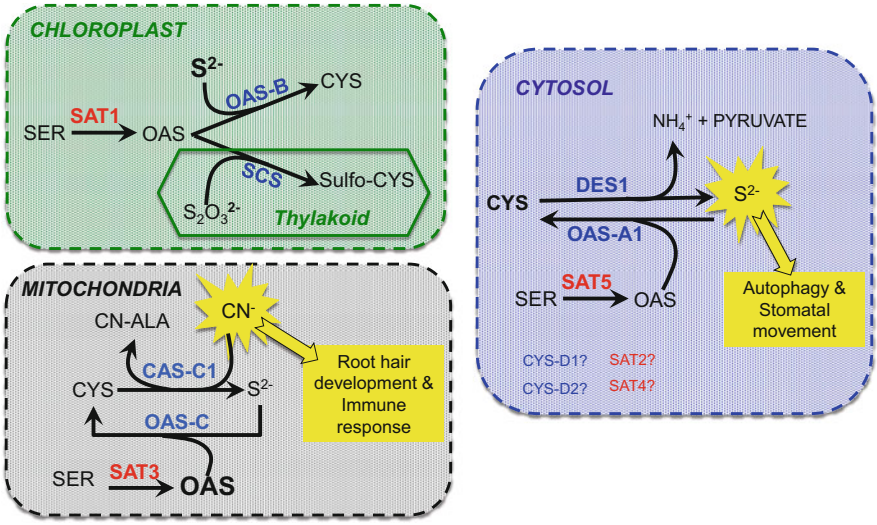

Fig. 3 Biosynthesis of cysteine in *Arabidopsis* cells and processes regulated by cytosolic sulfide and mitochondrial cyanide. Members of the SAT enzyme family are colored in *red* and the members of the OASTL enzyme family are colored in *blue*

catalyzes the formation of β-cyanoalanine and sulfide from cysteine and cyanide (Hatzfeld et al. 2000; Yamaguchi et al. 2000). The sulfide produced is then used by the authentic OAS-C as a substrate for the synthesis of cysteine, which in turn is used by CAS-C1, creating a cyclic pathway in the mitochondria (Álvarez et al. 2012c). The mitochondrial cyanide levels modulated by the CAS-C1 enzyme have been recently demonstrated to play a signaling role in essential plant processes (García et al. 2010; García et al. 2013), as described below in detail.

The other members of the OASTL family are much less abundant and were initially identified by sequence homology and considered to play auxiliary functions to the major isoforms. The *Arabidopsis* chloroplasts also contain, in addition to OAS-B, SCS (former CS26, At3g03630), an *S*-sulfocysteine synthase that catalyzes the incorporation of thiosulfate into OAS to form *S*-sulfocysteine, instead of sulfide, which is catalyzed by OAS-B (Fig. 3). This activity, although known in bacteria, was only recently demonstrated in plants and shown to play an essential role in chloroplast function (Bermúdez et al. 2010). Another important difference between these two enzymes is their localization inside the chloroplast; while OAS-B is a stromal protein, SCS is located in the thylakoid lumen (Bermúdez et al. 2012). Detailed photosynthetic characterization of SCS null mutants has shown that this enzyme is essential for the adequate performance of the photosynthetic apparatus under long-day growth conditions, and it is suggested that SCS functions as a sensor of the chloroplast redox state (Bermúdez et al. 2012; Gotor and Romero 2013).

An additional three minor OASTL proteins are present in the cytosol of *Arabidopsis*: CYS-D1 (At3g04940) and CYS-D2 (At5g28020), which have not

been studied in detail, and DES1 (former CS-LIKE, At5g28030), which has recently been the focus of intense investigation. DES1 exhibits L-cysteine desulfhydrase activity, catalyzing the desulfuration of cysteine to sulfide plus ammonia and pyruvate, instead of the synthesis of cysteine like an authentic OASTL (Álvarez et al. 2010). Thus, DES1 functions in opposition to OAS-A1 in the cytosol; both enzymes are responsible for the regulation of the homeostasis of cysteine and the generation of sulfide for the signaling of important plant processes (Álvarez et al. 2010; Álvarez et al. 2012a; Álvarez et al. 2012b; Gotor et al. 2015; Gotor et al. 2013; Romero et al. 2014; Romero et al. 2013), as will be described in detail in the next sections.

Although it was originally thought that each cell compartment where protein synthesis occurs requires the in situ production of cysteine (Lunn et al. 1990), the viability of the major chloroplastic OASTL mutant in *Arabidopsis* demonstrated that the synthesis of cysteine in chloroplasts can be compensated by its synthesis in other compartments. In fact, the analysis of different T-DNA insertion mutants defective in different members of the SAT and OASTL families in *Arabidopsis* has allowed a deep knowledge of the biosynthesis of cysteine inside cells. It is now well established that the substrates for the synthesis of cysteine are produced in the mitochondria, which are the main source of OAS, and in the chloroplasts, where sulfide is produced by the photosynthetic reduction of sulfate, while cysteine is mainly synthesized in the cytosol (Haas et al. 2008; Heeg et al. 2008; López-Martín et al. 2008a; Watanabe et al. 2008a; Watanabe et al. 2008b; Krueger et al. 2009). However, in the case of a mutation affecting any one of the SAT or OASTL isoforms, members of the other compartments can compensate for the synthesis of cysteine, and the resulting mutants are viable, suggesting efficient transport mechanisms of sulfide, OAS, and cysteine among the cell compartments.

The amount of cysteine measured in the cytosol was higher than 300 μM, while in the other compartments, the estimated concentration was below 10 μM (Krueger et al. 2009), corroborating the observation that the cytosol is the major site for the synthesis of cysteine. However, cysteine can be highly toxic at higher concentrations due to its high reactivity, originating reactive species of sulfur and oxygen (Park and Imlay 2003), and in consequence, it is very important to regulate the cysteine homeostasis in the cytosol. This precise regulation has been proposed to be performed by the action of the enzymes OAS-A1 (catalyzing cysteine synthesis) and DES1 (catalyzing cysteine degradation). This suggestion was based on the phenotypes of the corresponding deficient mutants, which show opposite impacts on plant metabolism. The OAS-A1 null mutants with reduced cysteine content show enhanced production of reactive oxygen species (ROS) (López-Martín et al. 2008a), while the DES1 null mutants with increased cysteine content show a significant reduction in ROS (Álvarez et al. 2010). Therefore, the level of cysteine in the cytosol determines the redox state and consequently influences processes regulated by redox signaling (López-Martín et al. 2008b). Thus, the levels of cysteine affect plant responses to abiotic stresses such as cadmium stress, in which the OAS-A1 null mutants show increased sensitivity, and the DES1 deficient mutants exhibit enhanced resistance (López-Martín et al. 2008a; Álvarez

et al. 2010). Similarly, the levels of cysteine alter the plant immunity, and the OAS-A1 and DES1 null mutants show decreased and enhanced resistance to pathogens, respectively (Álvarez et al. 2012a).

4 Signaling by Cysteine-Related Molecules

As above described, an intense investigation of the enzymes involved in the biosynthesis of cysteine in *A. thaliana* has developed since the beginning of the new century. An important body of knowledge has emerged, although the main focus has been on the OASTL enzymes considered as authentic, and always in the context of their involvement in the sulfate assimilation pathway. However, recent research focused on determining the specific roles of the other OASTLs and the metabolites they produce has provided new perspectives on their functions. Examples include the less-abundant cytosolic DES1 with L-cysteine desulfhydrase activity and the major mitochondrial enzyme CAS-C1 with β-cyanoalanine synthase activity. The most significant finding has been to highlight the importance of molecules related to cysteine, such as sulfide and cyanide, as signaling molecules regulating essential plant processes (Romero et al. 2014; Gotor et al. 2015). As described below, this research has provided insight into the role of the sulfide specifically generated from cysteine in the cytosol, acting as a signaling molecule regulating autophagy and the stomatal movement. Moreover, the discrete accumulation of cyanide, maintained by the levels of cysteine in the mitochondria, modulates root hair development and the plant immune system.

Therefore, a change in concept has been proposed for cysteine-related molecules, from metabolic function to a new concept of signaling molecules. When we compare sulfide and cyanide with already recognized signaling molecules such as nitric oxide, hydrogen peroxide, and ethylene, we observe important similarities (Table 1). All of these molecules are of low molecular weight and are gaseous but can be dissolved in water depending on pH, and therefore they can cross membranes and be maintained in different cell compartments to perform their signaling roles. All show high or moderate chemical reactivity that allows them to modify protein activity, making them perfect for signaling molecules. In addition, we can

Table 1 Characteristics of signaling molecules

	Hydrogen sulfide	Hydrogen cyanide	Nitric oxide	Hydrogen peroxide	Ethylene
Chemical formula	H–S–H	HC≡N	N=O	HO–OH	$H_2C=CH_2$
Molecular mass (g mol^{-1})	34.08	27.03	30.01	34.01	28.05
Chemical reactivity	Very high	Moderate	Very high	Very high	Moderate
Toxicity/signaling	YES/yes	YES/yes	YES/YES	YES/YES	yes/YES

consider that all molecules (in some cases suggested and in others proved) present a duality between their toxicity and their signaling role. Thus, above a threshold concentration, all of these molecules are toxic to life, but below a threshold concentration, they are recognized as important signaling molecules. In conclusion, both sulfide and cyanide meet all the requirements for signaling, although in the case of sulfide, intense research has been conducted in animal and plant systems, and sulfide is already accepted as a regulator of essential life processes. However, the evidence for cyanide is currently emerging, and more investigation remains necessary to emphasize its importance in plant systems. In the following sections, a more detailed description of the roles of these molecules, mainly in *Arabidopsis*, will be provided.

4.1 Sulfide

Hydrogen sulfide is endogenously produced and metabolized by cells in a precise and regulated manner. In animal systems, it is already recognized as an important signaling molecule, comparable to carbon monoxide and nitric oxide (Gadalla and Snyder 2010; Wang 2012). Emerging data in plant systems have recently changed the concept of sulfide from a toxic molecule to a signaling molecule of the same importance as nitric oxide and hydrogen peroxide (García-Mata and Lamattina 2013; Calderwood and Kopriva 2014).

As described above, the plant chloroplast is the main source of sulfide, which is produced in the sulfate reduction stage of the photosynthetic assimilation pathway. This chloroplastic sulfide was thought to reach the cytosol for the synthesis of cysteine by diffusion across the chloroplast membranes. However, at the basic pH of the chloroplast stroma under illumination (Wu and Berkowitz 1992), hydrogen sulfide is dissociated and is present in the anionic form instead of a gaseous molecule and therefore is unable to permeate the chloroplast envelope (Kabil and Banerjee 2010). In mitochondria, sulfide is also produced during the synthesis of β-cyanoalanine catalyzed by CAS-C1 and is then used by OAS-C as a substrate in a cyclic pathway. Similarly to the chloroplast, the pH of the mitochondrial stroma is basic in metabolically active cells due to the pumping of protons out of the matrix (Santo-Domingo and Demaurex 2012), and sulfide is predominantly present in its ionic form. Consequently, an active transporter is required for sulfide to reach the plant cytosol, and this transporter is yet undiscovered, although a bacterial hydrosulfide ion channel has been described (Czyzewski and Wang 2012).

In the cytosol of *Arabidopsis*, it was demonstrated that one of the low-abundance proteins of the OASTL family (previously designated as CS-LIKE) catalyzes the degradation of cysteine instead of its synthesis. The enzymatic characterization of the recombinant protein and its structural features showed that this protein, renamed DES1, is a novel L-cysteine desulfhydrase that catalyzes the desulfuration of cysteine to sulfide, ammonia, and pyruvate (Álvarez et al. 2010; Gotor et al. 2010) (Fig. 3). The function of DES1 and its impact on plant metabolism

was revealed by the detailed characterization of the DES1 null mutants. The deficiency of DES1 avoids the generation of sulfide in the cytosol, affecting plant responses to abiotic stress conditions, plant immunity, the regulation of the leaf senescence, the progress of autophagy, and the modulation of abscisic acid-dependent stomatal closure (Álvarez et al. 2010; Álvarez et al. 2012a; Álvarez et al. 2012b; Romero et al. 2013; Scuffi et al. 2014). Therefore, DES1 is considered to be responsible for the release of sulfide in the cytosol for signaling purposes.

The role of the sulfide generated by the DES1 enzyme as a signaling molecule has been deeply studied in several essential processes for plant performance. Thus, experimental evidence showed that a mutation in *DES1* leads to premature leaf senescence, observed at the phonotypical, cellular, and transcriptional levels. A faster developmental program was observed in the null mutant plants grown side by side with the wild type (Romero et al. 2014). Additionally, an accumulation of the senescence-associated vacuoles was detected in mesophyll protoplasts from DES1 null mutants. The mutant transcriptional profile was also compatible with early senescence, showing the increased expression of genes coding for senescence-associated proteins and protein proteases and the des-regulation of the ubiquitin-dependent degradation pathway (Álvarez et al. 2012b). Importantly, the exogenous application of sulfide clearly rescues the early senescence phenotype of the mutants, and thus, the mesophyll protoplasts prepared from sulfide-treated mutant plants showed no detectable senescence-associated vacuoles and also provoked the reversal of all alterations of the transcriptional profile (Álvarez et al. 2012b).

The signaling nature of the sulfide molecule and the essential role of the DES1 enzyme in the generation of this sulfide in the cytosol were also inferred in the regulation of the process of autophagy upon detailed characterization of the DES1 null mutants. Autophagy is a conserved mechanism present in eukaryotic cells, consisting of the digestion of cell contents for recycling, in the majority of the cases with a survival role. Various types of autophagy have been described, but the most studied in plants is characterized by the synthesis of double-membrane structures called autophagosomes in the cytosol. In these structures, the cytosolic contents are sequestrated, transported to the plant vacuole, and released inside for degradation by vacuolar hydrolases (Thompson and Vierstra 2005; Bassham et al. 2006). The proteins involved in autophagy are referred to as ATGs, and the ATG8 protein has been extensively studied for monitoring autophagy in plants. ATG8 is covalently conjugated to the lipid phosphatidylethanolamine (PE), and this adduct is required for the formation of autophagosomes, remaining bound to this structure. Therefore, the accumulation and lipidation of ATG8 is a marker of autophagy activation (Bassham 2015). The deficiency of DES1 was shown to induce the accumulation and lipidation of ATG8, which were observable by immunoblot analysis and at the transcriptional level. Because the mutation of DES1 disrupts the capacity of the cytosol to generate sulfide, restoring this capacity by exogenous sulfide application or genetic complementation clearly reverses the induction of autophagy (Álvarez et al. 2012b). Therefore, it was concluded that sulfide functions in the cytosol as a signaling molecule in autophagy through negative regulation and that DES1 is responsible for generating this signaling sulfide (Gotor et al. 2013; Romero

et al. 2013). Moreover, the role of sulfide as a repressor of autophagy is independent of nutrient conditions because it can also inhibit autophagy under dark-induced carbon starvation, a condition unrelated to sulfur metabolism (Álvarez et al. 2012b).

Another key process for plant development in which sulfide has been reported as a signaling molecule is the abscisic acid (ABA)-dependent stomatal movement. This participation was first demonstrated by exogenous applications of different sulfide donor molecules (García-Mata and Lamattina 2010; Lisjak et al. 2010). Very recently, the use of the DES1 null mutants has allowed deeper study of the participation of the sulfide generated by DES1 and its cross talk with nitric oxide in the ABA-dependent signaling network in guard cells. The null mutants are unable to close the stomata in the presence of ABA, but the exogenous application of sulfide or the genetic complementation restores this defect. By using different mutants affecting the ABA perception and signaling, it was concluded that sulfide acts upstream of the ABA receptor and that DES1 is required for ABA-dependent NO production. Therefore, DES1 is a novel component of ABA signaling in guard cells, in which NO is downstream of sulfide in the stomatal closure (Scuffi et al. 2014). In addition to the cross talk of sulfide-generated DES1 with ABA and nitric oxide, cross talk with other hormones regulating plant developmental processes, such as auxins, has recently been suggested (Laureano-Marín et al. 2014).

The involvement of sulfide in many other processes in plant systems has been demonstrated, such as tolerance to a vast array of plant stresses, ranging from heavy metal stress to salinity, hypoxia, and drought (Zhang et al. 2008; Zhang et al. 2010; Jin et al. 2011; Cheng et al. 2013; Christou et al. 2013), thus allowing plant viability. Moreover, sulfide has also been implicated in regulating other essential processes for adequate plant performance, such as photosynthesis (Chen et al. 2011).

Deciphering the mechanism of action of sulfide and its molecular targets has become an interesting area of investigation. Two putative mechanisms have been proposed based on the nucleophilic properties of this molecule. The first mechanism proposes that sulfide acts as a reducer of oxygen and other ROS, thus reducing the cellular oxidative stress (Kabil and Banerjee 2010). Interestingly, the effect of sulfide-mediated tolerance and protection against several plant stresses was demonstrated to be produced by increasing the antioxidant defenses (Zhang et al. 2008).

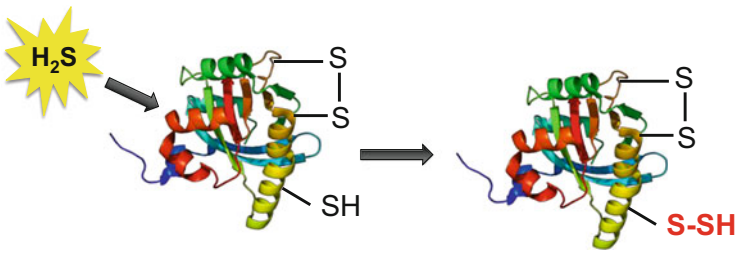

Fig. 4 Post-translational modification of protein cysteine residues by *S*-sulfhydration

Thus, although it has not been demonstrated, the action of sulfide as a ROS scavenger is considered a possible mechanism.

The second proposed mechanism involves the post-translational modification of cysteine residues in proteins, altering the thiol group (-SH) to form a persulfide group (-SSH) (Mustafa et al. 2009). This process is called *S*-sulfhydration (Fig. 4). The chemical process behind this method is a matter of debate with respect to the form in which the thiol group reacts with the molecule of hydrogen sulfide. In any case, the molecular targets of *S*-sulfhydration are proteins, and the change of a thiol to a persulfide modifies the chemical reactivity and specific functions of the proteins, as demonstrated in animals (Mustafa et al. 2009; Mustafa et al. 2011) and very recently in plant systems (Aroca et al. 2015). Effectively, *S*-sulfhydration has been demonstrated endogenously in *Arabidopsis* using a modified biotin switch method, previously used in mammals. This study revealed that the sulfide added through this modification reversibly regulates the function of plant proteins, and the presence of an *S*-sulfhydration-modified cysteine residue on cytosolic ascorbate peroxidase was demonstrated (Aroca et al. 2015).

4.2 Cyanide

Cyanide is extensively present in organisms such as bacteria, fungi, insects, and plants, despite its high toxicity due to its high reactivity, especially in mitochondria, where cyanide is a potent inhibitor of the cytochrome c oxidase, thus blocking the respiratory chain (Cooper and Brown 2008). Cyanogenic plants synthesize cyanogenic compounds that release cyanide against herbivores (Conn 2008). In non-cyanogenic plants such as *Arabidopsis*, cyanide is produced during the biosynthesis of the hormone ethylene, which is involved in the regulation of numerous developmental processes and in the responses to stress conditions (Bleecker and Kende 2000). Additionally, cyanide originates in the biosynthesis of the phytoalexin camalexin, which is formed when *Arabidopsis* plants are infected by a large variety of microorganisms (Glawischnig 2007). Therefore, cyanide is produced in significant amounts under certain conditions in the plant and should be maintained below the toxic concentration threshold.

In *A. thaliana*, cyanide is mainly detoxified by the mitochondrial CAS-C1 (former CYS-C1) enzyme, a member of the OASL family, through the conversion of cyanide and cysteine into β-cyanoalanine and sulfide, as described above. CAS-C1 acts together with the authentic mitochondrial OASTL, OAS-C, which provides the required substrate for cysteine and eliminates the produced sulfide, which is also toxic to the cytochrome c oxidase. Thus, a cyclic pathway for cyanide detoxification exists in the mitochondria (Álvarez et al. 2012c) (Fig. 3).

Different reports have suggested that cyanide at non-toxic levels performs regulatory roles in different physiological processes, not only in plants but also in animal systems, where, for example, it has been hypothesized to act as neuromodulator (Cipollone and Visca 2007). Restricted to plants, exogenously

applied cyanide was demonstrated to be a regulator of seed germination and dormancy release in various plants (Cohn and Hughes 1986; Bethke et al. 2006) and to play a role in plant resistance to viral and fungal pathogens (Chivasa and Carr 1998; Seo et al. 2011). The investigation of the CAS-C1 null mutant has provided insight into the role of endogenously produced cyanide in *Arabidopsis*.

The mutation of CAS-C1 (and also of OAS-C, which acts jointly) leads to a significant accumulation of cyanide, mainly in the root tissues, but is not toxic for the plant, which is perfectly viable. The increased level of cyanide correlates with very interesting phenotypic characteristics, suggesting that cyanide plays a signaling role. Both null mutants show hairless root phenotypes that are rescued by genetic complementation, thereby confirming that the phenotypes are specific to the mutations (García et al. 2010; Álvarez et al. 2012c). In addition, this characteristic phenotype of the absence of root hairs can be obtained in wild type roots by the exogenous application of cyanide and reversed by the addition of a cyanide antidote used when humans are poisoned (García et al. 2010).

Another important aspect that is altered in the CAS-C1 null mutants and correlated with the accumulation of cyanide levels is the plant response to pathogens. The mutants show an increased susceptibility to necrotrophic fungus and increased tolerance to biotrophic bacteria and viruses. This altered response is rescued by genetic complementation and by the application of a cyanide antidote, reinforcing the conclusion that it is dependent on cyanide. In addition, cyanide accumulates differently during compatible and incompatible plant–bacteria interactions (García et al. 2013). Because the null mutants exhibit an induced alternative oxidase respiration, an accumulation of ROS and an induced salicylic acid-dependent pathway, it was hypothesized that cyanide might generate a mitochondrial signal, unknown to date, that could modulate the plant immune system (García et al. 2014).

All the described findings suggest the existence of a signaling mechanism dependent on mitochondrial cyanide that regulates the root hair development process and the immune responses. The underlying mechanisms of the mode of action and specific targets of cyanide remain open questions and constitute a new area for investigation. However, by analogy with *S*-sulfhydration and based on chemical reports, the existence of a new post-translational modification of proteins, consisting of the direct incorporation of a molecule of cyanide into the thiol group of cysteine, could be a possibility. Thus, protein cyanylation can be achieved in vitro by treatments with cyanylating chemicals that have been used for the determination of disulfide structure in proteins (Qi et al. 2001), although the natural cyanylation of proteins with biological relevance has not yet been proven.

5 Concluding Remarks

Sulfur-containing compounds are essential for life, and the entry point of sulfur into the metabolism is through cysteine synthesized by plants during photosynthetic sulfate assimilation. This pathway has been intensively studied, and the emergence of functional genomics and of *omics* technology has produced a dramatic breakthrough in knowledge of the plant sulfur metabolism. The current research on plant sulfur is focused on signaling. A change of the concept of sulfur compounds and related molecules has been raised, focusing not on sulfur metabolism, but instead on signaling roles. Specifically, two molecules related to cysteine, namely cytosolic sulfide and mitochondrial cyanide, have been shown to act as signaling molecules, regulating essential plant processes. Recent research has provided insight into the role of the sulfide, specifically generated from cysteine in the cytosol by the enzyme L-cysteine desulfhydrase DES1, as a signaling molecule regulating the process of autophagy and the abscisic acid-dependent stomatal movement. Moreover, in the mitochondria, cysteine plays a central role in maintaining discrete concentrations of cyanide with signaling purposes. The non-toxic accumulation of cyanide is essential for proper root hair development and for the full induction of plant responses to pathogens by modulating the plant immune system. This novel view of cysteine-related molecules with a signaling fate reveals new and highly interesting areas for potential investigation, such as the specific function, targets, and regulation of these molecules involved in the signaling and control of different plant processes, as well as the underlying mechanisms.

Acknowledgments This work was funded in part by the European Regional Development Fund through the Ministerio de Economia y Competitividad (grant MOLCIS, no. BIO2013-44648-P). A. M. L.-M. and L. A.-A. thank the Ministerio de Economia y Competitividad for fellowship support through the program of Formación de Personal Investigador. A. A. thanks the Consejo Superior de Investigaciones Científicas for support through the postdoctoral program of the Junta de Ampliación de Estudios.

References

Álvarez C, Calo L, Romero LC, García I, Gotor C (2010) An O-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. Plant Physiol 152(2):656–669. doi:10.1104/pp.109.147975

Álvarez C, Bermudez MA, Romero LC, Gotor C, García I (2012a) Cysteine homeostasis plays an essential role in plant immunity. New Phytol 193(1):165–177. doi:10.1111/j.1469-8137.2011.03889.x

Álvarez C, García I, Moreno I, Pérez-Pérez ME, Crespo JL, Romero LC, Gotor C (2012b) Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in Arabidopsis. Plant Cell 24(11):4621–4634. doi:10.1105/tpc.112.105403

Álvarez C, García I, Romero LC, Gotor C (2012c) Mitochondrial sulfide detoxification requires a functional isoform O-acetylserine(thiol)lyase C in Arabidopsis thaliana. Mol Plant 5 (6):1217–1226. doi:10.1093/mp/sss043

Aroca Á, Serna A, Gotor C, Romero LC (2015) S-sulfhydration: a cysteine posttranslational modification in plant systems. Plant Physiol 168(1):334–342. doi:10.1104/pp.15.00009

Balk J, Schaedler TA (2014) Iron cofactor assembly in plants. Annu Rev Plant Biol 65:125–153. doi:10.1146/annurev-arplant-050213-035759

Bassham DC (2015) Methods for analysis of autophagy in plants. Methods 75:181–188. doi:10.1016/j.ymeth.2014.09.003

Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K (2006) Autophagy in development and stress responses of plants. Autophagy 2(1):2–11

Bermúdez MA, Páez-Ochoa MA, Gotor C, Romero LC (2010) Arabidopsis S-sulfocysteine synthase activity is essential for chloroplast function and long-day light-dependent redox control. Plant Cell 22(2):403–416. doi:10.1105/tpc.109.071985

Bermúdez MA, Galmés J, Moreno I, Mullineaux PM, Gotor C, Romero LC (2012) Photosynthetic adaptation to length of day is dependent on S-sulfocysteine synthase activity in the thylakoid lumen. Plant Physiol 160(1):274–288. doi:10.1104/pp.112.201491

Bethke PC, Libourel IG, Reinohl V, Jones RL (2006) Sodium nitroprusside, cyanide, nitrite, and nitrate break Arabidopsis seed dormancy in a nitric oxide-dependent manner. Planta 223 (4):805–812. doi:10.1007/s00425-005-0116-9

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18. doi:10.1146/annurev.cellbio.16.1.1

Bonner ER, Cahoon RE, Knapke SM, Jez JM (2005) Molecular basis of cysteine biosynthesis in plants: structural and functional analysis of O-acetylserine sulfhydrylase from Arabidopsis thaliana. J Biol Chem 280(46):38803–38813

Borlinghaus J, Albrecht F, Gruhlke MC, Nwachukwu ID, Slusarenko AJ (2014) Allicin: chemistry and biological properties. Molecules 19(8):12591–12618. doi:10.3390/molecules190812591

Buchanan BB, Balmer Y (2005) Redox regulation: a broadening horizon. Annu Rev Plant Biol 56:187–220

Calderwood A, Kopriva S (2014) Hydrogen sulfide in plants: from dissipation of excess sulfur to signaling molecule. Nitric Oxide 41:72. doi:10.1016/j.niox.2014.02.005

Chen J, Wu FH, Wang WH, Zheng CJ, Lin GH, Dong XJ, He JX, Pei ZM, Zheng HL (2011) Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. J Exp Bot 62(13):4481–4493. doi:10.1093/jxb/err145

Cheng W, Zhang L, Jiao C, Su M, Yang T, Zhou L, Peng R, Wang R, Wang C (2013) Hydrogen sulfide alleviates hypoxia-induced root tip death in Pisum sativum. Plant Physiol Biochem 70:278–286. doi:10.1016/j.plaphy.2013.05.042

Chivasa S, Carr JP (1998) Cyanide restores N gene-mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. Plant Cell 10(9):1489–1498

Christou A, Manganaris GA, Papadopoulos I, Fotopoulos V (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. J Exp Bot 64(7):1953–1966. doi:10.1093/jxb/ert055

Cipollone R, Visca P (2007) Is there evidence that cyanide can act as a neuromodulator? IUBMB Life 59(3):187–189. doi:10.1080/15216540600981768

Cohn MA, Hughes JA (1986) Seed dormancy in red rice : v. Response to azide, hydroxylamine, and cyanide. Plant Physiol 80(2):531–533

Conn EE (2008) Our work with cyanogenic plants. Annu Rev Plant Biol 59:1–19. doi:10.1146/annurev.arplant.59.032607.092924

Cooper CE, Brown GC (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism

and physiological significance. J Bioenerg Biomembr 40(5):533–539. doi:10.1007/s10863-008-9166-6
Czyzewski BK, Wang DN (2012) Identification and characterization of a bacterial hydrosulphide ion channel. Nature 483(7390):494–497. doi:10.1038/nature10881
Davidian JC, Kopriva S (2010) Regulation of sulfate uptake and assimilation--the same or not the same? Mol Plant 3(2):314–325. doi:10.1093/mp/ssq001
Dinkova-Kostova AT, Kostov RV (2012) Glucosinolates and isothiocyanates in health and disease. Trends Mol Med 18(6):337–347. doi:10.1016/j.molmed.2012.04.003
Dixon DP, Lapthorn A, Edwards R (2002) Plant glutathione transferases. Genome Biol 3(3): Review S3004
Droux M, Ruffet ML, Douce R, Job D (1998) Interactions between serine acetyltransferase and O-acetylserine (thiol) lyase in higher plants--structural and kinetic properties of the free and bound enzymes. Eur J Biochem 255(1):235–245
Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155(1):2–18. doi:10.1104/pp.110.167569
Francois JA, Kumaran S, Jez JM (2006) Structural basis for interaction of O-acetylserine sulfhydrylase and serine acetyltransferase in the Arabidopsis cysteine synthase complex. Plant Cell 18(12):3647–3655
Gadalla MM, Snyder SH (2010) Hydrogen sulfide as a gasotransmitter. J Neurochem 113 (1):14–26. doi:10.1111/j.1471-4159.2010.06580.x
García I, Castellano JM, Vioque B, Solano R, Gotor C, Romero LC (2010) Mitochondrial {beta}-cyanoalanine synthase is essential for root hair formation in Arabidopsis thaliana. Plant Cell 22 (10):3268–3279. doi:10.1105/tpc.110.076828
García I, Rosas T, Bejarano ER, Gotor C, Romero LC (2013) Transient transcriptional regulation of the CYS-C1 gene and cyanide accumulation upon pathogen infection in the plant immune response. Plant Physiol 162(4):2015–2027. doi:10.1104/pp.113.219436
García I, Gotor C, Romero LC (2014) Beyond toxicity: a regulatory role for mitochondrial cyanide. Plant Signal Behav 9, e27612
García I, Romero LC, Gotor C (2015) Cysteine homeostasis. In: D'Mello JPF (ed) Amino acids in higher plants. CAB International, Wallingford, pp 219–233
García-Mata C, Lamattina L (2010) Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytol 188(4):977–984. doi:10.1111/j.1469-8137.2010.03465.x
García-Mata C, Lamattina L (2013) Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. Plant Sci 201–202:66–73. doi:10.1016/j.plantsci.2012.11.007
Glawischnig E (2007) Camalexin. Phytochemistry 68(4):401–406. doi:10.1016/j.phytochem.2006.12.005
Gotor C, Romero LC (2013) S-sulfocysteine synthase function in sensing chloroplast redox status. Plant Signal Behav 8(3), e23313
Gotor C, Álvarez C, Bermúdez MA, Moreno I, García I, Romero LC (2010) Low abundance does not mean less importance in cysteine metabolism. Plant Signal Behav 5(8):1028–1030. doi:10.1105/tpc.109.071985
Gotor C, García I, Crespo JL, Romero LC (2013) Sulfide as a signaling molecule in autophagy. Autophagy 9(4):609–611. doi:10.4161/auto.23460
Gotor C, Laureano-Marín AM, Moreno I, Aroca Á, García I, Romero LC (2015) Signaling in the plant cytosol: cysteine or sulfide? Amino Acids 47(10):2155–2164. doi:10.1007/s00726-014-1786-z
Gutiérrez-Marcos JF, Roberts MA, Campbell EI, Wray JL (1996) Three members of a novel small gene-family from Arabidopsis thaliana able to complement functionally an Escherichia coli mutant defective in PAPS reductase activity encode proteins with a thioredoxin-like domain and "APS reductase" activity. Proc Natl Acad Sci U S A 93(23):13377–13382

Haas FH, Heeg C, Queiroz R, Bauer A, Wirtz M, Hell R (2008) Mitochondrial serine acetyltransferase functions as a pacemaker of cysteine synthesis in plant cells. Plant Physiol 148(2):1055–1067

Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K, Saito K (2000) beta-Cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and Arabidopsis. Plant Physiol 123(3):1163–1171

Heeg C, Kruse C, Jost R, Gutensohn M, Ruppert T, Wirtz M, Hell R (2008) Analysis of the Arabidopsis O-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis. Plant Cell 20(1):168–185

Herrmann J, Ravilious GE, McKinney SE, Westfall CS, Lee SG, Baraniecka P, Giovannetti M, Kopriva S, Krishnan HB, Jez JM (2014) Structure and mechanism of soybean ATP sulfurylase and the committed step in plant sulfur assimilation. J Biol Chem 289(15):10919–10929. doi:10.1074/jbc.M113.540401

Hirai MY, Sugiyama K, Sawada Y, Tohge T, Obayashi T, Suzuki A, Araki R, Sakurai N, Suzuki H, Aoki K, Goda H, Nishizawa OI, Shibata D, Saito K (2007) Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proc Natl Acad Sci U S A 104(15):6478–6483. doi:10.1073/pnas.0611629104

Howarth JR, Domínguez-Solís JR, Gutiérrez-Alcalá G, Wray JL, Romero LC, Gotor C (2003) The serine acetyltransferase gene family in Arabidopsis thaliana and the regulation of its expression by cadmium. Plant Mol Biol 51(4):589–598

Jez JM, Dey S (2013) The cysteine regulatory complex from plants and microbes: what was old is new again. Curr Opin Struct Biol 23(2):302–310. doi:10.1016/j.sbi.2013.02.011

Jin Z, Shen J, Qiao Z, Yang G, Wang R, Pei Y (2011) Hydrogen sulfide improves drought resistance in Arabidopsis thaliana. Biochem Biophys Res Commun 414(3):481–486. doi:10.1016/j.bbrc.2011.09.090

Kabil O, Banerjee R (2010) Redox biochemistry of hydrogen sulfide. J Biol Chem 285 (29):21903–21907. doi:10.1074/jbc.R110.128363

Kawashima CG, Berkowitz O, Hell R, Noji M, Saito K (2005) Characterization and expression analysis of a serine acetyltransferase gene family involved in a key step of the sulfur assimilation pathway in Arabidopsis. Plant Physiol 137(1):220–230

Kawashima CG, Yoshimoto N, Maruyama-Nakashita A, Tsuchiya YN, Saito K, Takahashi H, Dalmay T (2009) Sulphur starvation induces the expression of microRNA-395 and one of its target genes but in different cell types. Plant J 57(2):313–321. doi:10.1111/j.1365-313X.2008.03690.x

Khan MS, Haas FH, Samami AA, Gholami AM, Bauer A, Fellenberg K, Reichelt M, Hansch R, Mendel RR, Meyer AJ, Wirtz M, Hell R (2010) Sulfite reductase defines a newly discovered bottleneck for assimilatory sulfate reduction and is essential for growth and development in Arabidopsis thaliana. Plant Cell 22(4):1216–1231. doi:10.1105/tpc.110.074088

Kopriva S, Koprivova A (2004) Plant adenosine 5′-phosphosulphate reductase: the past, the present, and the future. J Exp Bot 55(404):1775–1783. doi:10.1093/jxb/erh185

Koprivova A, Kopriva S (2014) Molecular mechanisms of regulation of sulfate assimilation: first steps on a long road. Front Plant Sci 5:589. doi:10.3389/fpls.2014.00589

Krueger S, Niehl A, López-Martín MC, Steinhauser D, Donath A, Hildebrandt T, Romero LC, Hoefgen R, Gotor C, Hesse H (2009) Analysis of cytosolic and plastidic serine acetyltransferase mutants and subcellular metabolite distributions suggests interplay of the cellular compartments for cysteine biosynthesis in Arabidopsis. Plant Cell Environ 32 (4):349–367. doi:10.1111/j.1365-3040.2008.01928.x

Laureano-Marín AM, García I, Romero LC, Gotor C (2014) Assessing the transcriptional regulation of L-cysteine desulfhydrase 1 in Arabidopsis thaliana. Front Plant Sci 5:683. doi:10.3389/fpls.2014.00683

Lee BR, Koprivova A, Kopriva S (2011) The key enzyme of sulfate assimilation, adenosine 5′-phosphosulfate reductase, is regulated by HY5 in Arabidopsis. Plant J 67(6):1042–1054. doi:10.1111/j.1365-313X.2011.04656.x

Lisjak M, Srivastava N, Teklic T, Civale L, Lewandowski K, Wilson I, Wood ME, Whiteman M, Hancock JT (2010) A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. Plant Physiol Biochem 48(12):931–935. doi:10.1016/j.plaphy.2010.09.016

López-Martín MC, Becana M, Romero LC, Gotor C (2008a) Knocking out cytosolic cysteine synthesis compromises the antioxidant capacity of the cytosol to maintain discrete concentrations of hydrogen peroxide in Arabidopsis. Plant Physiol 147(2):562–572

López-Martín MC, Romero LC, Gotor C (2008b) Cytosolic cysteine in redox signaling. Plant Signal Behav 3(10):880–881

Lunn JE, Droux M, Martin J, Douce R (1990) Localization of ATP sulfurylase and O-acetylserine (thiol)lyase in spinach leaves. Plant Physiol 94(3):1345–1352

Martin MN, Tarczynski MC, Shen B, Leustek T (2005) The role of 5′-adenylylsulfate reductase in controlling sulfate reduction in plants. Photosynth Res 86(3):309–323

Maruyama-Nakashita A, Nakamura Y, Watanabe-Takahashi A, Inoue E, Yamaya T, Takahashi H (2005) Identification of a novel cis-acting element conferring sulfur deficiency response in Arabidopsis roots. Plant J 42(3):305–314. doi:10.1111/j.1365-313X.2005.02363.x

Maruyama-Nakashita A, Nakamura Y, Tohge T, Saito K, Takahashi H (2006) Arabidopsis SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism. Plant Cell 18 (11):3235–3251. doi:10.1105/tpc.106.046458

Miret JA, Munne-Bosch S (2014) Plant amino acid-derived vitamins: biosynthesis and function. Amino Acids 46(4):809–824. doi:10.1007/s00726-013-1653-3

Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH (2009) H2S signals through protein S-sulfhydration. Sci Signal 2(96):72. doi:10.1126/scisignal.2000464

Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, Amzel LM, Berkowitz DE, Snyder SH (2011) Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circ Res 109(11):1259–1268. doi:10.1161/CIRCRESAHA.111.240242

Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35(2):454–484. doi:10.1111/j.1365-3040.2011.02400.x

Park S, Imlay JA (2003) High levels of intracellular cysteine promote oxidative DNA damage by driving the fenton reaction. J Bacteriol 185(6):1942–1950

Qi J, Wu J, Somkuti GA, Watson JT (2001) Determination of the disulfide structure of sillucin, a highly knotted, cysteine-rich peptide, by cyanylation/cleavage mass mapping. Biochemistry 40 (15):4531–4538

Rausch T, Wachter A (2005) Sulfur metabolism: a versatile platform for launching defence operations. Trends Plant Sci 10(10):503–509

Ravilious GE, Jez JM (2012) Structural biology of plant sulfur metabolism: from assimilation to biosynthesis. Nat Prod Rep 29(10):1138–1152. doi:10.1039/c2np20009k

Rea PA (2012) Phytochelatin synthase: of a protease a peptide polymerase made. Physiol Plant 145(1):154–164. doi:10.1111/j.1399-3054.2012.01571.x

Rennenberg H, Herschbach C (2014) A detailed view on sulphur metabolism at the cellular and whole-plant level illustrates challenges in metabolite flux analyses. J Exp Bot 65 (20):5711–5724. doi:10.1093/jxb/eru315

Richau KH, Kaschani F, Verdoes M, Pansuriya TC, Niessen S, Stuber K, Colby T, Overkleeft HS, Bogyo M, Van der Hoorn RA (2012) Subclassification and biochemical analysis of plant papain-like cysteine proteases displays subfamily-specific characteristics. Plant Physiol 158 (4):1583–1599. doi:10.1104/pp.112.194001

Romero LC, García I, Gotor C (2013) L-Cysteine Desulfhydrase 1 modulates the generation of the signaling molecule sulfide in plant cytosol. Plant Signal Behav 8(5), e24007. doi:10.4161/psb.24007

Romero LC, Aroca MÁ, Laureano-Marín AM, Moreno I, García I, Gotor C (2014) Cysteine and cysteine-related signaling pathways in Arabidopsis thaliana. Mol Plant 7(2):264–276. doi:10.1093/mp/sst168

Rotte C, Leustek T (2000) Differential subcellular localization and expression of ATP sulfurylase and 5′-adenylylsulfate reductase during ontogenesis of Arabidopsis leaves indicates that cytosolic and plastid forms of ATP sulfurylase may have specialized functions. Plant Physiol 124(2):715–724

Santo-Domingo J, Demaurex N (2012) Perspectives on: SGP symposium on mitochondrial physiology and medicine: the renaissance of mitochondrial pH. J Gen Physiol 139 (6):415–423. doi:10.1085/jgp.201110767

Scuffi D, Álvarez C, Laspina N, Gotor C, Lamattina L, García-Mata C (2014) Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. Plant Physiol 166(4):2065–2076. doi:10.1104/pp.114.245373

Seo S, Mitsuhara I, Feng J, Iwai T, Hasegawa M, Ohashi Y (2011) Cyanide, a coproduct of plant hormone ethylene biosynthesis, contributes to the resistance of rice to blast fungus. Plant Physiol 155(1):502–514. doi:10.1104/pp.110.162412

Setya A, Murillo M, Leustek T (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5′-adenylylsulfate reductase. Proc Natl Acad Sci U S A 93(23):13383–13388

Takahashi H (2010) Regulation of sulfate transport and assimilation in plants. In: Kwang WJ (ed) Int Rev Cell Mol Biol 281:129–159. Academic Press. doi:10.1016/S1937-6448(10)81004-4

Takahashi H, Kopriva S, Giordano M, Saito K, Hell R (2011) Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. Annu Rev Plant Biol 62:157–184. doi:10.1146/annurev-arplant-042110-103921

Thompson AR, Vierstra RD (2005) Autophagic recycling: lessons from yeast help define the process in plants. Curr Opin Plant Biol 8(2):165–173. doi:10.1016/j.pbi.2005.01.013

Trivedi MV, Laurence JS, Siahaan TJ (2009) The role of thiols and disulfides on protein stability. Curr Protein Pept Sci 10(6):614–625

Wang R (2012) Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 92(2):791–896. doi:10.1152/physrev.00017.2011

Watanabe M, Kusano M, Oikawa A, Fukushima A, Noji M, Saito K (2008a) Physiological roles of the beta-substituted alanine synthase gene family in Arabidopsis. Plant Physiol 146 (1):310–320

Watanabe M, Mochida K, Kato T, Tabata S, Yoshimoto N, Noji M, Saito K (2008b) Comparative genomics and reverse genetics analysis reveal indispensable functions of the serine acetyltransferase gene family in Arabidopsis. Plant Cell 20(9):2484–2496

Wawrzynska A, Lewandowska M, Sirko A (2010) Nicotiana tabacum EIL2 directly regulates expression of at least one tobacco gene induced by sulphur starvation. J Exp Bot 61 (3):889–900. doi:10.1093/jxb/erp356

Wirtz M, Hell R (2006) Functional analysis of the cysteine synthase protein complex from plants: structural, biochemical and regulatory properties. J Plant Physiol 163(3):273–286

Wirtz M, Hell R (2007) Dominant-negative modification reveals the regulatory function of the multimeric cysteine synthase protein complex in transgenic tobacco. Plant Cell 19(2):625–639

Wu W, Berkowitz GA (1992) Stromal pH and photosynthesis are affected by electroneutral K and H exchange THROUGH chloroplast envelope ion channels. Plant Physiol 98(2):666–672

Yamaguchi Y, Nakamura T, Kusano T, Sano H (2000) Three Arabidopsis genes encoding proteins with differential activities for cysteine synthase and beta-cyanoalanine synthase. Plant Cell Physiol 41(4):465–476

Yi H, Galant A, Ravilious GE, Preuss ML, Jez JM (2010) Sensing sulfur conditions: simple to complex protein regulatory mechanisms in plant thiol metabolism. Mol Plant 3(2):269–279. doi:10.1093/mp/ssp112

Yoshimoto N, Inoue E, Watanabe-Takahashi A, Saito K, Takahashi H (2007) Posttranscriptional regulation of high-affinity sulfate transporters in Arabidopsis by sulfur nutrition. Plant Physiol 145(2):378–388. doi:10.1104/pp.107.105742

Zhang H, Hu LY, Hu KD, He YD, Wang SH, Luo JP (2008) Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. J Integr Plant Biol 50 (12):1518–1529. doi:10.1111/j.1744-7909.2008.00769.x

Zhang H, Tan ZQ, Hu LY, Wang SH, Luo JP, Jones RL (2010) Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. J Integr Plant Biol 52(6):556–567. doi:10.1111/j.1744-7909.2010.00946.x

Plant Vacuolar Sorting: An Overview

Bruno Peixoto, Susana Pereira, and José Pissarra

Contents

Abstract Eukaryotic cells have developed membrane-bound organelles, connected between themselves in a complex and tightly regulated network – the endomembrane system. Despite being less well understood when compared to the animal and yeast models, plant cells have begun to reveal an intricate and dense network of endomembranes. Particularly diverse is the network of pathways revolving around the vacuole, especially when comparing plant and non-plant models. This dynamic, pleiomorphic and multifunctional organelle is essential for correct plant growth and development, compartmentalizing different components, from proteins to secondary metabolites. In this review we will provide an historical perspective of what has been discovered relating vacuolar sorting, and the potential biotech applications of such findings.

B. Peixoto • S. Pereira (✉) • J. Pissarra
Faculty of Sciences, Department of Biology, BioISI – Biosystems & Integrative Sciences Institute, University of Porto, Rua do Campo Alegre, s/n°, 4169-007 Porto, Portugal
e-mail: bruno.peixoto@fc.up.pt; mspereir@fc.up.pt; jpissarr@fc.up.pt

Progress in Botany (2017) 78: 67–94, DOI 10.1007/124_2016_6,
Published online: 2 June 2016

List of Abbreviations

AP	Aspartic proteinase
BFA	Brefeldin A
BP-80	80 kDa proaleurein-binding protein
CCV	Clathrin coated vesicle
CPY	Carboxypeptidase Y
ctVSD	C-terminal vacuolar sorting determinant
Cvt	Cytosol-to-vacuole targeting pathway
DCB	Dichlorobenzonitrile
EM	Electron microscopy
ER	Endoplasmic reticulum
ERvt	ER to the vacuole targeting pathway
EST	Expressed sequence tag
GEF	Guanine nucleotide exchange factor
LV	Lytic vacuole
M6P	Mannose-6-phosphate
PA Domain	Protease associated domain
PB	Protein body
PI3P	Phosphatidylinositol 3-phosphate
PM	Plasma membrane
PSI	Plant-specific insert
PSV	Protein storage vacuole
psVSD	Physical structure vacuolar sorting determinant
PVC	Prevacuolar compartment
RMR	Receptor-homology-region-transmembrane-domain-RING-H2
SAPLIP	Saposin-like protein
SNARE	Soluble NSF attachment protein receptor
ssVSD	Sequence-specific vacuolar sorting determinant
TGN	*trans* Golgi Network
TIP	Tonoplast intrinsic protein
VSR	Vacuolar sorting receptor

1 Introduction

Though often unnoticed, plants play vital roles in our lives. Whole industrial sectors, as varied as food and feed, textile, construction, fuels and even the pharmaceutical sectors, derive their raw materials from plants. In more recent years, these versatile organisms have also been exploited as biofactories for the

recombinant production of vaccines, antibiotics and vitamins. Plant biology has been advancing rather rapidly, and plant biologists find themselves at the point where they must study plants from a holistic point of view, integrating physiology and metabolism with the subcellular architecture and their associated dynamics underlying each individual cellular component.

Understanding plants at the molecular level – how are their proteins synthesized? What functions do they serve? When and where are these proteins accumulated to serve their function? And in which way are these proteins interacting with each other, for the ultimate purpose of homeostatic maintenance of the whole organism is becoming fundamental in our quest to further explore these organisms as the rich sources of materials and wealth that they have already proven to be.

In essence, a eukaryotic cell is an integrated system comprised of several membrane-delimited compartments. These endomembranes serve the function of delimiting different physical and chemical sub-regions within the cell, allowing for much more diversified metabolic reactions to occur. Thus, understanding the functional organization of the endomembrane system, and how these cells create, maintain and erase their chemical landscapes at the most fundamental levels might allow for future manipulation of such processes by the biotechnological sector.

At present, most studies focusing on the plant endomembrane system attempt to either functionally characterize these compartments, identifying key pathways, vesicle carriers or molecular machinery responsible for the selective transport of cargo molecules between compartments, allowing for the arrival of all proteins at their site of action in a timely fashion. Our group is no exception, and our focus during the last decade has been on the study of two aspartic proteinases. Originally isolated from *Cynara cardunculus* L. (cardoon), cardosins share substantial sequence similarity (approximately 70%), but accumulate in different subcellular compartments in the native system. This curious dynamic led us to try and comprehend the subtleties of cardosin trafficking in plant systems, which have proven themselves a very rich model to study vacuolar sorting in plant cells.

Plants are sessile organisms, and thus must be capable of incredible adaptability in order to survive an ever-changing environment. By studying the plant endomembrane system, we have started to better understand the molecular bases behind plant plasticity and adaptation to all sorts of stressors, be they biotic or abiotic in nature. Such knowledge may also prove useful for the future design and engineering of tailor-made plants, better prepared to both face specific environmental challenges, or better optimized for obtaining different sets of end-products (food vs fuel strains, for example).

Focusing on the plant vacuole and the sorting of its proteins, this paper will review what is currently known about this subject, from an historical perspective and up to the latest findings in the study of the plant endomembrane system, namely in terms of RAB GTPases, SNAREs and sorting receptors and determinants. We will explore some of the chemical tools currently under development and the rise of "chemical genomics" in plant biology, present a study case, and explore how the

application of this body of knowledge through biotechnology might one day benefit all of mankind.

2 Plant Vacuoles and Function

The vacuole is an integral part of the plant endomembrane system, occupying a large percentage of these cells' volume (up to 90%). Delineated by a membrane (the tonoplast), these organelles are incredibly dynamic. Vacuolar morphology has been a hot topic for some time, and still attracts the attention of many researchers worldwide, as it changes continuously during a cell's life cycle and developmental cycles (Zhang et al. 2014). Vacuoles play several functions in plant cell physiology, most notably as a storage point for solutes, ions and water. Due to the osmotic water uptake, these organelles serve as a hydrostatic skeleton, which combined with the rigid cell wall, drives cell growth and cell volume regulation. The plant vacuole is also a key player in plant defence, whether by facilitating cytoplasm detoxification from harmful molecules, or by accumulating toxic compounds to be released against predators. The plant vacuole may also serve as a plant lysosome, hydrolysing proteins received either by endocytic or autophagic processes. This lytic vacuole (LV) is mostly present in plant vegetative tissues. In seeds, most vacuoles are of a different type – protein storage vacuoles (PSVs) accumulate reserve proteins for the embryo to mobilize during the early phases of seed germination. Both types of vacuoles may co-exist, particularly during seed germination, when LVs fuse with PSVs, for the degradation of the storage proteins, and the mobilization of free amino acids.

The tonoplast is impermeable to water, ions and other metabolites. For transporting these compounds, the vacuolar membrane is adorned with transmembrane enzymes, transporters and channels. These proteins' activities are modulated by the chemical landscape of the cell's cytosol, regulating water and solute exchange between the two compartments, maintaining cellular homeostasis. Classically, LVs and PSVs have been distinguished by the aquaporin isoforms that ornament their tonoplast – tonoplast intrinsic proteins (TIPs). Lytic vacuoles would have a γ-TIP enriched membrane, whereas protein storage vacuoles would instead accumulate α-TIP in their tonoplasts (Vitale and Raikhel 1999; Paris et al. 1996). We have since then come to realize the real picture to be somewhat more complex, as overlapping of both markers and vacuolar remodelling has been observed (Olbrich et al. 2007; Bolte et al. 2011). Vacuolar remodelling is particularly evident during seed germination, due to the massive amount of morphological changes that can be observed in a short amount of time. During this remodelling, PSV tonoplasts are converted into the central vacuole membrane (Maeshima et al. 1994), but this system is far from being perfectly understood, as evidenced by the complexity unearthed in recent studies of *Arabidopsis thaliana* vacuome remodelling during seed germination (Bolte et al. 2011).

3 Plant Vacuolar Sorting: Receptors and Determinants

From a simplified perspective, the transport of cargo molecules between any two organelles can occur via two main mechanisms – either by protein–protein or protein–lipid interactions. These interactions are responsible for the formation and shaping of either vesicular or tubular structures that interconnect different organelles, mediating the transfer of cargo between them (Bonifacino and Glick 2004). This exchange of material may result in the gradual change of the compartments' biochemical compositions, forming the basis of organelle maturation and differentiation events (Luini 2011). The aforementioned protein and lipid bases of the sorting machinery are not linearly transported in a single direction; they must be continuously recycled back and forth between any two adjacent organelles, in order to maintain the basic cellular architecture from collapsing during normal cellular metabolism. This means that some components of the endomembrane system must be capable of bidirectional movement between organelles (De Marcos Lousa et al. 2012).

The principles behind protein trafficking and vesicular transport were historically laid out by a mix of electron microscopy (EM) and analytical biochemistry. One such discovery was the identification of the mammalian mannose-6-phosphate (M6P) receptors, responsible for the lysosomal transport of soluble proteins (Hoflack and Kornfeld 1985).

Lytic vacuoles are the plant equivalents to the mammalian lysosomes, accumulating a wide range of hydrolytic enzymes. Early studies quickly led to the understanding that, unlike the mammalian systems, plants produced no mannose 6-phosphate residues. Instead, vacuolar sorting information appeared encoded by short peptide sequences, rather than modified glycan structures (Shinshi et al. 1988; Voelker et al. 1989; Bednarek et al. 1990; Matsuoka and Nakamura 1991; Neuhaus et al. 1991; Holwerda et al. 1992). It is by interacting with these peptides that proteins contained in the endomembrane system are segregated from the default passive bulk-flow pathway, towards their final destinations.

The observation that a series of vacuolar proteins were enriched in pea (*Pisum sativum*) clathrin coated vesicles (CCVs) inspired the very first studies with plant vacuolar sorting receptor (VSR) proteins (Harley and Beevers 1989; Kirsch et al. 1994). With the exponential growth of available expressed sequence tags (ESTs) and sequenced genomes, it became apparent that these VSR proteins were encoded by large gene families. Arabidopsis alone has seven such genes, distributed by three different classes, all of them predicted to have very similar topologies (Fig. 1), and a possibly highly redundant biological function, as single mutants for most of these VSR proteins fail to produce any discernible phenotypes during plant development and growth (De Marcos Lousa et al. 2012). Nonetheless, Shimada et al. (2003) detected partial secretion of vacuolar cargo in *vsr1* mutant lines. Further work some years later, yielded confirmatory results that VSRs have a broad specificity in terms of vacuolar cargo recognition and sorting (Craddock et al. 2008). This interaction with sorting determinants is conditional, with pH and Ca^{2+} concentrations playing

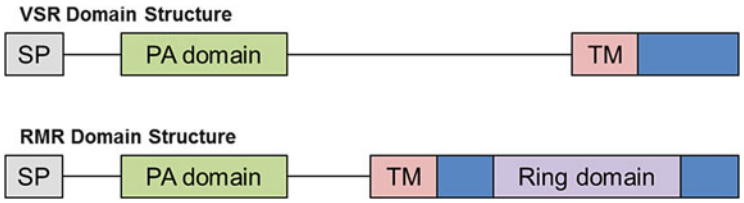

Fig. 1 Graphical representation of the domain structure of plant VSR and RMR proteins. Both receptor proteins are characterized by the presence of an amino-terminally located signal peptide (SP), responsible for inserting the proteins into the endomembrane system, a conserved protease associated domain (PA domain), which has been shown to be important for VSR–ligand interaction, and a transmembrane (TM) domain, which anchors the proteins to the lipid membranes. The RMR proteins are also characterized by the presence of a RING domain, which is inserted into a highly conserved area (represented by the *violet boxes*)

an important role in the regulation of ligand binding (Kirsch et al. 1994; Cao et al. 2000; Watanabe et al. 2002, 2004; Suen et al. 2010).

Luo et al. (2014) have been trying to understand the molecular mechanisms behind VSR-VSD interaction. By resolving the crystallographic structures of the protease-associated (PA) VSR1 domain alone and complexed with a VSD-containing peptide ($_{1}$ADS**NPIR**PVT$_{10}$), they were able to identify the occurrence of large structural changes of the VSR upon ligand binding. The main conclusions drawn by this study were that (1) hydrogen bonds were formed at the PA binding cavity in order to accept the ligand peptide; (2) binding of cargo induced a 180° flip of the VSR PA C-terminus region, which became stabilized by a novel hydrogen bond between His-181 and Glu-24. Also very relevant is that (3) the residues preceding the NPIR motif are important for cargo recognition and trafficking – the peptide's Ser-3 residue was identified as particularly relevant in this recognition process, as mutational analysis of this residue resulted in considerable missorting in vivo. (4) The invariant Arg-95 residue of the VSR PA domain is crucial for cargo binding, as it has been shown to interact with the previously identified peptide Ser-3 and (5) the swivel motion of the C-terminal tail is needed for receptor-cargo recognition (Luo et al. 2014).

The authors didn't fail to point out the observable homology between the VSR PA domain and the luminal region of the receptor-homology-region-transmembrane-domain-RING-H2 (RMR) proteins. The sequence of the binding loop appears conserved among both VSR and RMR proteins, with the RGxCxF consensus sequence, raising the possibility that RMR receptor–cargo interaction could be similar to what was observed for VSR1. How the NPIR domain interacts with the VSR receptor remains elusive; however, as this study shows a clearly disordered state of these residues upon binding between the PA domain and the preceding peptide residues. It remains possible that the PA and central domains of the VSR molecule each recognize a different sequence motif within vacuolar VSDs – the PA domain would be responsible for binding the NPIR-preceding residues, whereas the NPIR motif itself could interact with the central domain (Luo et al. 2014). Despite the open questions about how VSR–VSD interactions occur at the biophysical

level, this work appears as an important landmark for the understanding of vacuolar sorting in plant systems.

The similarity between the VSR amino-terminal 100 amino acids and the lumenal RMR protein sequence has led to the initial hypothesis that these novel receptor proteins could be involved in the sorting of vacuolar proteins (Cao et al. 2000). The first two plant RMR genes were identified due to this homology (Jiang et al. 2000), but it is currently known that the Arabidopsis genome contains a total of five such genes (AtRMR1-5). They all share high sequence similarity, and are characterized as type I integral membrane proteins, with a typical N-terminal signal peptide and a PA domain, in similarity to VSR topology (Fig. 1) (Wang et al. 2011). Although the conservation of the PA-TM-RING region is highly conserved not only amongst plant proteins, but also between Arabidopsis and animal proteins, raising the possibility of a highly conserved biological function for these receptors, the fact remains that RMR RING-H2 domain functions remain to be properly elucidated in plant systems (Wang et al. 2011).

In order to further understand these plant proteins, the intracellular localization and trafficking of RMRs have been studied in different plant cells and tissue types (Jiang et al. 2000; Park et al. 2005, 2007; Hinz et al. 2007). Jiang et al. (2000) observed tomato seed PSVs and detected the presence of RMR proteins within these organelles, particularly at the crystalloid. Further analysis revealed that this intravacuolar structure presented a high lipid to protein ratio, fuelling the idea that these crystalloids are composed of integral membrane and soluble proteins, packed within an array of lipid bilayers (Jiang et al. 2000). Curiously, even plants without a well-defined crystalloid structure inside the PSV were shown to still contain an internal network of cross-linked integral membrane proteins, including RMR proteins (Gillespie et al. 2005). Dissection of the trafficking pathway followed by these receptors showed them to possess complex glycans, a strong indication of passage through the Golgi Complex, but the receptor's final destination differs from that of the VSR reporter BP-80, which is known to accumulate in the prevacuolar compartment (PVC) (Jiang et al. 2000).

Regarding potential cargoes, Park et al. (2007) determined that the AtRMR2 receptor bound specifically to C-terminal vacuolar sorting signals, but only when these residues were exposed at the C-terminal region of the peptide. Contrary to what is observed for VSRs, this binding was found to not be pH dependent (Park et al. 2007). These observations strongly suggest that these RMR receptor proteins could be involved in the sorting of cargo to the protein storage vacuole. It is important to note that the accumulation of the RMR proteins at the PSV crystalloid would effectively remove these receptors from the endomembrane system after just one round of sorting – the aggregation model of storage protein sorting, by which a single receptor protein would interact with an aggregate of several storage protein molecules during dense vesicle-mediated sorting, could explain how a "one-round receptor" would remain efficient (Hillmer et al. 2001). In fact, Wang et al. (2011) postulated that such trapping of the RMR proteins could in fact be necessary, should the plant RING-H2 domain possess ubiquitin ligase activity, as has been

demonstrated for the mammalian homologues (Bocock et al. 2009; Kriegel et al. 2009; Wang et al. 2011).

An initial classification of vacuolar sorting determinants (VSDs) separated these signals into N-terminal, C-terminal and internal signals. Some of these determinants are easily swapped between proteins, whereas others seem to be context-dependent. Sequence-specific sorting signals (ssVSDs) allow little variation of the conserved Asn-Pro-Ile-Arg (NPIR) sequence. This VSD is typically located at the amino-terminal region of the protein, directing it towards the lytic vacuole, and can be found in proteins such as barley (*Hordeum vulgare*) proaleurain or sweet potato (*Ipomoea batatas*) sporamin. C-terminal signals (ctVSDs) are generally responsible for sorting of proteins towards the protein storage vacuole and must be exposed at the carboxyl terminus of the peptide in order for them to function properly. ctVSDs can be found in proteins such as cardoon's (*Cynara cardunculus*) cardosins, tobacco (*Nicotiana tabacum*) chitinase or Brazil nut (*Bertholletia excelsa*) 2S storage albumin. No homologous sequence or size has been so far identified for these determinants, but one common characteristic among them is that they are rich in hydrophobic amino acids. The third group of VSDs is slightly more complicated to characterize, as these signals are dependent on their tertiary structure – physical structure VSDs (psVSDs). Typically found in storage proteins, such as common bean (*Phaseolus vulgaris*) phytohemagglutinin, or broad bean (*Vicia faba*) B-type legumin, these sorting signals are present in the protein sequence and the residues that comprise them might be scattered along the protein's primary structure, coming together after correct three-dimensional folding is completed (Neuhaus and Rogers 1998; Jolliffe et al. 2005; Zouhar and Rojo 2009). Traditionally, in order for a sequence of residues to be considered a true VSD, two conditions must be met – the peptide must be both necessary and sufficient for vacuolar sorting. Many examples of VSDs that comply with these prerequisites have been described in the past (Nakamura and Matsuoka 1993; Vitale and Raikhel 1999; Robinson et al. 2005; Zouhar and Rojo 2009). However, some proteins have been identified carrying more than one VSD, often of different types. The C-terminus of soybean's β-conglycinin α-subunit withholds two functional sorting signals – a typical ctVSD and a sequence-specific sorting signal (Nishizawa et al. 2006). Cardosin A is another such example – this aspartic proteinase (AP) also possesses a typical ctVSD at the carboxy terminus, and an internal signal sequence – the plant-specific insert (PSI) – both perfectly capable of efficiently redirecting a secretory mCherry marker, towards the vacuole (Pereira et al. 2013). This PSI sequence has revealed itself as an unusual VSD sequence – approximately 100 residues-long and present in all typical plant APs, this domain is removed during the proteolytic maturation of these enzymes. Highly similar to mammalian saposins, this member of the saposin-like proteins (SAPLIPs) has been proposed to play a similar role in AP vacuolar sorting as the one described for mammalian saposin C and cathepsin D. The formation of a saposin C – cathepsin D complex is thought to be behind the M6P-independent lysosomal sorting of this mammalian enzyme (reviewed in Simões and Faro 2004).

The simultaneous presence of two VSDs in the same protein could be a strategy developed by plant cells to regulate vacuolar targeting of proteins under different physiological conditions, or tissue types. Another not entirely incompatible explanation is that two sorting signals might have an additive effect, resulting in a much greater sorting efficiency (Holkeri and Vitale 2001; Nishizawa et al. 2006). Studies with phaseolin have shown that the C-terminal peptide undergoes homotypic interactions and could be partially substituted by a C-terminal Cys residue (Pompa et al. 2010). This study has raised the question about the importance of multiple VSDs in assembled homo-oligomeric complexes. The ratio between number of VSDs and protein size is also suggested to play a role in the issue of sorting efficiency (Nishizawa et al. 2006), but so far this question is far from being clearly understood.

4 Plant Vacuolar Sorting: SNAREs

SNAREs (*N*-ethylmaleimide-sensitive factor adaptor protein receptors) are small proteins capable of forming a coiled-coil structure, interacting with other SNARE proteins via hetero-oligomeric interactions, forming highly stable SNARE complexes responsible for membrane fusion (Fig. 2) (Di Sansebastiano and Piro 2014). Particularly relevant in vesicle trafficking regulation, these proteins are nonetheless important regulators of many other signalling networks, due to their direct role in the exo- and endocytosis of all sorts of membrane proteins, such as receptor and channel proteins (Di Sansebastiano and Piro 2014). SNAREs have been classified in

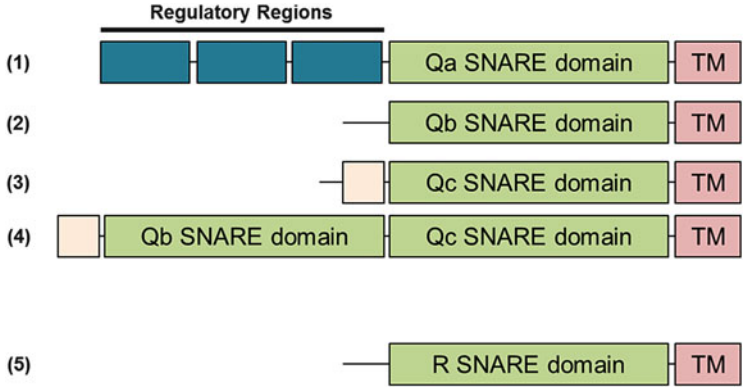

Fig. 2 Graphical representation of the domain structure of the different SNARE classes. (*1–4*) t-SNAREs are comprised by a transmembrane (TM) domain and a SNARE domain. (*1*) Qa-SNAREs are classified as "syntaxin-like" and possess a series of regulatory domains, prior to the SNARE domain (*dark blue boxes*). (*4*) SNAP25-like t-SNARE is comprised by two SNARE domains, belonging to the Qb and Qc groups. (*5*) Graphical representation of a typical R-SNARE

several ways, but the most commonly employed classification nowadays takes into account the amino acid present at the centre of the SNARE motif. Q-SNAREs have a conserved glutamine residue at this location, whereas R-SNAREs possess a conserved arginine residue instead (Fasshauer et al. 1998). There are three types of Q-SNAREs: Qa-, Qb- and Qc-SNAREs, whereas R-SNAREs can have either a short N-terminal regulatory region (designated as brevins), or a long regulatory region (designated as longins). Curiously enough, plants seem to only possess longins (Uemura et al. 2005).

A typical plant SNARE complex will generally consist of Qa-, Qb- and Qc-SNAREs at the target membrane, and R-SNAREs on the vesicle membrane (Sutter et al. 2006). The large number of plant SNAREs (estimated at 65 for Arabidopsis) may very well be the basis of the distinct secretory and vacuolar trafficking steps of plant systems (Sanderfoot 2007), and some authors argue that the multiplication of SNAREs and other membrane trafficking key component proteins is a prerequisite for the support of increasingly more complex body plans and life cycles (Fujimoto and Ueda 2012). Some of these SNARE proteins have been localized to post-Golgi compartments – particularly relevant for vacuolar sorting, SYP21/PEP12 and SYP22/VAM3 Qa-SNAREs have been found to localize at the late endosome (Sanderfoot 2007). Knock-out *syp22* mutants show pleiotropic phenotypes, such as semi-dwarfism, late flowering and an underdeveloped leaf vascular network (Ohtomo et al. 2005; Ueda et al. 2006; Shirakawa et al. 2009; Ebine et al. 2012). Although *syp21* lines fail to develop any observable phenotype, overexpression of SYP21 results in the homotypic fusion of PVC bodies and the partial secretion of vacuolar proteins (Foresti et al. 2006), perhaps by inhibiting anterograde trafficking at the PVC-vacuole route. Double mutants *syp21 syp22* are gametophytic lethal, but knocked-down SYP21 levels in a *syp22* background have been shown to result in impaired vacuolar transport of 12S globulin and 2S albumin, pointing to a possibly redundant function between the two SYP2 members in vacuolar transport of proteins (Shirakawa et al. 2010; Uemura et al. 2010).

The Qb-VTI11 SNARE is a SYP22 and SYP21 binding partner. This Qb-SNARE is essential for phosphatidylinositol 3-phosphate (PI3P)-mediated vacuolar biogenesis, and loss of its function in the *zig-1* mutant line results in defective gravitropic responses, zig-zag shaped inflorescences and altered vacuolar morphologies (Sanderfoot et al. 2001; Morita et al. 2002; Hashiguchi et al. 2010; Saito et al. 2011). SYP5 members are Qc-SNARES that interact with SYP22 and are localized to the tonoplast and TGN. Despite their degree of similarity, their function differs, in that SYP51 is required for GFP-chitinase transport, whereas SYP52 is involved in transporting Aleurain-GFP (De Benedictis et al. 2013). SYP51/52 has also been reported to act as interfering SNAREs (i-SNAREs) when overexpressed, accumulating at the tonoplast level (Uemura et al. 2004). i-SNAREs are a novel functional class of SNAREs, which are thought capable of inhibiting membrane fusion by direct competition with fusogenic SNARE pins, effectively forming nonfusogenic complexes (Di Sansebastiano 2013). Sorting processes would then be modulated by the different levels of these SNAREs – an example is that VSR proteins appear diverted to the plasma membrane in germinating pollen tubes (Wang et al. 2011),

where SYP5 members are found in particularly elevated levels (Lipka et al. 2007; De Benedictis et al. 2013).

Members of the VAMP71 family (AtVAMP711-AtVAMP714) have been associated with stomatal aperture control during drought stress (Leshem et al. 2010). This would implicate this protein family in membrane fusion events at the tonoplast or vesicle budding from the vacuole, processes responsible for the control of vacuolar size and tonoplast surface area. The R-SNARE AtVAMP727 has been found to dual-localize at the endosome/PVC and plasma membrane (PM) (Uemura et al. 2004; Ebine et al. 2008, 2011). VAMP713 has already been localized to the tonoplast (De Benedictis et al. 2013), and it is known that VAMP713 is also a member of the SYP22 complex (Ebine et al. 2011). The R-SNARE VAMP727 has also been shown to associate with the SYP22, VTI11 and SYP5 complex (Ebine et al. 2008). The *atsyp22*/*atvamp727* double mutant secretes storage vacuolar cargo such as 2S albumin, pointing towards an important role of these proteins in vacuolar trafficking. Vacuolar morphology was also severely affected in this mutant (Ebine et al. 2008). It is interesting to note that VAMP727 is present and well conserved in seed plants, but absent from the currently sequenced mosses, raising the hypothesis that vacuolar trafficking pathways might have diversified in the higher plants. Further studying this R-SNARE might prove valuable in understanding not only the point of evolutionary divergence of vacuolar sorting pathways, but also to better understand higher plants' vacuolar plasticity and trafficking routes. The results herein described have been summarized in Table 1, for easier reading.

5 Plant Vacuolar Sorting: RAB GTPases

RAB GTPases are the largest family of proteins in the Ras superfamily, and their main biological function is to serve as molecular switches, regulating the targeting and tethering of transport carriers to target membranes (Fig. 3). To do this, they cycle between a GTP-bound active state and a GDP-bound inactive state. Activation of RAB GTPases begins with membrane attachment, and the exchange of the bound GDP for a GTP molecule, effectively priming the RAB protein for action. This exchange is catalysed by guanine nucleotide exchange factors (GEFs). Once activated, GTP-bound RAB GTPases will interact with effector molecules, inducing downstream reactions such as tethering of transport carriers to target membranes (Grosshans et al. 2006). Similarly to the SNARE proteins, RAB GTPases are also incredibly diverse proteins, and are also thought to be the basis behind endomembrane system diversification events (Dacks and Fields 2007; Gurkan et al. 2007; Elias 2010). Some authors also believe that RAB GTPases, in tandem with certain SNARE proteins at specific subcellular localizations, may provide specificity to the membrane fusion events (Stenmark and Olkkonen 2001; Zerial and McBride 2001; Rehman et al. 2008), as GTP hydrolysis enables the SNARE complex syntaxin to bind the vesicles during docking. In this way, it would be feasible to assume that processes involving particular SNARE complexes

Table 1 Overview of the different SNARE proteins explored in this review

SNARE type	SNARE sub-type	Name	Subcellular localization	Described phenotypes (KO/OE/KD)	References
Q-SNARE	Qa-SNARE	SYP21/ PEP12	Late endosome	**SYP21 OE** Homotypic fusion of PVC bodies Partial secretion of vacuolar proteins **SYP21 KD/SYP22 KO** Impaired vacuolar transport of 12S globulin and 2S albumin **SYP21 KO/SYP22 KO** Gametophytic lethal	Foresti et al. (2006), Sanderfoot (2007), Shirakawa et al. (2010), and Uemura et al. (2010)
		SYP22/ VAM3	Late endosome	**SYP22 KO** Semi-dwarfism Late flowering Underdeveloped leaf vascular network **SYP21 KD/SYP22 KO** Impaired vacuolar transport of 12S globulin and 2S albumin **SYP21 KO/SYP22 KO** Gametophytic lethal **SYP22 KO/VAMP727 KO** Secreted storage vacuolar cargo Aberrant vacuolar morphology	Ebine et al. (2012), Ohtomo et al. (2005), Sanderfoot (2007), Shirakawa et al. (2009), and Ueda et al. (2006)
	Qb-SNARE	VTI11		**VTI11 KO (*zig-1* mutant)** Defective gravitropic responses Zig-zag shaped inflorescences Altered vacuolar morphologies	Hashiguchi et al. (2010), Morita et al. (2002), Saito et al. (2011), and Sanderfoot et al. (2001)
	Qc –SNARE/ i-SNARE	SYP51	Tonoplast/ TGN	–	De Benedictis et al. (2013) and Uemura et al. (2004)
		SYP52	Tonoplast/ TGN	–	De Benedictis et al. (2013) and Uemura et al. (2004)

R-SNARE	VAMP713	Tonoplast	–	De Benedictis et al. (2013)
	VAMP727	Endosome/ PVC Plasma membrane	**SYP22 KO/VAMP727 KO** Secreted storage vacuolar cargo Aberrant vacuolar morphology	Ebine et al. (2008), Ebine et al. (2011), and Uemura et al. (2004)

In the "Described Phenotypes" Section, KO stands for "Knock out", OE stands for "Overexpressed" and KD stands for "Knock down"

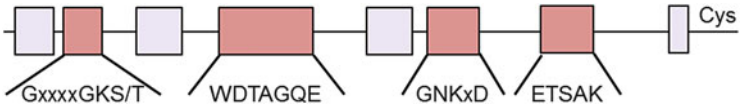

Fig. 3 Graphical representation of a typical RAB GTPase. *Brown boxes* represent the sequence motifs conserved in RAB GTPases. These motifs are indicated below the boxes, in the single-letter amino acid code, and are involved in nucleotide binding and hydrolysis. Some of these residues have been mutated in order to generate constitutively active (GTP-bound) or inactive (GDP-bound) mutant RAB GTPases, that have been employed for the functional study of these proteins. *Light violet boxes* represent the residues conserved in each functional subclass. They differ between subclasses and are used for the identification of all RAB GTPases into the different subgroups. At the carboxy-terminus, the cysteine (Cys) residues are indicated. These Cys residues are involved in membrane attachment of the RAB GTPases and are highly conserved

(comprising specific syntaxins), could be capable of coordinating specific RAB GTPases (Rehman et al. 2008).

The *Arabidopsis thaliana* genome contains 57 RAB GTPases, classified into eight different groups (RABA-RABH). These plant groups are highly similar to the animal RAB1, RAB2, RAB5-8, RAB11 and RAB18 groups (Rutherford and Moore 2002; Vernoud et al. 2003). When comparing land plants to other eukaryotic organisms, it becomes easy to realize that the RABA/RAB11 group is particularly enriched in genes, with 26 out of the 57 Arabidopsis genes, belonging to this group. This RABA group can be further sub-divided into 6 sub-groups: RABA1-RABA6 (Rutherford and Moore 2002). These RABA members have been shown to localize around the *trans* Golgi network (TGN) in the past (Ueda et al. 1996; de Graaf et al. 2005; Chow et al. 2008; Szumlanski and Nielsen 2009). The great diversity of RABA members in land plants might suggest this group to be responsible for plant-unique functions, such as cell-plate formation (Chow et al. 2008), or normal tip growth in pollen tubes and root hairs (Preuss et al. 2004; de Graaf et al. 2005; Szumlanski and Nielsen 2009).

In terms of endosomal and vacuolar trafficking, RAB5 and RAB7 are characterized as particularly relevant in yeast and animal cells, while also being present in plant systems. *Arabidopsis thaliana* possesses orthologous genes for all these RAB GTPases, plus a plant-specific RAB5 member – ARA6/RABF1 (Rutherford and Moore 2002). RABF1, RABF2a and RABF2b are all localized to distinct endosomal populations, despite being considerably overlapping, and they regulate separate trafficking pathways (Ueda et al. 2004; Haas et al. 2007; Ebine et al. 2011). Both RABF2a and RABF2b are involved in the vacuolar trafficking pathway, whereas RABF1 is responsible for mediating the flow of transport towards the plasma membrane (Kotzer et al. 2004; Viotti et al. 2010; Ebine et al. 2011). Despite its role in the trafficking of proteins towards the plasma membrane, RABF1 has also been implicated in the vacuolar sorting of soluble cargo and the recycling of vacuolar sorting receptor molecules from the late endosome to the TGN, implying multifunctional roles for this RAB in different plant species or tissues (Bottanelli et al. 2011, 2012). Some of the eight RAB7-related GTPases found in Arabidopsis (RABG1, RABG2 and RABG3a-f) have been detected at the tonoplast and the

multivesicular body (Rutherford and Moore 2002; Saito and Ueda 2009; Cui et al. 2014). These RABs have been implicated in plant responses against biotic and abiotic stress conditions. Overexpression of RABG3e results in increased vacuolar concentrations of sodium, conferring tolerance to salt and osmotic stress. This RAB GTPase has also been shown to become upregulated upon superoxide or salicylic acid treatment, or during infection (Mazel et al. 2004). Utilizing a dominant-negative mutant of RABG3f (RABG3f [T22N]), Cui et al. (2014) were successful in causing phenotypic alterations of the multivesicular body and vacuole – these organelles became enlarged and deformed, soluble vacuolar cargoes became missorted to the extracellular space, and storage proteins failed to be properly degraded during germination. These observations all point towards a critical role that RABG3 plays during the vacuolar transport of soluble cargo in Arabidopsis. At this point in time, an Arabidopsis sextuple *rabg3a,b,c,d,e,f* mutant has been generated and analysed – this mutant exhibits semi-dwarfism in the earliest developmental stages (Ebine et al. 2014). RABG activity is therefore important for the proper development of the Arabidopsis model.

The similarity of phenotypes obtained upon impairment of RABF or RABG proteins suggests they might both be active in the same trafficking pathway. However, vacuolar morphology appears more sensitive to mutations in RABG-related molecular components, whereas the RABF-related machineries appear to affect particularly the transport of soluble cargo to the protein storage vacuole (Ebine et al. 2014). It is also relevant to note that *rabf* and *rabg* mutations result in antagonistic effects in a *vti1/zig-1* mutant background. Overexpression of dominant-negative mutants of either RABF or RABG proteins also results in differential transport of membrane proteins in tobacco (Bottanelli et al. 2011). All these observations seem to point towards a differential function for these two GTPases in the plant vacuolar pathways, against what has been previously observed in non-plant systems. An overview of all the described RAB GTPases, their subcellular localizations, as well as their putative physiological functions in plant cells has been compiled in Table 2.

6 Chemical Genomics as a Novel Tool for the Study of the Plant Endomembrane System

Historically, drug discovery has been a very long and hard process, with most of the lead compounds being discovered by methodical biochemical and molecular biology analysis – as most biological targets were not known, luck played a huge role in the discovery of a chemical structure capable of producing a positive phenotypic effect. One such example is that of aspirin, which was employed by the medical community for its biological effects, long before the scientific community could understand its molecular mechanism (Robert et al. 2009). Nowadays, the strategy has been vastly improved, and the drug discovery process is no longer such a hit-

Table 2 An overview of the different RAB GTPases explored in this review, as well as their subcellular localizations and putative physiological functions

RAB group	Mammalian homologues	RAB sub-group	Subcellular localization	Putative physiological functions	References
RABA	–	RABA1	TGN	Cell-plate formation Tip growth (pollen tubes and root hairs)	Chow et al. (2008), de Graaf et al. (2005), Preuss et al. (2004), Szumlanski and Nielsen (2009), Ueda et al. (1996), and Vernoud et al. (2003)
	RAB11	RABA2			
	–	RABA3			
	–	RABA4			
	–	RABA5			
	–	RABA6			
RABF	–	RABF1/ ARA6	Endosomes	Sorting of proteins towards the plasma membrane Vacuolar sorting of soluble cargo Recycling of vacuolar sorting receptors	Bottanelli et al. (2011), Bottanelli et al. (2012), Ebine et al. (2011), Haas et al. (2007), Kotzer et al. (2004), Ueda et al. (2004), Vernoud et al. (2003), and Viotti et al. (2010)
	RAB5	RABF2a-b	Endosomes	Vacuolar sorting	
RABG	–	RABG1	Tonoplast and MVB	–	Bottanelli et al. (2011), Cui et al. (2014), Ebine et al. (2014), Mazel et al. (2004), Rutherford and Moore (2002), Saito and Ueda (2009), and Vernoud et al. (2003)
	–	RABG2		–	
	RAB7	RABG3a-f		Vacuolar transport of soluble cargo	

and-miss strategy. Chemists will rely on general rules, such as the Lipinski rule of five (Lipinski et al. 1997) when designing lead compounds. These leads will then be structurally altered in the slightest ways, creating a vast library of chemical compounds based upon the same lead compound. These libraries can then be employed for high-throughput screens, allowing the researcher to choose the structural scaffold with the highest biological activity, the most precise point of action, the lowest toxicity or any other desirable characteristic (Robert et al. 2009). Chemical genomics is based on different principles, when compared with drug design. For chemical genomics studies, researchers are no longer interested in the screening of drugs, but rather they search for compounds capable of eliciting an observable biological effect that may be used for understanding that organism's basic physiology. An obvious difference is that the chemical libraries are not collections of similar compounds, but rather a collection of widely different chemical structures and lead scaffolds. Furthermore, plant biologists are less interested in

certain chemical characteristics, such as high potency or metabolic turnover. Reversibility of effects is desirable, but not mandatory for all applications (Robert et al. 2009).

The most widely employed probe reagents are small organic molecules, and despite its insipience, plant chemical genomic screen projects have been returning some very interesting molecules for basic physiological research. Some of these molecules affect cell wall biogenesis, such as the herbicides dichlorobenzonitrile (DCB), isoxaben (*N*-[3(1-ethyl-1-methylpropyl)-5-isoxazolyl]) and CGA325_615 (Peng et al. 2001).

Classical genetic approaches have proven limited for the study of the plant endomembrane system, particularly the study of vesicular trafficking. The *vacuoless 1* (*vcl1*) Arabidopsis mutant is one of the rare cases of limited success, leading to defective vacuole biogenesis, but also embryo lethality, indicating the importance of this organelle for plant organisms (something not observed in yeast, for example). Gene-redundancy is also a hurdle that usually results in no observable phenotype for many T-DNA insertion lines. Chemical genomics can, therefore, be a powerful tool for overcoming these common limitations, and some work has been done in this area already (Robert et al. 2009). In 2004 Zouhar et al. screened a library of 4800 compounds in yeast. Of these compounds, 14 resulted in the aberrant secretion of the commonly vacuolar carboxypeptidase Y (CPY) enzyme, effectively mimicking the *vps* phenotype (Bowers and Stevens 2005). Of these 14 compounds, 2 (Sortin1 and Sortin2) were shown to have an effect in Arabidopsis – reversible effects were detected on vacuole biogenesis, root development and CPY secretion, with no detectable phenotype in any other organelles (Zouhar et al. 2004). More recently, Rosado et al. (2011) have further expanded these results, coming to the understanding that sortin1-hypersensitive Arabidopsis mutants exhibited not only drastic vacuolar morphological alterations, but also defects in flavonoid accumulation, thus linking both vacuolar-trafficking defects and flavonoid metabolism, all the while proving the usefulness of chemical compounds in the elucidation of plant biological responses that have been difficult to dissect by conventional genetics (Rosado et al. 2011). Other examples of already characterized compounds, well accepted by the community as useful chemical tools include wortmannin, a phosphoinositide 3-kinase inhibitor, brefeldin A (BFA), an inhibitor of vesicular transport, coronatine, a bacterial phytotoxin and other variations of the E-64 protease inhibitor (Murphy et al. 2005; Samaj et al. 2006; Kolodziejek and van der Hoorn 2010; Wasternack and Kombrink 2010). Despite the enormous potential of the chemical biology methods for screening basic plant physiology, some processes remain unprobed by these methodologies – current strategies employ either single-celled (pollen or suspension cultures) or seedlings grown in microplates, making the study of end-of-cycle events, such as flowering and seed formation hard, if not impossible, to investigate. Alternative model organisms such as the duckweeds Lemneae and Wolffieae sp. have been suggested, as they include some of the smallest flowering plants and can be easily cultivated in liquid cultures in microplates, and might be the key for the chemical biological study of subjects as diverse as endomembrane trafficking or flowering control (Serrano et al. 2015).

7 Alternative Sorting Routes

Recent advances in the study of plant autophagy have led to the discovery of alternative pathways for proteins to reach the vacuole. Macroautophagy is a process generally associated with stress and starvation-induced bulk degradation of cellular components, though it may also be a highly selective process, capable of targeting malfunctioning organelles such as mitochondria, peroxisomes, protein aggregates, pathogens or even specific proteins (Floyd et al. 2012; Li and Vierstra 2012). Selective autophagy has thus been identified as the major mechanism behind the delivery of several resident vacuolar proteins (Michaeli et al. 2014). Of these processes, the cytosol-to-vacuole targeting pathway (Cvt) of yeast is the most well studied and better understood (Scott et al. 1996), being responsible for the transport of at least two hydrolases (aminopeptidase 1 and α-mannosidase) from the cytosol to the vacuole. The selectivity of this mechanism is determined by the core autophagy machinery, namely Atg19, which bridges between both hydrolases, and the Atg11 and Atg8 autophagic proteins mediating the formation of the Cvt vesicle (Lynch-Day and Klionsky 2010).

Another alternative vacuolar sorting route is present mainly during seed development, during the biosynthesis of storage proteins. These storage proteins may be transported directly from the endoplasmic reticulum (ER) to the vacuole (ERvt route), bypassing the Golgi Complex entirely, an hypothesis which was formulated due to the careful study of these storage proteins through electron microscopy observations of wheat seeds (Levanony et al. 1992; Galili et al. 1993). This sorting pathway begins with the aggregation of the proteins inside the ER, budding and formation of ER-derived protein bodies (PBs) and their subsequent internalization into the storage vacuoles by an autophagy-like mechanism (Hara-Nishimura et al. 1998; Robinson et al. 2005; Herman 2008; Ibl and Stoger 2011; Wang et al. 2011), although it is still currently unknown, whether this process utilizes the same molecular machinery.

8 Plant Aspartic Proteinases: Cardosins as a Study Case

Cardosins are aspartic proteinases (APs) belonging to the A1 family of APs, that have been identified in *Cynara cardunculus* L. The two main isoforms of these enzymes – cardosins A and B are found in the floral tissues of this plant, and despite their high sequence similarity, cardosin A is found to accumulate in the protein storage vacuoles of the stigmatic papillae, whereas cardosin B is detected in the extracellular matrix of the stigma and style transmitting tissue (Ramalho-Santos et al. 1997; Vieira et al. 2001; Duarte et al. 2008). Curiously enough, when expressed in heterologous systems, cardosin A accumulated in different vacuole types, in a tissue-dependent manner – lytic vacuoles in vegetative tissues, or protein storage vacuoles in *Arabidopsis thaliana* seedlings. Alternatively, cardosin B is no

longer secreted in tobacco leaf epidermal cells, accumulating in the large central vacuole instead (da Costa et al. 2010). These enzymes are synthesized as zymogens (preprocardosins) – inactive precursors that suffer a series of proteolytic cleavages during their vacuolar sorting, resulting in their timely activation when reaching the vacuolar lumen (Duarte et al. 2008). In cardoon seeds however, preprocardosin A can be detected in protein bodies (PBs), its mature form appearing at a later time, in the central lytic vacuole, as it engulfs the remaining PBs. Cardosin A tissue-specific dual localization could be explained by the presence of two distinct vacuolar sorting determinants in the enzyme's primary structure – a typical ctVSD, and an internal signal, the PSI. Cardosin A's PSI domain is capable of the COPII independent vacuolar sorting of a fluorescent mCherry reporter in *Nicotiana tabacum* leaf epidermal cells, as demonstrated in 2013 by Pereira and collaborators (Pereira et al. 2013). The authors postulate that a PSI-mediated COPII independent vacuolar sorting mechanism could be relevant in metabolically active organs, such as floral and seed tissues, where protein storage vacuoles are the predominant vacuole type. These direct ER-vacuole routes have been postulated to be important for the plants' rapid adaptation to the ever-changing environment around them (Xiang et al. 2013). Adding to the complexity of the cardosin model, Duarte and collaborators observed the presence of endoglycosidase-H-sensitive intermediate forms of cardosin A, and an inability for dominant-inhibitory RAB GTPases to completely inhibit this enzyme's processing events. Furthermore, secretion assays in tobacco protoplasts revealed the secretion of an intermediate form of cardosin A, raising the hypothesis that this enzyme might be transiently secreted and quickly re-internalized, prior to accumulation in the vacuolar lumen (Duarte et al. 2008). By analysing all the results obtained so far, we have developed a model of cardosin trafficking, which might follow different routes in a tissue-specific manner (Fig. 4) (Reviewed in Pereira et al. 2014).

9 Bringing Vacuoles to the Marketplace: Biotechnological Implications

Plants are the basis for the production of many different economically and socially relevant products, from food and feed, to bioactive secondary metabolites with medicinal applications. These organisms have been classified as ideal bioreactors for the production of relevant macromolecules, but the main efforts have been directed at dissecting enzymatic machineries and metabolic pathways – the transport mechanisms are generally overlooked and might be responsible for significant losses in productivity. In this regard, Di Sansebastiano et al. (2014) have been working on the classical problem of artemisinin production. Artemisinin is a sesquiterpene lactone endoperoxide with anti-malarial properties that occurs in the plant *Artemisia annua*. A wide array of strategies have been employed to increase the plants' production of this compound, such as overexpression of

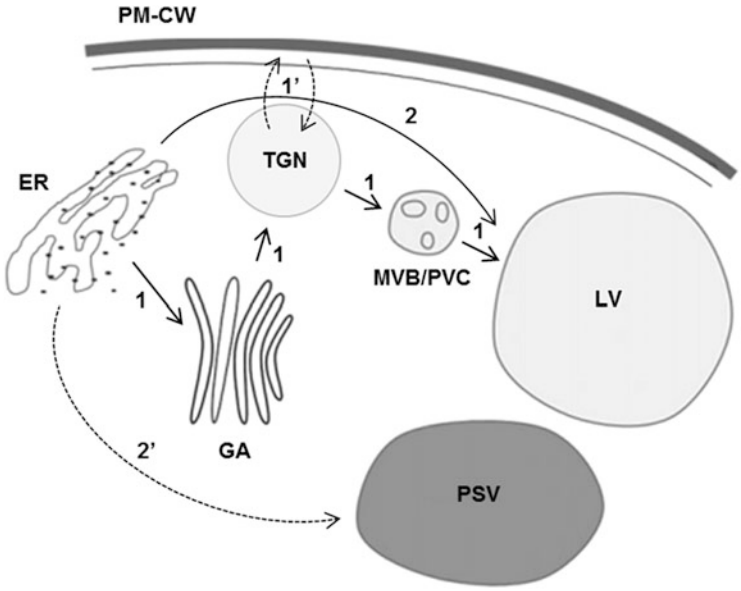

Fig. 4 A putative model of cardosin trafficking. (*1*) COPII-dependent sorting between the ER and Golgi has been demonstrated for both cardosins A and B in *Nicotiana tabacum* leaf epidermis, mediated by the enzymes' C-termini VSDs. RABF2b [S24N] blockage of post-Golgi trafficking results in secretion of both cardosins and their C-terminal peptides, suggesting these proteins must pass through the PVC, prior to vacuolar accumulation. Secretion assays with tobacco protoplasts have shown an intermediate, not fully matured form of cardosin A becoming secreted, raising the hypothesis that these enzymes might be transiently secreted during their transit to the vacuole (*1′*). This hypothesis remains insufficiently substantiated at this time, and further work will have to be done in order to confirm or disprove this idea. (*2*) Cardosin A (but not cardosin B) PSI-mediated sorting appears both COPII and RABF2b-independent, hinting at the possibility that this VSD shuttles proteins directly from the ER towards the lytic vacuole. (*2′*) It is hypothesized that this PSI-mediated GA-bypass is tissue specific and might be prevalent in seeds, as unprocessed cardosin A has been detected at the protein storage vacuole in dormant seeds, becoming fully maturated during germination upon vacuolar lumen acidification due to LV-PSV fusion events (adapted from Pereira et al. 2013)

enzymes (Nafis et al. 2011; Tang et al. 2014), inhibition of competitive biosynthetic pathways (Chen et al. 2011), transcription factor regulated modulation (Lu et al. 2013; Tang et al. 2014) and biomass (Banyai et al. 2011), or specialized cell density increments (Maes and Goossens 2010). Success has been limited, in part because the accumulated artemisinin is unstable and quickly degraded. Knowing that plant cells will sometimes generate MVB-derived small membranous compartments for the accumulation and delivery of phenylpropanoids to the cell periphery, Di Sansebastiano employed a truncated AtSYP51, whose N-terminal portion had been removed, to artificially induce the formation of a stable compartment generated by ER- and endocytosis-derived membranes, where the artemisinin metabolite became stabilized and accumulated for longer periods (Di Sansebastiano et al. 2014). By applying what is now known about vacuolar sorting and biogenesis

mechanisms, such an engineering approach might allow for plant organisms to become even more versatile biofactories, especially when complemented with the more classical approaches of gene overexpression or silencing. The same approach might also be applicable to the production of recombinant protein products, overcoming the problems associated with traditional strategies that rely on ER accumulation of recombinant protein, usually inducing ER stress due to protein overload, or the accumulation in less stable environments, such as the protease-enriched extracellular space or vacuoles.

Another important area of plant biotechnology is the development of improved crops and plant species, better capable of resisting environmental stresses. In fact, a study with Arabidopsis expressing a RABF1/ARA6 [Q93L] GTP-bound mutant transgene, found these plants to be resistant to salt stress, as tested by supplementing their growth media with NaCl. In fact, these plants showed no phenotype even when in the presence of 100 mM NaCl (Ebine et al. 2011), pointing towards an important function of these proteins in the plant stress response. This process is not limited to Arabidopsis, as RABF1 induction has also been demonstrated during the salt stress response of the halophyte *Mesembryanthemum crystallinum* plant (Bolte et al. 2000). Also in rice (*Oryza sativa*) and *Pennisetum glaucum,* RAB7 accumulated upon cold, salt and dehydration stresses, and also upon ABA treatment, hinting at important functions for this GTPase in a variety of stress responses (Nahm et al. 2003; Agarwal et al. 2008). Overexpression of Arabidopsis RAB7 also resulted in increased sodium content in the shoots, with Na^+ ions becoming trapped in the vacuole as a means of reducing the associated toxicity effects (Mazel et al. 2004). These reports are representative of how, by better understanding the molecular mechanisms behind vacuolar sorting, we may end up capable of manipulating cellular responses, in order to better engineer different plant species optimized for different ends.

10 Conclusions and Future Perspectives

In 2013 the Nobel Prize for Medicine was awarded to James E. Rothman, the researcher responsible for understanding the way SNARE proteins interact and form complexes. This prize was shared with two other scientists – Randy W. Scheckman and Thomas C. Südhof, for their contributions to the body of knowledge in vesicle trafficking. This prize is indicative of the relevance behind this area of study. In plant models, these mechanisms are behind a huge variety of different responses – from vacuolar biogenesis and protein accumulation, to plasticity towards different environmental stressors. In this regard, a better understanding of the plant endomembrane system and of their vacuolar systems in particular will undoubtedly result in novel ways to design better, more productive and hardier plants better suited for feeding an ever increasing population in a world undergoing climate change. But this knowledge will unlock even more possibilities, as plants are the providers of not only food, but also more valuable compounds, with

industrial and medicinal applications. Subcellular engineering is an exciting new possibility for increasing yields of both recombinant protein and secondary metabolite production in plants bred for the sole purpose of being used as bioreactors. In order for these exciting possibilities to be achieved, more will have to be known at the most fundamental levels – the high-throughput discovery of chemical compounds through chemical genomics projects will no doubt prove invaluable in accelerating further discoveries in this field, particularly by better defining targets worth studying in greater depth, or by allowing the transient exploration of otherwise lethal phenotypes. Better understanding the cross-roads where both RAB GTPases and SNAREs intersect in terms of stress sensing and response, as well as how they interact with each other for generating the subcellular identity of different compartments is sure to be important for our understanding of plant cell physiology and development, as well as to open up the door for novel applications in the realm of subcellular engineering. This is definitely an exciting time to be working in this subject, as the near future is sure to show us.

Acknowledgments Work developed under the Strategic Project OE/BIA/UI4046/2014 of the BioISI – Biosystems & Integrative Sciences Institute, supported by FCT (Fundação para a Ciência e a Tecnologia) funding.

References

Agarwal PK, Jain P, Jha B, Reddy MK, Sopory SK (2008) Constitutive over-expression of a stress-inducible small GTP binding protein PgRab7 from *Pennisetum glaucum* enhances abiotic stress tolerance in transgenic tobacco. Plant Cell Rep 27:105–115

Banyai W, Mii M, Supaibulwatana K (2011) Enhancement of artemisinin content and biomass in *Artemisia annua* by exogenous GA3 treatment. Plant Growth Regul 63:45–54

Bednarek SY, Wilkins TA, Dombrowski JE, Raikhel NV (1990) A carboxyl-terminal propeptide is necessary for proper sorting of barley lectin to vacuoles of tobacco. Plant Cell 2:1145–1155

Bocock J, Carmicle S, Chhotani S, Ruffolo MR, Chu H, Erickson AH (2009) The PA-TM-RING protein RING finger protein 13 is an endosomal integral membrane E3 ubiquitin ligase whose RING finger domain is released to the cytoplasm by proteolysis. FEBS J 276:1860–1877

Bolte S, Schiene K, Dietz KJ (2000) Characterization of a small GTP-binding protein of the rab 5 family in *Mesembryanthemum crystallinum* with increased level of expression during early salt stress. Plant Mol Biol 42:923–936

Bolte S, Lanquar V, Soler MN, Beebo A, Satiat-Jeunemaitre B, Bouhidel K, Thomine S (2011) Distinct lytic vacuolar compartments are embedded inside the protein storage vacuole of dry and germinating *Arabidopsis thaliana* seeds. Plant Cell Physiol 52:1142–1152

Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. Cell 116:153–166

Bottanelli F, Foresti O, Hanton S, Denecke J (2011) Vacuolar transport in tobacco leaf epidermis cells involves a single route for soluble cargo and multiple routes for membrane cargo. Plant Cell 23:3007–3025

Bottanelli F, Gershlick DC, Denecke J (2012) Evidence for sequential action of Rab5 and Rab7 GTPases in prevacuolar organelle partitioning. Traffic 13:338–354

Bowers K, Stevens TH (2005) Protein transport from the late Golgi to the vacuole in the yeast Saccharomyces cerevisiae. Biochim Biophys Acta 1744:438–454

Cao X, Rogers SW, Butler J, Beevers L, Rogers JC (2000) Structural requirements for ligand binding by a probable plant vacuolar sorting receptor. Plant Cell 12:493–506

Chen JL, Fang HM, Ji YP, Pu GB, Guo YW, Huang LL, Du ZG, Liu BY, Ye HC, Li GF, Wang H (2011) Artemisinin biosynthesis enhancement in transgenic *Artemisia annua* plants by downregulation of the β-caryophyllene synthase gene. Planta Med 77:1759–1765

Chow CM, Neto H, Foucart C, Moore I (2008) Rab-A2 and Rab-A3 GTPases define a trans-golgi endosomal membrane domain in Arabidopsis that contributes substantially to the cell plate. Plant Cell 20:101–123

Craddock CP, Hunter PR, Szakacs E, Hinz G, Robinson DG, Frigerio L (2008) Lack of a vacuolar sorting receptor leads to non-specific missorting of soluble vacuolar proteins in Arabidopsis seeds. Traffic 9:408–416

Cui Y, Zhao Q, Gao C, Ding Y, Zeng Y, Ueda T, Nakano A, Jiang L (2014) Activation of the Rab7 GTPase by the MON1-CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in Arabidopsis. Plant Cell 26(5):2080–2097

da Costa DS, Pereira S, Moore I, Pissarra J (2010) Dissecting cardosin B trafficking pathways in heterologous systems. Planta 232(6):1517–1530

Dacks JB, Fields MC (2007) Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. J Cell Sci 120:2977–2985

De Benedictis M, Bleve G, Faraco M, Stigliano E, Grieco F, Piro G, Dalessandro G, Di Sansebastiano GP (2013) AtSYP51/52 functions diverge in the post-Golgi traffic and differently affect vacuolar sorting. Mol Plant 6:916–930

de Graaf BH, Cheung AY, Andreyeva T, Levasseur K, Kieliszewski M, Wu HM (2005) Rab11 GTPase-regulated membrane trafficking is crucial for tip-focused pollen tube growth in tobacco. Plant Cell 17:2564–2579

De Marcos Lousa C, Gershlick DC, Denecke J (2012) Mechanisms and concepts paving the way towards a complete transport cycle of plant vacuolar sorting receptors. Plant Cell 24:1714–1732

Di Sansebastiano GP (2013) Defining new SNARE functions: the i-SNARE". Front Plant Sci 4:99

Di Sansebastiano GP, Rizzello F, Durante M, Caretto S, Nisi R, De Paolis A, Faraco M, Montefusco A, Piro G, Mita G (2014) Subcellular compartmentalization in protoplastos from *Artemisia annua* cell cultures: engineering attempts using a modified SNARE protein. J Biotechnol 202:146–152

Duarte P, Pissarra J, Moore I (2008) Processing and trafficking of a single isoform of the aspartic proteinase cardosin A on the vacuolar pathway. Planta 227:1255–1268

Ebine K, Okatani Y, Uemura T, Goh T, Shoda K, Niihama M, Morita MT, Spitzer C, Otegui MS, Nakano A, Ueda T (2008) A SNARE complex unique to seed plants is required for protein storage vacuole biogenesis and seed development of *Arabidopsis thaliana*. Plant Cell 20:3006–3021

Ebine K, Fujimoto M, Okatani Y, Nishiyama T, Goh T, Ito E, Dainobu T, Nishitani A, Uemura T, Sato MH, Thordal-Christensen H, Tsutsumi N, Nakano A, Ueda T (2011) A membrane trafficking pathway regulated by the plant-specific RAB GTPase ARA6. Nat Cell Biol 13(7): U853–U859

Ebine K, Uemura T, Nakano A, Ueda T (2012) Flowering time modulation by a vacuolar SNARE via FLOWERING LOCUS C in *Arabidopsis thaliana*. PLoS One 7, e42239

Ebine K, Inoue T, Ito J, Ito E, Uemura T, Goh T, Abe H, Sato K, Nakano A, Ueda T (2014) Plant vacuolar trafficking occurs through distinctly regulated pathways. Curr Biol 24:1375–1382

Elias M (2010) Patterns and processes in the evolution of the eukaryotic endomembrane system. Mol Membr Biol 27:469–489

Fasshauer D, Sutton RB, Brunger AT, Jahn R (1998) Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc Natl Acad Sci U S A 95:15781–15786

Floyd BE, Morriss SC, Macintosh GC, Bassham DC (2012) What to eat: evidence for selective autophagy in plants. J Integr Plant Biol 54:907–920

Foresti O, Da Silva LL, Denecke J (2006) Overexpression of the *Arabidopsis* syntaxin PEP12/SYP21 inhibits transport from the prevacuolar compartment to the lytic vacuole in vivo. Plant Cell 18:2275–2293

Fujimoto M, Ueda T (2012) Conserved and plant-unique mechanisms regulating plant post-Golgi traffic. Front Plant Sci 3(197):1–10

Galili G, Altschuler Y, Levanony H (1993) Assembly and transport of seed storage proteins. Trends Cell Biol 3:437–442

Gillespie JE, Rogers SW, Deery M, Dupree P, Rogers JC (2005) A unique family of proteins associated with internalized membranes in protein storage vacuoles of the Brassicaceae. Plant J 41:429–441

Grosshans BL, Ortiz D, Novick P (2006) Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A 103:11821–11827

Gurkan C, Koulov AV, Balch WE (2007) An evolutionary perspective on eukaryotic membrane trafficking. Adv Exp Med Biol 607:73–83

Haas TJ, Sliwinski MK, Martinez DE, Preuss M, Ebine K, Ueda T, Nielsen E, Odorizzi G, Otegui MS (2007) The Arabidopsis AAA ATPase SKD1 is involved in multivesicular endosome function and interacts with its positive regulator LYST-INTERACTING PROTEIN5. Plant Cell 19:1295–1312

Hara-Nishimura I, Shimada T, Hatano K, Takeuchi Y, Nishimura M (1998) Transport of storage proteins to protein storage vacuoles is mediated by large precursor-accumulating vesicles. Plant Cell 10:825–836

Harley SM, Beevers L (1989) Coated vesicles are involved in the transport of storage proteins during seed development in *Pisum sativum* L. Plant Physiol 91:674–678

Hashiguchi Y, Niihama M, Takahashi T, Saito C, Nakano A, Tasaka M, Morita MT (2010) Loss-of-function mutations of retromer large subunit genes suppress the phenotype of an Arabidopsis zig mutant that lacks Qb-SNARE VTI11. Plant Cell 22:159–172

Herman EM (2008) Endoplasmic reticulum bodies: solving the insoluble. Curr Opin Plant Biol 11:672–679

Hillmer S, Movafeghi A, Robinson DG, Hinz G (2001) Vacuolar storage proteins are sorted in the *cis*-cisternae of the pea cotyledon Golgi apparatus. J Cell Biol 152:41–50

Hinz G, Colanesi S, Hillmer S, Rogers JC, Robinson DG (2007) Localization of vacuolar transport receptors and cargo proteins in the Golgi apparatus of developing *Arabidopsis* embryos. Traffic 8:1452–1464

Hoflack B, Kornfeld S (1985) Lysosomal enzyme binding to mouse P388D1 macrophage membranes lacking the 215-kDa mannose 6-phosphate receptor: evidence for the existence of a second mannose 6-phosphate receptor. Proc Natl Acad Sci U S A 82:4428–4432

Holkeri H, Vitale A (2001) Vacuolar sorting determinants within a plant storage protein trimer act cumulatively. Traffic 2:737–741

Holwerda BC, Padgett HS, Rogers JC (1992) Proaleurain vacuolar targeting is mediated by short contiguous peptide interactions. Plant Cell 4:307–318

Ibl V, Stoger E (2011) The formation, function and fate of protein storage compartments in seeds. Protoplasma 249:379–392

Jiang L, Phillips TE, Rogers SW, Rogers JC (2000) Biogenesis of the protein storage vacuole crystalloid. J Cell Biol 150:755–769

Jolliffe NA, Craddock CP, Frigerio L (2005) Pathways for protein transport to seed storage vacuoles. Biochem Soc Trans 33:1016–1018

Kirsch T, Paris N, Butler JM, Beevers L, Rogers JC (1994) Purification and initial characterization of a potential plant vacuolar targeting receptor. Proc Natl Acad Sci U S A 91:3403–3407

Kolodziejek I, Van Der Hoorn RA (2010) Mining the active proteome in plant science and biotechnology. Curr Opin Biotechnol 21:225–233

Kotzer AM, Brandizzi F, Neuman U, Paris N, More I, Hawes C (2004) AtRabF2b (Ara7) acts on the vacuolar trafficking pathway in tobacco leaf epidermal cells. J Cell Sci 117:6377–6389

Kriegel MA, Rathinam C, Flavell RA (2009) E3 ubiquitin ligase GRAIL controls primary T cell activation and oral tolerance. Proc Natl Acad Sci U S A 106:16770–16775

Leshem Y, Golani Y, Kaye Y, Levine A (2010) Reduced expression of the v-SNAREs AtVAMP71/AtVAMP7C gene family in *Arabidopsis* reduces drought tolerance by suppression of abscisic acid-dependent stomatal closure. J Exp Bot 61:2615–2622

Levanony H, Rubin R, Altschuler Y, Galili G (1992) Evidence for a novel route of wheat storage proteins to vacuoles. J Cell Biol 119:1117–1128

Li F, Vierstra RD (2012) Autophagy: a multifaceted intracellular system for bulk and selective recycling. Trends Plant Sci 17:526–537

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23:3–25

Lipka V, Kwon C, Panstruga R (2007) SNARE-ware: the role of SNARE-domain proteins in plant biology. Annu Rev Cell Dev Biol 23:147–174

Lu X, Zhang L, Zhang F, Jiang W, Shen Q, Zhang L, Lv Z, Wang G, Tang K (2013) AaORA, a trichome-specific AP2/ERF transcription factor of *Artemisia annua*, is a positive regulator in the artemisinin biosynthetic pathway and in disease resistance to *Botrytis cinerea*. New Phytol 198:1191–1202

Luini A (2011) A brief history of the cisternal progression-maturation model. Cell Logist 1:6–11

Luo F, Fong YH, Zeng Y, Shen J, Jiang L, Wong K (2014) How vacuolar sorting receptor proteins interact with their cargo proteins: crystal structures of Apo and cargo-bound forms of the protease-associated domain from an *Arabidopsis* vacuolar sorting receptor. Plant Cell 26:3693–3708

Lynch-Day MA, Klionsky DJ (2010) The Cvt pathway as a model for selective autophagy. FEBS Lett 584:1359–1366

Maes L, Goossens A (2010) Hormone-mediated promotion of trichome initiation in plants is conserved but utilizes species and trichome-specific regulatory mechanisms. Plant Signal Behav 5:205–207

Maeshima M, Haranishimura I, Takeuchi Y, Nishimura M (1994) Accumulation of vacuolar H^+-pyrophosphatase and H^+-ATPase during reformation of the central vacuole in germinating pumpkin seeds. Plant Physiol 106:61–69

Matsuoka K, Nakamura K (1991) Propeptide of a precursor to a plant vacuolar protein required for vacuolar targeting. Proc Natl Acad Sci U S A 88:834–838

Mazel A, Leshem Y, Tiwari BS, Levine A (2004) Induction of salt and osmotic stress tolerance by overexpression of an intracellular vesicle trafficking protein AtRab7 (AtRabG3e). Plant Physiol 134:118–128

Michaeli S, Avin-Wittenberg T, Galili G (2014) Involvement of autophagy in the direct ER to vacuole protein trafficking route in plants. Front Plant Sci 5:134

Morita MT, Kato T, Nagafusa K, Saito C, Ueda T, Nakano A, Tasaka M (2002) Involvement of the vacuoles of the endodermis in the early process of shot gravitropism in Arabidopsis. Plant Cell 14:47–56

Murphy AS, Bandyopadhyay A, Holstein SE, Peer WA (2005) Endocytotic cycling of PM proteins. Annu Rev Plant Biol 56:221–251

Nafis T, Akmal M, Ram M, Alam P, Ahlawat S, Mohd A, Abdin MZ (2011) Enhancement of artemisinin content by constitutive expression of the HMG-CoA reductase gene in high-yielding strain of *Artemisia annua* L. Plant Biotechnol Rep 5:53–60

Nahm MY, Kim SW, Yun D, Lee SY, Cho MJ, Bahk JD (2003) Molecular and biochemical analyses of OsRAB7, a rice Rab7 homolog. Plant Cell Physiol 44:1341–1349

Nakamura K, Matsuoka K (1993) Protein targeting to the vacuole in plant cells. Plant Physiol 101:1–5

Neuhaus JM, Rogers J (1998) Sorting of proteins to vacuoles in plant cells. Plant Mol Biol 38:127–144

Neuhaus JM, Sticher L, Meins F Jr, Boller T (1991) A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. Proc Natl Acad Sci U S A 88:10362–10366

Nishizawa K, Maruyama N, Utsumi S (2006) The C-terminal region of α' subunit of soybean β-conglycinin contains two types of vacuolar sorting determinants. Plant Mol Biol 62:111–125

Ohtomo I, Ueda H, Shimada T, Nishiyama C, Komoto Y, Hara-Nishimura I, Takahashi T (2005) Identification of na allele of VAM3/SYP22 that confers a semi-dwarf phenotype in *Arabidopsis thaliana*. Plant Cell Physiol 46:1358–1365

Olbrich A, Hillmer S, Hinz G, Oliviusson P, Robinson DG (2007) Newly formed vacuoles in root meristems of barley and pea seedlings have characteristics of both protein storage and lytic vacuoles. Plant Physiol 145:1383–1394

Paris N, Stanley CM, Jones RL, Rogers JC (1996) Plant cells contain two functionally distinct vacuolar compartments. Cell 85:563–572

Park M, Lee D, Lee G-J, Hwang I (2005) AtRMR1 functions as a cargo receptor for protein trafficking to the protein storage vacuole. J Cell Biol 170:757–767

Park JH, Oufattole M, Rogers JC (2007) Golgi-mediated vacuolar sorting in plant cells: RMR proteins are sorting receptors for the protein aggregation/membrane internalization pathway. Plant Sci 172:728–745

Peng L, Xiang F, Roberts E, Kawagoe Y, Greve LC, Kreuz K, Delmer DP (2001) The experimental herbicide CGA 325'615 inhibits synthesis of crystalline cellulose and causes accumulation of non-crystalline beta-1,4-glucan associated with CesA protein. Plant Physiol 126:981–992

Pereira C, Pereira S, Satiat-Jeunemaitre B, Pissarra J (2013) Cardosin A contains two vacuolar sorting signals using different vacuolar routes in tobacco epidermal cells. Plant J 76:87–100

Pereira C, Pereira S, Pissarra J (2014) Delivering of proteins to the plant vacuole – an update. Int J Mol Sci 15:7611–7623

Pompa A, de Marchis F, Vitale A, Arcioni S, Bellucci M (2010) An engineered C-terminal disulphide bond can partially replace the phaseolin vacuolar sorting signal. Plant J 61:782–791

Preuss ML, Serna J, Falbel TG, Bednarek SY, Nielsen E (2004) The Arabidopsis RabGTPase RabA4b localizes to the tips of growing root hair cells. Plant Cell 16:1589–1603

Ramalho-Santos M, Pissarra J, Veríssimo P, Pereira S, Salema R, Pires E, Faro CJ (1997) Cardosin A, na abundant aspartic proteinase, accumulates in protein storage vacuoles in the stigmatic papillae of *Cynara cardunculus* L. Planta 203:204–212

Rehman RU, Stigliano E, Lycett G, Sticher L, Franchesca S, Dalessandro G, Di Sansebastiano GP (2008) Tomato Rab11a characterization evidenced a difference between SYP121 dependent and SYP122-dependent exocytosis. Plant Cell Physiol 49(5):751–766

Robert S, Raikhel NV, Hicks GR (2009) Powerful partners: Arabidopsis and chemical genomics. Arabidopsis Book 7, e0109, http://doi.org/10.1199/tab.0109

Robinson DG, Oliviusson P, Hinz G (2005) Protein sorting to the storage vacuoles of plants: a critical appraisal. Traffic 6:615–625

Rosado A, Hicks GR, Norambuena L, Rogachev I, Meir S, Pourcel L, Zouhar J, Brown MQ, Boirsdore MP, Puckrin RS, Cutler SR, Rojo E, Aharoni A, Raikhel NV (2011) Sortin1-hypersensitive mutants link vacuolar-trafficking defects and flavonoid metabolism in Arabidopsis vegetative tissues. Chem Biol 18:187–197

Rutherford S, Moore I (2002) The Arabidopsis Rab GTPase family: another enigma variation. Curr Opin Plant Biol 5:518–528

Saito C, Ueda T (2009) Chapter 4: Functions of RAB and SNARE proteins in plant life. Int Rev Cell Mol Biol 274:183–233

Saito C, Uemura T, Awai C, Tominaga M, Ebine K, Ito J, Ueda T, Abe H, Morita MT, Tasaka M et al (2011) The occurrence of 'bulbs', a complex configuration of the vacuolar membrane, is affected by mutations of vacuolar SNARE and phospholipase in Arabidopsis. Plant J 68:64–73

Samaj J, Muller J, Beck M, Bohm N, Menzel D (2006) Vesicular trafficking, cytoskeleton and signalling in root hairs and pollen tubes. Trends Plant Sci 11:594–600

Sanderfoot A (2007) Increases in the number of SNARE genes parallels the rise of multicellularity among the green plants. Plant Physiol 144:6–17

Sanderfoot AA, Kovaleva V, Bassham DC, Raikhel NV (2001) Interactions between syntaxins identify at least five SNARE complexes within the Golgi/prevacuolar system of the Arabidopsis cell. Mol Biol Cell 12:3733–3743

Sansebastiano GD, Piro G (2014) The SNARE proteins (in plants) beyond the Noble Prize. J Plant Biochem Physiol 2:2

Scott SV, Hefner-Gravink A, Morano KA, Noda T, Ohsumi Y, Klionsky DJ (1996) Cytoplasm-to-vacuole targeting and autophagy employ the same machinery to deliver proteins to the yeast vacuole. Proc Natl Acad Sci U S A 93:12304–12308

Serrano M, Kombrink E, Meesters C (2015) Considerations for designing chemical screening strategies in plant biology. Front Plant Sci 6:131

Shimada T, Fuji K, Tamura K, Kondo M, Nishimura M, Hara-Nishimura I (2003) Vacuolar sorting receptor for seed storage proteins in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 100:16095–16100

Shinshi H, Wenzler H, Neuhaus JM, Felix G, Hofsteenge J, Meins F (1988) Evidence for N- and C-terminal processing of a plant defense-related enzyme: primary structure of tobacco prepro-beta-1,3-glucanase. Proc Natl Acad Sci U S A 85:5541–5545

Shirakawa M, Ueda H, Shimada T, Nishiyama C, Hara-Nishimura I (2009) Vacuolar SNAREs function in the formation of the leaf vascuolar network by regulating auxin distribution. Plant Cell Physiol 50:1319–1328

Shirakawa M, Ueda H, Shimada T, Koumoto Y, Shimada TL, Kondo M, Takahashi T, Okuyama Y, Nishimura M, Hara-Nishimura I (2010) Arabidopsis Qa-SNARE SYP2 proteins localized to different subcellular regions function redundantly in vacuolar protein sorting and plant development. Plant J 64:924–935

Simões I, Faro C (2004) Structure and function of plant aspartic proteinases. Eur J Biochem 271:2067–2075

Stenmark H, Olkkonen VM (2001) The Rab GTPase family. Genome Biol 2, Reviews 3007. doi:10.1186/gb-2001-2-5-reviews3007

Suen PK, Shen J, Sun SSM, Jiang L (2010) Expression and characterization of two functional vacuolar sorting receptor (VSR) proteins, BP-80 and AtVSR4 from culture media of transgenic tobacco BY-2 cells. Plant Sci 179:68–76

Sutter JU, Campanoni P, Blatt MR, Paneque M (2006) Setting SNAREs in a different wood. Traffic 7:627–638

Szumlanski AL, Nielsen E (2009) The Rab GTPase RabA4d regulates pollen tube tip growth in Arabidopsis thaliana. Plant Cell 21:526–544

Tang K, Shen Q, Yan T, Fu X (2014) Transgenic approach to increase artemisinin content in *Artemisia annua* L. Plant Cell Rep 33:605–615

Ueda T, Matsuda N, Anai T, Tsukaya H, Uchimiya H, Nakano A (1996) An Arabidopsis gene isolated by a novel method for detecting genetic interaction in yeast encodes the GDP dissociation inhibitor of Ara4 GTPase. Plant Cell 8:2079–2091

Ueda T, Uemura T, Sato MH, Nakano A (2004) Functional differentiation of endosomes in Arabidopsis cells. Plant J 40:783–789

Ueda H, Nishiyama C, Shimada T, Koumoto Y, Hayashi Y, Kondo M, Takahashi T, Ohtomo I, Nishimura M, Hara-Nishimura I (2006) AtVAM3 is required for normal specification of idioblasts, myrosin cells. Plant Cell Physiol 47:164–175

Uemura T, Ueda T, Ohniwa RL, Nakano A, Takeyasu K, Sato MH (2004) Systematic analysis of SNARE molecules in *Arabidopsis*: dissection of the post-Golgi network in plant cells. Cell Struct Funct 29:49–65

Uemura T, Sato MH, Takeyasu K (2005) The longin domain regulates subcellular targeting of VAMP7 in Arabidopsis thaliana. FEBS Lett 579:2842–2846

Uemura T, Morita MT, Ebine K, Okatani Y, Yano D, Saito C, Ueda T, Nakano A (2010) Vacuolar/pre-vacuolar compartment Qa-SNAREs VAM3/SYP22 and PEP12/SYP21 have interchangeable functions in Arabidopsis. Plant J 64:864–873

Vernoud V, Horton AC, Yang Z, Nielsen E (2003) Analysis of the small GTPase gene superfamily of Arabidopsis. Plant Physiol 131:1191–1208

Vieira M, Pissarra J, Veríssimo P, Castanheira P, Costa Y, Pires E, Faro C (2001) Molecular cloning and characterization of cDNA encoding cardosin B, na aspartic proteinase accumulating extracellularly in the transmitting tissue of *Cynara cardunculus* L. Plant Mol Biol 45:529–539

Viotti C, Bubeck J, Stierhof YD, Krebs M, Langhans M, van den Berg W, van Dongen W, Richter S, Geldener N, Takano J et al (2010) Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. Plant Cell 22:1344–1357

Vitale A, Raikhel NV (1999) What do proteins need to reach different vacuoles? Trends Plant Sci 4:149–155

Voelker TA, Herman EM, Chrispeels MJ (1989) In vitro mutated phytohemagglutinin genes expressed in tobacco seeds: role of glycans in protein targeting and stability. Plant Cell 1:95–104

Wang H, Rogers JC, Jiang L (2011) Plant RMR proteins: unique vacuolar sorting receptors that couple ligand sorting with membrane internalization. FEBS J 278:59–68

Wasternack C, Kombrink E (2010) Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem Biol 5:63–77

Watanabe E, Shimada T, Kuroyanagi M, Nishimura M, Hara-Nishimura I (2002) Calcium-mediated association of a putative vacuolar sorting receptor PV72 with a propeptide of 2S albumin. J Biol Chem 277:8708–8715

Watanabe E, Shimada T, Tamura K, Matsushima R, Koumoto Y, Nishimura M, Hara-Nishimura I (2004) An ER-localized form of PV72, a seed-specific vacuolar sorting receptor, interferes the transport of an NPIR-containing proteinase in Arabidopsis leaves. Plant Cell Physiol 45:9–17

Xiang L, Etxeberria E, van den Ende W (2013) Vacuolar protein sorting mechanisms in plants. FEBS J 280:979–993

Zerial M, McBride H (2001) Rab proteins as membrane organisers. Nat Rev Mol Cell Biol 2:107–117

Zhang C, Hicks GR, Raikhel NV (2014) Plant vacuole morphology and vacuolar trafficking. Front Plant Sci 5:476–485

Zouhar J, Rojo E (2009) Plant vacuoles: where did they come from and where are they heading? Curr Opin Plant Biol 12:677–684

Zouhar J, Hicks GR, Raikhel NV (2004) Sorting inhibitors (Sortins): chemical compounds to study vacuolar sorting in Arabidopsis. Proc Natl Acad Sci U S A 101:9497–9501

Reactive Nitrogen Species (RNS) in Plants Under Physiological and Adverse Environmental Conditions: Current View

Francisco J. Corpas

Contents

Abstract Nitric oxide ($^{\cdot}$NO) and derived molecules, referred to as reactive nitrogen species (RNS), have become a new area of plant research. These molecules are involved in almost all physiological plant processes, ranging from seed germination, development, senescence, stomatal movement, fruit ripening, and reproduction to mechanisms of response to adverse environmental conditions possibly associated with nitro-oxidative stress. $^{\cdot}$NO can perform a dual function depending on its rate of production; at low concentrations, it acts as a signal molecule and, at high concentrations, like a stress molecule. Although in some cases the simultaneous high $^{\cdot}$NO production with other reactive oxygen species (ROS) can be useful to the cells as mechanism of defense, for example, against pathogens. All these processes are usually mediated by the chemical interactions of $^{\cdot}$NO whose functions

F.J. Corpas
Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Apartado 419, 18080 Granada, Spain
e-mail: javier.corpas@eez.csic.es

Progress in Botany (2017) 78: 97–120, DOI 10.1007/124_2016_3,
Published online: 3 June 2016

are affected by other molecules. It is worth pointing out that the post-translational modifications of target proteins caused by nitration and *S*-nitrosylation have been best described in plants. However, ·NO can also regulate gene expression through direct interaction with DNA or through interaction with transcription factors. This review provides a comprehensive overview of the role played by RNS in the physiology of plants and their involvement in the mechanism of response to a diverse range of adverse environmental conditions.

1 Introduction

Endogenously generated nitric oxide (·NO) is currently one of the most studied bioactive gas molecules in plant cells. This is due to its involvement in a wide spectrum of plant physiological processes including seed germination, primary and lateral root growth, flowering, pollen tube growth regulation, stomatal movement, fruit ripening, and senescence; nevertheless, new functions of ·NO are gradually being added to the list. This key signaling molecule in different intracellular processes also participates in the mechanism of response to biotic and abiotic stresses (Neill et al. 2003; Lamotte et al. 2005; Besson-Bard et al. 2008; Corpas et al. 2011; Domingos et al. 2015; Corpas and Barroso 2015a). However, nitric oxide's versatility depends on its chemical properties, enabling it to interact with many molecules that affect its biochemical interactions and consequently its functions in plant cells (Pfeiffer et al. 1999).

2 Nitric Oxide and Reactive Nitrogen Species

The gasotransmitter nitric oxide, also referred to as nitrogen monoxide, is a free radical whose π orbital contains an unpaired electron represented by a dot on the N atom (·NO); however, for simplification purposes, this dot is omitted in many publications. Nitric oxide has a family of NO-derived molecules generally referred to as reactive nitrogen species (RNS). Table 1 shows the most representative RNS including radical and non-radical molecules. Thus, ·NO can react with many inorganic and organic molecules such as peptides, proteins, lipids, and nucleotides; this reactivity explains its numerous biochemical interactions. Figure 1 shows a simple model of ·NO metabolism in plant cells. ·NO in both its gas phase and aqueous solution form can react with O_2 to form dinitrogen trioxide (N_2O_3) and nitrogen dioxide (·NO_2). In aqueous solution, N_2O_3 and ·NO_2 produce stoichiometric amounts of nitrite (NO_2^-) and nitrate (NO_3^-). Nitrogen dioxide is 1.5 times more soluble in a lipid membrane than in water (Signorelli et al. 2011). ·NO is also

Table 1 Main reactive nitrogen species (RNS) including inorganic and organic molecules

Non-radicals	Radicals
Inorganic molecules	
Nitroxyl anion (NO^-)	Nitric oxide ($^{\cdot}NO$)
Nitrosonium cation (NO^+)	Nitrogen dioxide ($^{\cdot}NO_2$)
Nitrous acid (HNO_2)	
Dinitrogen trioxide (N_2O_3)	
Dinitrogen tetroxide (N_2O_4)	
Peroxynitrite ($ONOO^-$)	
Peroxynitrous acid (ONOOH)	
Organic molecules	
Nitrotyrosine (Tyr-NO_2)	Lipid peroxyl radicals ($LOO^{\cdot}$)
Nitrosoglutathione (GSNO)	
Nitrosothiols (SNOs)	
Nitro-γ-tocopherol	
Nitro-fatty acids (FA-NO_2)	

able to react with the superoxide radical ($O_2^{\cdot -}$) to yield peroxynitrite ($ONOO^-$), with the rate constant (~ 10^{10} M^{-1} s^{-1}) for this reaction being considerably very high (Estévez and Jordán 2002), virtually ensuring that $ONOO^-$ will be formed in any plant cell or tissue where both radicals are present simultaneously. This chemical reaction is very fast given a rate constant of 2×10^9 M^{-1} s^{-1} for $O_2^{\cdot -}$ dismutation by the CuZn superoxide dismutase (SOD) enzyme (Gray and Carmichael 1992). The production site of $ONOO^-$ must therefore be associated with the sources of $O_2^{\cdot -}$ and $^{\cdot}NO$ including the main plant cell organelles, such as chloroplasts, mitochondria, and peroxisomes (Blokhina and Fagerstedt 2010; Corpas and Barroso 2014). Peroxynitrite, a powerful oxidant, plays a highly important role as it can mediate nitration processes and cause cellular injury (Szabó et al. 2007; Corpas et al. 2009a; Arasimowicz-Jelonek and Floryszak-Wieczorek 2011; Calcerrada et al. 2011; Berton et al. 2012; Szuba et al. 2015).

Nevertheless, one of the most controversial issues with respect to higher plants is the way in which $^{\cdot}NO$ is endogenously generated in the cell. At least two main enzymatic pathways for generating endogenous $^{\cdot}NO$, using the amino acid L-arginine and/or the nitrite-dependent pathway as well as non-enzymatic $^{\cdot}NO$ generation, have been described (Wojtaszek 2000). The involvement of at least one of these pathways in a specific process is supported by experimental data although it is important to note that, depending on the plant species, developmental stage, and/or environment conditions involved, the participation of both pathways cannot be ruled out. In the case of L-arginine-dependent nitric oxide synthase (NOS) activity, there is a strong biochemical evidence of the presence of this activity in plants, which requires the presence of all the co-factors of animal NOS using NADPH as an electron donor (see Corpas et al. 2009b); new data also confirm the existence of an NOS-like protein in the green alga *Ostreococcus tauri* (Foresi et al. 2010). Additionally, L-arginine is a precursor of the biosynthesis of polyamines which,

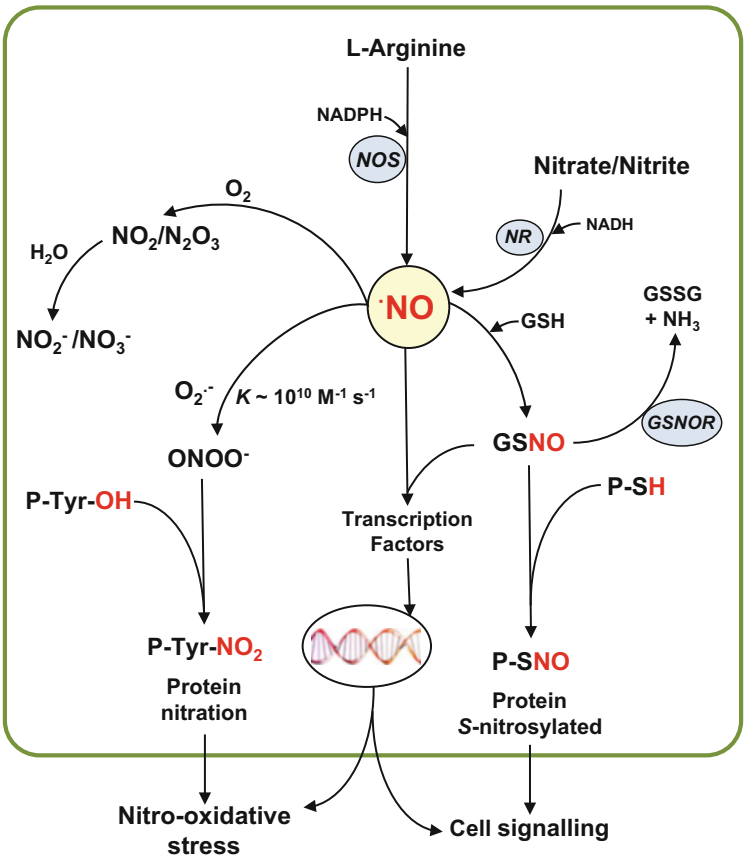

Fig. 1 Graphic model of nitric oxide (NO) metabolism in plant cells. L-Arginine-dependent nitric oxide synthase (NOS) and nitrate reductase (NR) generate NO which can react with reduced glutathione (GSH) in the presence of O_2 to form *S*-nitrosoglutathione (GSNO) through a process of *S*-nitrosylation. This metabolite can be converted by the enzyme GSNO reductase (GSNOR) into oxidized glutathione (GSSG) and NH_3. GSNO and other *S*-nitrosothiols can interact with specific sulfhydryl (-SH) groups to produce *S*-nitrosylated proteins in a process called *S*-transnitrosation which can mediate signaling processes. Nitric oxide in the presence of oxygen is converted into dinitrogen trioxide (N_2O_3) and nitrogen dioxide (NO_2) which, in aqueous solutions, are transformed into nitrite and nitrate. Nitric oxide can also react very rapidly ($K \sim 10^{10}$ M^{-1} s^{-1}) with superoxide radicals (O_2^-) to generate peroxynitrite ($ONOO^-$), a powerful oxidant molecule that can mediate the tyrosine nitration of proteins. Nitric oxide and related molecules could be part of cell signaling or nitro-oxidative stress processes

according to some experimental data, could be involved in ·NO biosynthesis (Tun et al. 2006; Wimalasekera et al. 2011a, b). The production of ·NO by the nitrite-dependent pathway depends on nitrate reductase (NR) activity which uses NADH as an electron donor instead of NADPH. This classic enzyme is involved in the nitrogen metabolism and has been widely accepted as an ·NO source candidate (Yamasaki et al. 1999; Rockel et al. 2002; Lozano-Juste and Leon 2010).

As mentioned previously, ·NO directly or indirectly performs its biological actions through different RNS by interacting with many other molecules. In the case of proteins, the most studied post-translational modifications (PTMs) in plant cells are nitration and *S*-nitrosylation (Fig. 1). On the other hand, regulation of gene expression by ·NO has also been reported to produce specific transcriptional responses (Begara-Morales et al. 2014a), indicating that ·NO is perceived differentially. This could occur through the impact of ·NO on transcription factors or through direct interaction with the DNA (Serpa et al. 2007; Tavares et al. 2014).

3 Post-translational Modifications Mediated by RNS

3.1 Nitration

Nitration is a chemical reaction that enables a nitro group (-NO_2) to be added to molecules including proteins, lipids, and nucleotide acids (Rubbo and Radi 2008). In proteins, some amino acids, such as tyrosine (Tyr), cysteine (Cys), methionine (Met), and tryptophan (Trp), are preferentially nitrated. However, in plants, most studies focus on tyrosine nitration (Tyr-NO_2), which involves adding a nitro group to one of the two equivalent ortho-carbons of the aromatic ring of tyrosine residues. This transforms Tyr into a negatively charged hydrophilic Tyr-NO_2 moiety and causes a marked shift in the hydroxyl group's local p*K*a from 10.1 in tyrosine to 7.5 in nitrotyrosine. This process depends on different intrinsic and extrinsic features such as protein structure, nitration mechanism, and the environmental compartments where the targeted protein is located (Corpas et al. 2013). These covalent changes may result in several potential effects such as protein function loss, gain, or no functional change, with loss of function being the most common. It is important to remember that there is a physiological nitration in plant cells; however, under internal or external adverse conditions, an increase in protein nitration or free nitrotyrosine could be regarded as a reliable marker of nitrosative stress (Corpas et al. 2007; Berton et al. 2012). Many new potential target proteins, which undergo this PTM in plant cells under physiological or stress conditions, have been identified (Corpas et al. 2015). However, this process is closely associated with the ROS metabolism and consequently with oxidative stress, therefore it should be called nitro-oxidative stress (Corpas and Barroso 2013). A good example of this connection is the way in which important antioxidant enzymes, such as catalase (Clark et al. 2000; Chaki et al. 2015a), ascorbate peroxidase (Begara-Morales et al. 2014b), monodehydroascorbate reductase (Begara-Morales et al. 2015), and SOD (Holzmeister et al. 2015), are negatively regulated by nitration (see Table 2).

A new promising area of plant research is the identification and characterization of nitro-fatty acids (Sánchez-Calvo et al. 2013). These molecules together with their signaling component have been under intense study in animal cells; they can

Table 2 Examples of identified plant enzymes involved in ROS metabolism which are post-translationally affected by either nitration and/or *S*-nitrosylation and their effects

Enzymes involved in ROS metabolism	Plant species	*S*-Nitrosylation	Nitration	Reference
Catalase	*Nicotiana tabacum, Capsicum annuum*	Inhibition	Inhibition	Clark et al. (2000), Ortega-Galisteo et al. (2012) and Chaki et al. (2015a)
Superoxide dismutase isozymes (MnSOD1, CuZn SOD3, FeSOD3)	*Arabidopsis thaliana*	No effect	Inhibition	Holzmeister et al. (2015)
Peroxiredoxin II E and F	*Arabidopsis thaliana*, *Pisum sativum*	Inhibition	NT	Romero-Puertas et al. (2007) and Camejo et al. (2015)
Enzymes of ascorbate–glutathione cycle				
Cytosolic ascorbate peroxidase (APX)	*Pisum sativum*	Increased activity	Decreased activity	Clark et al. (2000), Fares et al. (2014), Begara-Morales et al. (2014b), and Yang et al. (2015)
Dehydroascorbate reductase (DHAR)	*Arabidopsis thaliana*, *Solanum tuberosum*	NT	NT	Fares et al. (2014) and Kato et al. (2013)
Monodehydroascorbate reductase (MDAR)	*Pisum sativum*	Inhibition	Inhibition	Begara-Morales et al. (2015)
Glutathione reductase	*Pisum sativum*	No effect	No effect	Begara-Morales et al. (2015)
Superoxide-generating system				
NADPH oxidase also called respiratory burst oxidase homologue (RBOH)	*Arabidopsis thaliana*	Inhibition	NT	Yun et al. (2011)

NT not tested

trigger signaling cascades via covalent and reversible PTMs of susceptible nucleophilic amino acids in target proteins which have important physiological functions such as an anti-inflammatory mechanism (Trostchansky et al. 2013). However, there are very few experimental data on this area of research in plants (Fazzari et al. 2014).

3.2 S-*Nitrosylation*

S-Nitrosylation, more appropriately called *S*-nitrosation, consists of the covalent attachment of an $^{\cdot}NO$ group to the thiol (-SH) side chain of cysteine (Cys) present in

peptides or proteins in order to produce a family of NO-derived molecules called *S*-nitrosothiols (SNOs). This covalent modification is highly labile under physiological conditions as their stability depends on the presence of trace metal ions (such as copper and iron) or reducing agents (such as thiols and ascorbate) which enhance their degradation (Askew et al. 1995; Vanin et al. 1997), thus making it difficult to study SNOs in cells. Furthermore, this process, which is selective and reversible, can alter protein conformation and/or protein properties. Different biochemical approaches such as biotin switch assays, resin-assisted capture (SNO-RAC), fluorescence switch, or protein microarray-based analysis (Jaffrey 2005; Han et al. 2008; Foster et al. 2009; Wang and Xian 2011; Fares et al. 2014) combined with mass spectrometry techniques have facilitated the study of SNO modulation and identification of a significant number of potential protein targets in different plant species under physiological and stress conditions (Lindermayr et al. 2005; Vanzo et al. 2014; Chaki et al. 2015b).

4 Nitric Oxide in Plant Development

Plant development involves many phases including seed germination, plant growth and differentiation of cells, tissues, and organs, flower formation, fruit ripening, and senescence. In all these processes, the direct involvement of ·NO or indirectly through its interaction with different phytohormones (auxins, ethylene, and abscisic acids) and molecules such as ROS (superoxide radical, H_2O_2) has been described to some degree (Zhao 2007; Freschi 2013; Airaki et al. 2015; Corpas and Barroso 2015b; Sanz et al. 2015).

Many metabolic pathways are involved in seed germination and seedling establishment which begin with water imbibition, a catabolic and anabolic process using lipid mobilization to support the development of the new seedling during the transition from dark to light conditions until the seedling can initiate photosynthesis. ·NO acts as a stimulator of germination and photo-morphogenesis (Beligni and Lamattina 2000; Kopyra and Gwózdz 2003; Simontacchi et al. 2004; Gniazdowska et al. 2010). Moreover, an increasing amount of data shows that ·NO interacts with other molecules including calcium, H_2O_2, auxin, and gibberellins in the regulation of primary and lateral root growth, which eventually determines root architecture (Pagnussat et al. 2002; Correa-Aragunde et al. 2004; Guo et al. 2008; Fernández-Marcos et al. 2011; Duan et al. 2014; Sanz et al. 2015). During seedling development in different plant species such as pea, Arabidopsis, and pepper, cellular analyses employing specific NO-sensitive fluorophores and confocal laser scanning microscopy (CLSM) reveal that the steady-state levels of ·NO content change depending on cell type in the main organs including roots, stems, and leaves (Corpas et al. 2006, 2009a; Airaki et al. 2015). Furthermore, in some cases, a temporal correlation between organ development and ·NO production from L-arginine-dependent NOS activity has been described (Corpas et al. 2004, 2006). In addition, in the presence of other NO-derived molecules such as *S*-nitrosoglutathione and peroxynitrite, protein

nitration has also been reported to be modulated during organ development (Airaki et al. 2015; Begara-Morales et al. 2013), indicating that these organs have a highly active $^{\cdot}NO$ metabolism.

The enzyme nitrosoglutathione reductase (GSNOR) is a conserved protein in prokaryotes and eukaryotes which catalyzes the NADH-dependent reduction of GSNO to GSSG and NH_3 regulating the level of GSNO and consequently cellular SNO homeostasis (Sakamoto et al. 2002; Leterrier et al. 2011). This activity is necessary for normal development under optimal growth conditions (Lee et al. 2008; Kubienová et al. 2013; Xu et al. 2013). Analysis of GSNOR in *Arabidopsis thaliana* indicates that roots and leaves from the initial stages of development have higher activity levels (Espunya et al. 2006). The importance of GSNOR in plant development has also been demonstrated by genetic approaches, as the over-expressing and knock-down GSNOR gene in Arabidopsis plants show an atypical phenotype with a short-root system, which correlates with a lowering of intracellular GSH levels and an alteration in its spatial distribution in the roots (Lee et al. 2008; Chen et al. 2009; Leterrier et al. 2011; Kwon et al. 2012). Moreover, new experimental data have begun to elucidate the molecular mechanism involved in the relationship between $^{\cdot}NO$ and auxin, a phytohormone that regulates growth and development processes such as lateral root formation, cell division, and elongation, as $^{\cdot}NO$ has the capacity to inhibit auxin transport through a mechanism of *S*-nitrosylation (Fernández-Marcos et al. 2011; Terrile et al. 2012; Shi et al. 2015).

$^{\cdot}NO$ is also involved in the final development processes of senescence and fruit ripening. In senescent pea plants, $^{\cdot}NO$ content is down-regulated in leaves, which closely correlates with the lower L-arginine-dependent NOS activity detected as compared to that determined in the leaves of young plants (Corpas et al. 2004). However, a similar analysis in the root systems of both young and senescent plants reveals a different behavior, characterized by increased $^{\cdot}NO$ and $ONOO^{-}$ content and protein nitration (Begara-Morales et al. 2013), suggesting some kind of specificity in the function of $^{\cdot}NO$ and related molecules depending on the analyzed organ and stage of development, as was mentioned previously.

Nitric oxide is also involved in reproductive organs and has the capacity to repress floral transition in *Arabidopsis thaliana* by inhibiting *CONSTANS* and *GIGANTEA* gene expression (He et al. 2004) which promotes flowering and regulates photoperiodic flowering, respectively. The rate and orientation of pollen tube growth is regulated by the level of $^{\cdot}NO$ at the pollen tube tip which appears to be mediated by cGMP (Prado et al. 2004). On the other hand, $^{\cdot}NO$ is involved in pollen–pistil interactions, in which $^{\cdot}NO$ appears to influence the targeting of pollen tubes to the ovule's micropyle by modulating the action of its diffusible factors (McInnis et al. 2006; Prado et al. 2008). Analysis carried out during olive flower development shows that $^{\cdot}NO$ content is also modulated depending on the developmental stage and tissue, with an increase in $^{\cdot}NO$ production in pollen grains and tubes observed during the receptive phase in the stigma (Zafra et al. 2010).

Fruit ripening is another complex process that is regulated by ethylene production which is characterized by increased ROS production. $^{\cdot}NO$ has also been shown to interact with ethylene and the ROS metabolism (Leshem and Pinchasov 2000;

Manjunatha et al. 2010). During pepper fruit ripening, apart from the more apparent change in color from green to red, there are also significant biochemical adjustments. Thus, analysis of ·NO metabolism using proteomic approaches shows that, during ripening, the nitration of proteins such as catalase, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, a transketolase 1, a 20S proteasome alpha 6 subunit, or ferredoxin-dependent glutamate synthase 1 involved in redox, oxidative, protein, and carbohydrate metabolisms changes, with the antioxidant catalase being among the most affected. However, it has been shown that the application of exogenous ·NO gas can prevent protein nitration and delay fruit ripening (Chaki et al. 2015a).

5 Nitro-Oxidative Stress Under Abiotic Conditions

Adverse environmental conditions including drought, salinity, soil mineral toxicity, cold, and heat can limit agricultural production considerably. It has been well established that all these abiotic stresses may lead to oxidative stress characterized by uncontrollable overproduction of ROS that generates molecular damage in lipids, proteins, and nucleic acid. With the discovery of ·NO and NO-derived molecules, many researchers set out to show that these adverse conditions are also associated with nitrosative stress (Valderrama et al. 2007; Corpas et al. 2008, 2011). Thus, it has been proposed that an increase in protein nitration could be a reliable biomarker of a specific stress similar to protein oxidation which is a marker of oxidative stress (Corpas et al. 2007; Arasimowicz-Jelonek and Floryszak-Wieczorek 2011). However, given the metabolic interplay between these two families of molecules (ROS and RNS) in which many enzymes involved in ROS metabolism are targets of PTMs mediated by ·NO (see Table 2), nitro-oxidative stress would be a more appropriate term (Corpas et al. 2013).

5.1 Heavy Metals

Contamination by heavy metals such as cadmium, arsenic, lead, or mercury is an increasingly serious problem for the environment and consequently for agriculture and human health (World Health Organization 2007; Hernández et al. 2015). Although it has been well established that heavy metals usually trigger an oxidative stress response, recent analysis of the plant ·NO metabolism has demonstrated that they also induce nitro-oxidative stress.

Arsenic (As) is a metalloid naturally present in the environment, with arsenate (AsV) being the main arsenic species in aerobic soils and arsenite (AsIII) in soils under anaerobic and reducing conditions. In higher plants, inorganic arsenic can be accumulated in the form of arsenite (AsIII) throughout nodulin 26-like intrinsic (NIP) aquaporin channels or in the form of arsenate (AsV) throughout the

phosphate transporter system (Zhao et al. 2010). Various studies have demonstrated that arsenic triggers nitro-oxidative stress. For example, *Arabidopsis thaliana* seedlings exposed to 500 μM AsV trigger a significant increase in ·NO content, GSNOR activity, and protein tyrosine nitration as well as a concomitant decrease in glutathione and GSNO content (Leterrier et al. 2012). Curiously, the exogenous application of ·NO can alleviate arsenic-induced oxidative stress in different plant species by enhancing antioxidant defenses (Singh et al. 2009, 2013; Hasanuzzaman and Fujita 2013), thus corroborating the aforementioned cross talk between the families of ROS and RNS molecules.

Cadmium toxicity in plants is well established (Chmielowska-Bąk et al. 2014) and generally causes an augmentation of ROS production and consequently oxidative stress (Dixit et al. 2001). In addition, the ·NO metabolism is differentially affected under cadmium stress. In pea, 50 μM $CdCl_2$ provoked a lower GSNOR activity with a decrease in ·NO and GSNO content but accompanied with a rise of ROS production and an increase in salicylic acid, jasmonic acid, and ethylene (Barroso et al. 2006; Rodríguez-Serrano et al. 2006), indicating the presence of nitro-oxidative stress where ·NO plays an essential role as a signaling molecule (Arasimowicz-Jelonek et al. 2011) and participates in homeostasis in order to maintain the metabolic equilibrium in the presence of cadmium (Gill et al. 2013; Liu et al. 2015). In *Arabidopsis thaliana*, cadmium induces ·NO generation from NOS activity, which contributes to the inhibition of root growth partly caused by iron deprivation (Besson-Bard et al. 2009; Han et al. 2014). At the subcellular level, Cd stress in Arabidopsis has recently been shown to trigger the production of both $O_2^{\cdot-}$ and ·NO in peroxisomes with a concomitant generation of $ONOO^-$, thus corroborating reports that these organelles participate in the mechanism of response to this metal (Corpas and Barroso 2014).

5.2 Salinity

Over 6 % of the world's land mass has been estimated to be affected by either salinity or sodicity which negatively affect plant productivity by inhibiting plant growth, ion balance, and water relations (Hasegawa et al. 2000). As mentioned previously, salinity is commonly accompanied by oxidative stress, with an increasing number of studies pointing to the involvement of RNS in these processes (Valderrama et al. 2007; Molassiotis et al. 2010). Although its effects may vary somewhat depending on the plant species and the severity of the salinity treatment, salinity generally triggers the ·NO metabolism, which has been observed to cause an increase in ·NO production and the number of *S*-nitrosylated and nitrated proteins as well as a modulation in redox homeostasis and the antioxidant system (Valderrama et al. 2007; Tanou et al. 2009b, 2012; Manai et al. 2014a). Some studies have also demonstrated that ·NO appears to modulate and enhance the expression of Na^+/H^+ antiporter genes under high salinity conditions which contributes to mitigating the negative effects of sodium (Zhang et al. 2006; Lu et al. 2013; Chen et al. 2013).

Proteomic approaches have also identified a significant number of proteins affected by *S*-nitrosylation and nitration (Tanou et al. 2009a). Data on the ·NO metabolism under salinity stress by the application of exogenous ·NO appear to provide a certain level of resistance to salinity stress. Thus, in 5-month-old bitter orange (*Citrus aurantium*) trees, pretreatment of the root system with 100 μM sodium nitroprusside (SNP; a ·NO donor) induces considerable antioxidant resistance in the form of catalase, SOD, ascorbate peroxidase, and glutathione reductase (Tanou et al. 2009a, b). Similarly, tomato (*Solanum lycopersicum*) plants exposed simultaneously to 120 mM NaCl and a ·NO donor (100 μM or 300 μM SNP) through their root system show a decrease in NaCl-induced lipid oxidation in leaves, which was accompanied by an increase in the antioxidant system's SOD, APX, GR, and POD activities in roots and leaves and also increased ascorbate and proline content. It is also worth noting that a newly CuZnSOD is induced in roots, suggesting that the ·NO performs a regulatory function at the protein and gene levels of this antioxidant enzyme (Manai et al. 2014b). Tomato plants have also shown an increase in the activity of some enzymes such as nitrate reductase (NR) and nitrite reductase (NiR) involved in the nitrogen metabolism. On the other hand, genetic approaches using overexpression of rat neuronal NOS (nNOS) in rice show increases in both NOS activity and ·NO accumulation, resulting in improved tolerance of transgenic rice to salt and drought stresses (Cai et al. 2015).

5.3 *Drought or Water Stress*

Drought affects plants at many levels, ranging from the morphological to the molecular one. The visual symptoms of drought stress include the reduction of shoots (leaves and stem) and root proliferation which disturbs plant–water relations and reduces water-use efficiency. At the biochemical level, drought stress disturbs the balance between ROS production and antioxidant defenses, thus causing oxidative stress. As with other types of stress, ·NO plays a prominent role in response mechanisms (Santisree et al. 2015). For example, in the legume *Lotus japonicus* exposed to water stress, the spatial distribution of nitro-oxidative stress has been described; oxidative stress levels increased in leaves while nitrosative stress levels were higher in roots in which ·NO content increased whereas GSNO reductase activity diminished, which may explain the rise in protein tyrosine nitration (Signorelli et al. 2013). On the other hand, in white clover (*Trifolium repens*), ·NO mediates drought tolerance through the activation of antioxidant enzymes such as SOD, APX, and catalase (Peng et al. 2015).

Abscisic acid (ABA), defined as a stress plant hormone due to its rapid accumulation in response to water stress, plays a major role in the regulation of plant growth, development, and tolerance under stress conditions. Under water deficiency conditions, plants use a stomatal closure strategy. The movement of guard cells is highly regulated, involving molecules such as ABA, H_2O_2, NO, and NADPH (Neill et al. 2008; Gayatri et al. 2013; Leterrier et al. 2016). ·NO is

known to induce stomatal closure, although the interplay between the different players is complex. A recent in vitro analysis has demonstrated that ABA receptors are targeted by tyrosine nitration which reduces receptor activity (Castillo et al. 2015). Therefore, under stress conditions, the production of both $^{\cdot}NO$ and superoxide together with a concomitant generation of $ONOO^{-}$ could explain how $^{\cdot}NO$ limits ABA signaling through nitration. On the other hand, $^{\cdot}NO$ negatively regulates ABA signaling in guard cells by inhibiting open stomata 1 (OST1)/sucrose nonfermenting 1 (SNF1)-related protein kinase 2.6 (SnRK2.6) through *S*-nitrosylation, specifically in the Cys137 (Wang et al. 2015).

5.4 Low and High Temperature

Extreme temperatures are environmental stresses that affect crop production and quality, involving the expression of many genes as well as significant numbers of proteins and metabolites. New data have shown that $^{\cdot}NO$ metabolism is affected at different levels depending on the plant species or temperature intensity among other factors (Corpas et al. 2008; Piterková et al. 2013; Puyaubert and Baudouin 2014; Sehrawat and Deswal 2014).

For example, pepper plants exposed to low temperature (LT) for several days undergo significant changes in the metabolism of RNS and ROS together with an increase in both protein tyrosine nitration (NO_2-Tyr) and lipid peroxidation, indicating that LT induces nitro-oxidative stress (Airaki et al. 2012). In *Medicago sativa* under cold stress, the interaction of $^{\cdot}NO$, H_2O_2, and ABA mediates the induction of the *S*-adenosylmethionine synthetase (SAMS), an enzyme that catalyzes the formation of *S*-adenosylmethionine (SAM), a precursor of polyamines and ethylene, which is involved in cold tolerance (Guo et al. 2014). The C-repeat binding factor (CBF) is one of the most studied cold stress-signaling pathways in plants. Recently, tomato seedlings under LT stress have been shown to undergo dual regulation of the CBF by $^{\cdot}NO$ at the transcriptional and translational level through *S*-nitrosylation (Kashyap et al. 2015). Furthermore, in poplar tree, the existence of a feedback mechanism between GSNOR activity and protein *S*-nitrosylation has been shown which is regulated in response to cold stress (Cheng et al. 2015).

On the other hand, sunflower seedlings exposed to high temperature (HT) experience oxidative stress; this impairs the $^{\cdot}NO$ metabolism by lowering $^{\cdot}NO$ content and reducing GSNOR activity and gene expression with a concomitant accumulation of total SNOs including GSNO as well as peroxynitrite formation and increased protein nitration; ferredoxin–NADP reductase is one of these inhibited nitrated proteins that affect photosynthesis (Chaki et al. 2011). The identification and characterization of the GSNOR knock-out mutant in Arabidopsis through the use of genetic techniques demonstrate the involvement of this important gene under physiological and stress conditions including HT. For example, analysis of the mutant HOT5 (sensitive to hot temperatures) shows that GSNOR modulates

the intracellular level of SNOs, resulting in thermotolerance as well as regulation of plant growth and development (Lee et al. 2008). Similarly, the mutant paraquat resistant 2 (PAR2) which encodes a GSNOR has a higher level of $^{\cdot}$NO, shows an anti-cell death phenotype (Chen et al. 2009; Xu et al. 2013).

6 Nitric Oxide in Plant–Pathogen Interactions

Systemic acquired resistance (SAR) is a cellular mechanism of disease resistance that is induced in response to initial infection and that protects uninfected areas of the plant against potential secondary infections by related or unrelated pathogens. In this process, the RNS and ROS families of molecules interact with each other to provide an adequate response to different pathogen organisms including bacteria, fungi, nematodes, and insects (Prats et al. 2005; Chaki et al. 2009; Wünsche et al. 2011; Scheler et al. 2013; Wendehenne et al. 2014; Zhou et al. 2015).

As a model plant, *Arabidopsis thaliana* has been extensively used to study the many ways in which $^{\cdot}$NO and derived molecules are involved in plant–pathogen interactions (Yu et al. 2012; Chen et al. 2014; Kovacs et al. 2015). This plant's available genomic database information combined with the use of proteomic techniques have facilitated the identification of the protein targets of *S*-nitrosylation (Romero-Puertas et al. 2008; Chaki et al. 2015b) and nitration (Cecconi et al. 2009) during pathogen infection. This information has enabled researchers to gain a deeper understanding of the $^{\cdot}$NO metabolism in this type of interaction. For example, it has been well established that plants use oxidative burst to respond to pathogen attacks, in this case, membrane-bound NADPH oxidase (NOX), also called the respiratory burst oxidase homologue (RBOH), a key element in the generation of $O_2^{-\cdot}$. Interestingly, *S*-nitrosylation of AtRBOH type D at Cys890 has been shown to abolish its ability to synthesize $O_2^{-\cdot}$ (Yun et al. 2011). However, there is complementary information on other plant species. For example, the infection of a susceptible cultivar of sunflower seedlings with the fungus *Plasmopara halstedii,* causing downy mildew, has been observed to increase protein nitration accompanied by an augmentation in SNOs; however, in the resistant sunflower cultivar, with its higher SNO content, a redistribution of these SNOs in the penetration site has been observed in the presence of the pathogen, which prevents the spread of infection (Chaki et al. 2009), thus confirming the importance of SNOs under this type of biotic stress. On the other hand, in tobacco cells exposed to the fungal elicitin cryptogein, analysis of *S*-nitrosylated proteins has identified 11 candidates for *S*-nitrosylation including the cell division cycle 48 (CDC48) protein, a member of the AAA^+ ATPase family that mediates resistance to certain pathogens. However, *S*-nitrosylation abolishes NtCDC48 ATPase activity (Astier et al. 2012). In tobacco cells exposed to cryptogein, $^{\cdot}$NO has also been observed to require NtRBOHD activity to induce cell death (Kulik et al. 2015).

7 Nitric Oxide in Plant Beneficial Interactions

Plants can also establish specific and beneficial interactions with other organisms, in which $^{\cdot}$NO and other elements have been found to act as signal molecules. In this context, the most studied interaction is the symbiosis between Legume–Rhizobium, characterized by the formation of differentiated organs called nodules, where $^{\cdot}$NO is required for the optimal establishment of the symbiotic interaction (Meilhoc et al. 2011; del Giudice et al. 2011; Hichri et al. 2015). Root nodules, enabling atmospheric N_2 to be fixed to ammonia, have two critical elements: leghemoglobin (Lb) and nitrogenase. Lb is an oxygen carrier whose function is to prevent the presence of O_2 which reduces the activity of oxygen-sensitive nitrogenase, the enzyme responsible for fixing atmospheric nitrogen to ammonia. Interestingly, nitrogenase activity is inhibited by $^{\cdot}$NO, indicating that $^{\cdot}$NO levels in rhizobia are a determining factor in efficient symbiotic processes (Cabrera et al. 2011). A rise in ROS and RNS has also been observed in senescent nodules which cause nitro-oxidative stress, leading to a reduction in the ability of symbiotic leghemoglobins to scavenge oxygen due to the modifications mediated by these ROS/RNS (Sainz et al. 2013). However, the formation of nitrated leghemoglobins during normal metabolism has been detected in functional nodules which, it has been suggested, may act as a sink for toxic peroxynitrite to protect nodule functionality (Sainz et al. 2015).

Another group of bacteria, *Azospirillum brasilense,* has the capacity to produce $^{\cdot}$NO from ammonia. When this bacterium interacts with tomato roots, the $^{\cdot}$NO produced stimulates lateral root formation (Creus et al. 2005; Molina-Favero et al. 2008). $^{\cdot}$NO is also generated in other interactions such as those between plant roots and arbuscular mycorrhizal fungi where $^{\cdot}$NO is produced in the roots of *Medicago truncatul*a when they come in contact with the exudates of the fungus *Gigaspora margarita* (Calcagno et al. 2012).

8 Conclusions and Future Perspectives

Since it was discovered that $^{\cdot}$NO could be endogenously produced in plant cells, the number of biochemical and physiological actions found to be mediated by this free radical has increased continually (Corpas and Barroso 2015a). It is now well established that $^{\cdot}$NO is responsible for a wide spectrum of actions which are based on a complex network of biochemical interactions with a diverse range of molecules, both small and large, such as metals, superoxide radicals, glutathione, proteins, lipids, and nucleic acid, that affect its structure and functions. In this context, although certain authors have begun to regard $^{\cdot}$NO as a "new phytohormone," some important issues still need to be addressed by the scientific community. For example, it is necessary to determine how and where $^{\cdot}$NO is produced in specific processes and also to identify the molecules which are targets under

physiological and stress conditions, with nitro-fatty acids being a good example of these. A major effort is therefore being made to determine the way in which $^{\cdot}$NO can be sensed in order to control the concentrations of RNS. On the other hand, research into the beneficial effects of applying exogenous $^{\cdot}$NO to treat plants against certain environmental stresses has been increasing. In fact, many studies have shown that exogenous $^{\cdot}$NO can ameliorate oxidative damage caused by stress by activating a diverse range of antioxidant systems and can even modulate physiological processes such as senescence and fruit ripening which are good examples of the biotechnological applications of $^{\cdot}$NO. Nevertheless, it is important to keep in mind than production levels of $^{\cdot}$NO into cells may determine its physiological function because when it is produced at low level, NO could have signaling functions but when it is overproduced it may have toxic effects.

Acknowledgements Work in my laboratory is supported by ERDF co-financed grants from the Ministry of Science and Innovation (Recupera 2020-20134R056) and the Junta de Andalucía (group BIO192) in Spain.

References

Airaki M, Leterrier M, Mateos RM, Valderrama R, Chaki M, Barroso JB, del Río LA, Palma JM, Corpas FJ (2012) Metabolism of reactive oxygen species and reactive nitrogen species in pepper (*Capsicum annuum* L.) plants under low temperature stress. Plant Cell Environ 35:281–295

Airaki M, Leterrier M, Valderrama R, Chaki M, Begara-Morales JC, Barroso JB, del Río LA, Palma JM, Corpas FJ (2015) Spatial and temporal regulation of the metabolism of reactive oxygen and nitrogen species during the early development of pepper (*Capsicum annuum*) seedlings. Ann Bot 116:679–693

Arasimowicz-Jelonek M, Floryszak-Wieczorek J (2011) Understanding the fate of peroxynitrite in plant cells--from physiology to pathophysiology. Phytochemistry 72:681–688

Arasimowicz-Jelonek M, Floryszak-Wieczorek J, Gwóźdź EA (2011) The message of nitric oxide in cadmium challenged plants. Plant Sci 181:612–620

Askew SC, Barnett DJ, McAninly J, Williams DLH (1995) Catalysis by Cu^{2+} of nitric oxide release from *S*-nitrosothiols (RSNO). J Chem Soc Perkin Trans 2:741–745

Astier J, Besson-Bard A, Lamotte O, Bertoldo J, Bourque S, Terenzi H, Wendehenne D (2012) Nitric oxide inhibits the ATPase activity of the chaperone-like AAA^{+} ATPase CDC48, a target for S-nitrosylation in cryptogein signalling in tobacco cells. Biochem J 447:249–260

Barroso JB, Corpas FJ, Carreras A, Rodríguez-Serrano M, Esteban FJ, Fernández-Ocaña A, Chaki M, Romero-Puertas MC, Valderrama R, Sandalio LM, del Río LA (2006) Localization of S-nitrosoglutathione and expression of S-nitrosoglutathione reductase in pea plants under cadmium stress. J Exp Bot 57:1785–1793

Begara-Morales JC, Chaki M, Sánchez-Calvo B, Mata-Pérez C, Leterrier M, Palma JM, Barroso JB, Corpas FJ (2013) Protein tyrosine nitration in pea roots during development and senescence. J Exp Bot 64:1121–1134

Begara-Morales JC, Sánchez-Calvo B, Luque F, Leyva-Pérez MO, Leterrier M, Corpas FJ, Barroso JB (2014a) Differential transcriptomic analysis by RNA-Seq of GSNO-responsive genes between Arabidopsis roots and leaves. Plant Cell Physiol 55:1080–1095

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Padilla MN, Carreras A, Corpas FJ, Barroso JB (2014b) Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and S-nitrosylation. J Exp Bot 65:527–538

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. J Exp Bot 66:5983–5996

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210:215–221

Berton P, Domínguez-Romero JC, Wuilloud RG, Sánchez-Calvo B, Chaki M, Carreras A, Valderrama R, Begara-Morales JC, Corpas FJ, Barroso JB, Gilbert-López B, García-Reyes JF, Molina-Díaz A (2012) Determination of nitrotyrosine in *Arabidopsis thaliana* cell cultures with a mixed-mode solid-phase extraction cleanup followed by liquid chromatography time-of-flight mass spectrometry. Anal Bioanal Chem 404:1495–1503

Besson-Bard A, Pugin A, Wendehenne D (2008) New insights into nitric oxide signaling in plants. Annu Rev Plant Biol 59:21–39

Besson-Bard A, Gravot A, Richaud P, Auroy P, Duc C, Gaymard F, Taconnat L, Renou JP, Pugin A, Wendehenne D (2009) Nitric oxide contributes to cadmium toxicity in Arabidopsis by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake. Plant Physiol 149:1302–1315

Blokhina O, Fagerstedt KV (2010) Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. Physiol Plant 138:447–462

Cabrera JJ, Sánchez C, Gates AJ, Bedmar EJ, Mesa S, Richardson DJ, Delgado MJ (2011) The nitric oxide response in plant-associated endosymbiotic bacteria. Biochem Soc Trans 39:1880–1885

Cai W, Liu W, Wang WS, Fu ZW, Han TT, Lu YT (2015) Overexpression of rat neurons nitric oxide synthase in rice enhances drought and salt tolerance. PLoS One 10(6), e0131599

Calcagno C, Novero M, Genre A, Bonfante P, Lanfranco L (2012) The exudate from an arbuscular mycorrhizal fungus induces nitric oxide accumulation in *Medicago truncatula* roots. Mycorrhiza 22:259–269

Calcerrada P, Peluffo G, Radi R (2011) Nitric oxide-derived oxidants with a focus on peroxynitrite: molecular targets, cellular responses and therapeutic implications. Curr Pharm Des 17:3905–3932

Camejo D, Jiménez A, Palma JM, Sevilla F (2015) Proteomic identification of mitochondrial carbonylated proteins in two maturation stages of pepper fruits. Proteomics 15:2634–2642

Castillo MC, Lozano-Juste J, González-Guzmán M, Rodriguez L, Rodriguez PL, León J (2015) Inactivation of PYR/PYL/RCAR ABA receptors by tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in plants. Sci Signal 8(392):ra89.

Cecconi D, Orzetti S, Vandelle E, Rinalducci S, Zolla L, Delledonne M (2009) Protein nitration during defense response in *Arabidopsis thaliana*. Electrophoresis 30:2460–2468

Chaki M, Fernández-Ocaña AM, Valderrama R, Carreras A, Esteban FJ, Luque F, Gómez-Rodríguez MV, Begara-Morales JC, Corpas FJ, Barroso JB (2009) Involvement of reactive nitrogen and oxygen species (RNS and ROS) in sunflower-mildew interaction. Plant Cell Physiol 50:265–279

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV, López-Jaramillo J, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011) High temperature triggers the metabolism of S-nitrosothiols in sunflower mediating a process of nitrosative stress which provokes the inhibition of ferredoxin-NADP reductase by tyrosine nitration. Plant Cell Environ 34:1803–1818

Chaki M, Álvarez de Morales P, Ruiz C, Begara-Morales JC, Barroso JB, Corpas FJ, Palma JM (2015a) Ripening of pepper (*Capsicum annuum*) fruit is characterized by an enhancement of protein tyrosine nitration. Ann Bot 116:637–647

Chaki M, Shekariesfahlan A, Ageeva A, Mengel A, von Toerne C, Durner J, Lindermayr C (2015b) Identification of nuclear target proteins for *S*-nitrosylation in pathogen-treated *Arabidopsis thaliana* cell cultures. Plant Sci 238:115–126

Chen R, Sun S, Wang C, Li Y, Liang Y, An F, Li C, Dong H, Yang X, Zhang J, Zuo J (2009) The Arabidopsis PARAQUAT RESISTANT2 gene encodes an *S*-nitrosoglutathione reductase that is a key regulator of cell death. Cell Res 19:1377–1387

Chen J, Xiong DY, Wang WH, Hu WJ, Simon M, Xiao Q, Chen J, Liu TW, Liu X, Zheng HL (2013) Nitric oxide mediates root K+/Na + balance in a mangrove plant, Kandelia obovata, by enhancing the expression of AKT1-type K^+ channel and Na^+/H^+ antiporter under high salinity. PLoS One 8(8), e71543

Chen J, Vandelle E, Bellin D, Delledonne M (2014) Detection and function of nitric oxide during the hypersensitive response in Arabidopsis thaliana: where there's a will there's a way. Nitric Oxide 43:81–88

Cheng T, Chen J, Allah EFA, Wang P, Wang G, Hu X, Shi J (2015) Quantitative proteomics analysis reveals that *S*-nitrosoglutathione reductase (GSNOR) and nitric oxide signaling enhance poplar defense against chilling stress. Planta 242:1361–1390

Chmielowska-Bąk J, Gzyl J, Rucińska-Sobkowiak R, Arasimowicz-Jelonek M, Deckert J (2014) The new insights into cadmium sensing. Front Plant Sci 5:245

Clark D, Durner J, Navarre DA, Klessig DF (2000) Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. Mol Plant Microbe Interact 13:1380–1384

Corpas FJ, Barroso JB (2013) Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. New Phytol 199:633–635

Corpas FJ, Barroso JB (2014) Peroxynitrite (ONOO-) is endogenously produced in Arabidopsis peroxisomes and is overproduced under cadmium stress. Ann Bot 113:87–96

Corpas FJ, Barroso JB (2015a) Nitric oxide from a green perspective. Nitric Oxide 45:15–19

Corpas FJ, Barroso JB (2015b) Functions of nitric oxide (NO) in roots during development and under adverse stress conditions. Plants 4:240–252

Corpas FJ, Barroso JB, Carreras A, Quirós M, León AM, Romero-Puertas MC, Esteban FJ, Valderrama R, Palma JM, Sandalio LM, Gómez M, del Río LA (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. Plant Physiol 136:2722–2733

Corpas FJ, Barroso JB, Carreras A, Valderrama R, Palma JM, León AM, Sandalio LM, del Río LA (2006) Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 224:246–254

Corpas FJ, del Río LA, Barroso JB (2007) Need of biomarkers of nitrosative stress in plants. Trends Plant Sci 12:436–438

Corpas FJ, Chaki M, Fernández-Ocaña A, Valderrama R, Palma JM, Carreras A, Begara-Morales JC, Airaki M, del Río LA, Barroso JB (2008) Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. Plant Cell Physiol 49:1711–1722

Corpas FJ, Hayashi M, Mano S, Nishimura M, Barroso JB (2009a) Peroxisomes are required for in vivo nitric oxide accumulation in the cytosol following salinity stress of Arabidopsis plants. Plant Physiol 151:2083–2894

Corpas FJ, Palma JM, del Río LA, Barroso JB (2009b) Evidence supporting the existence of L-arginine-dependent nitric oxide synthase activity in plants. New Phytol 184:9–14

Corpas FJ, Leterrier M, Valderrama R, Airaki M, Chaki M, Palma JM, Barroso JB (2011) Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Sci 181:604–611

Corpas FJ, Palma JM, del Río LA, Barroso JB (2013) Protein tyrosine nitration in higher plants grown under natural and stress conditions. Front Plant Sci 4:29

Corpas FJ, Begara-Morales JC, Sánchez-Calvo B, Chaki C, Barroso JB (2015) Nitration and *S*-nitrosylation: two post-translational modifications (PTMs) mediated by reactive nitrogen species (RNS) and their role in signalling processes of plant cells. In: KJ Gupta, AU Igamberdiev (eds) Reactive oxygen and nitrogen species signaling and communication in

plants, signaling and communication in plants, vol 23. Springer International Publishing, Switzerland, pp 267–281. ISBN: 978-3-319-10078-4
Correa-Aragunde N, Graziano M, Lamattina L (2004) Nitric oxide plays a central role in determining lateral root development in tomato. Planta 218:900–905
Creus CM, Graziano M, Casanovas EM, Pereyra MA, Simontacchi M, Puntarulo S, Barassi CA, Lamattina L (2005) Nitric oxide is involved in the Azospirillum brasilense-induced lateral root formation in tomato. Planta 221:297–303
del Giudice J, Cam Y, Damiani I, Fung-Chat F, Meilhoc E, Bruand C, Brouquisse R, Puppo A, Boscari A (2011) Nitric oxide is required for an optimal establishment of the Medicago truncatula-Sinorhizobium meliloti symbiosis. New Phytol 191:405–417
Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J Exp Bot 52:1101–1109
Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Mol Plant 8:506–520
Duan X, Li X, Ding F, Zhao J, Guo A, Zhang L, Yao J, Yang Y (2014) Interaction of nitric oxide and reactive oxygen species and associated regulation of root growth in wheat seedlings under zinc stress. Ecotoxicol Environ Saf 113C:95–102
Espunya MC, Diaz M, Moreno-Romero J, Martinez MC (2006) Modification of intracellular levels of glutathione-dependent formaldehyde dehydrogenase alters glutathione homeostasis and root development. Plant Cell Environ 29:1002–1011
Estévez AG, Jordán J (2002) Nitric oxide and superoxide, a deadly cocktail. Ann N Y Acad Sci 962:207–211
Fares A, Nespoulous C, Rossignol M, Peltier JB (2014) Simultaneous identification and quantification of nitrosylation sites by combination of biotin switch and ICAT labeling. Methods Mol Biol 1072:609–620
Fazzari M, Trostchansky A, Schopfer FJ, Salvatore SR, Sánchez-Calvo B, Vitturi D, Valderrama R, Barroso JB, Radi R, Freeman BA, Rubbo H (2014) Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. PLoS One 9(1), e84884
Fernández-Marcos M, Sanz L, Lewis DR, Muday GK, Lorenzo O (2011) Nitric oxide causes root apical meristem defects and growth inhibition while reducing PINFORMED 1 (PIN1)-dependent acropetal auxin transport. Proc Natl Acad Sci U S A 108:18506–18511
Foresi N, Correa-Aragunde N, Parisi G, Caló G, Salerno G, Lamattina L (2010) Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 22:3816–3830
Foster MW, Forrester MT, Stamler JS (2009) A protein microarray-based analysis of S-nitrosylation. Proc Natl Acad Sci U S A 106:18948–18953
Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:398
Gayatri G, Agurla S, Raghavendra AS (2013) Nitric oxide in guard cells as an important secondary messenger during stomatal closure. Front Plant Sci 4:425
Gill SS, Hasanuzzaman M, Nahar K, Macovei A, Tuteja N (2013) Importance of nitric oxide in cadmium stress tolerance in crop plants. Plant Physiol Biochem 63:254–261
Gniazdowska A, Krasuska U, Debska K, Andryka P, Bogatek R (2010) The beneficial effect of small toxic molecules on dormancy alleviation and germination of apple embryos is due to NO formation. Planta 232:999–1005
Gray B, Carmichael AJ (1992) Kinetics of superoxide scavenging by dismutase enzymes and manganese mimics determined by electron spin resonance. Biochem J 281:795–802
Guo K, Xia K, Yang Z-M (2008) Regulation of tomato lateral root development by carbon monoxide and involvement in auxin and nitric oxide. J Exp Bot 59:3443–3452
Guo Z, Tan J, Zhuo C, Wang C, Xiang B, Wang Z (2014) Abscisic acid, H_2O_2 and nitric oxide interactions mediated cold-induced *S*-adenosylmethionine synthetase in *Medicago sativa*

subsp. falcata that confers cold tolerance through up-regulating polyamine oxidation. Plant Biotechnol J 12:601–612
Han P, Zhou X, Huang B, Zhang X, Chen C (2008) On-gel fluorescent visualization and the site identification of S-nitrosylated proteins. Anal Biochem 377:150–155
Han B, Yang Z, Xie Y, Nie L, Cui J, Shen W (2014) Arabidopsis HY1 confers cadmium tolerance by decreasing nitric oxide production and improving iron homeostasis. Mol Plant 7:388–403
Hasanuzzaman M, Fujita M (2013) Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. Ecotoxicology 22:584–596
Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499
He Y, Tang RH, Hao Y, Stevens RD, Cook CW, Ahn SM, Jing L, Yang Z, Chen L, Guo F, Fiorani F, Jackson RB, Crawford NM, Pei ZM (2004) Nitric oxide represses the Arabidopsis floral transition. Science 305:1968–1971
Hernández LE, Sobrino-Plata J, Montero-Palmero MB, Carrasco-Gil S, Flores-Cáceres ML, Ortega-Villasante C, Escobar C (2015) Contribution of glutathione to the control of cellular redox homeostasis under toxic metal and metalloid stress. J Exp Bot 66:2901–2911
Hichri I, Boscari A, Castella C, Rovere M, Puppo A, Brouquisse R (2015) Nitric oxide: a multifaceted regulator of the nitrogen-fixing symbiosis. J Exp Bot 66:2877–2887
Holzmeister C, Gaupels F, Geerlof A, Sarioglu H, Sattler M, Durner J, Lindermayr C (2015) Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. J Exp Bot 66:989–999
Jaffrey SR (2005) Detection and characterization of protein nitrosothiols. Methods Enzymol 396:105–118
Kashyap P, Sehrawat A, Deswal R (2015) Nitric oxide modulates *Lycopersicon esculentum* C-repeat binding factor 1 (LeCBF1) transcriptionally as well as post-translationally by nitrosylation. Plant Physiol Biochem 96:115–123
Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of *S*-nitrosylated proteins in potato plant. Physiol Plantarum 148:371–386
Kopyra M, Gwózdz EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. Plant Physiol Biochem 41:1011–1017
Kovacs I, Durner J, Lindermayr C (2015) Crosstalk between nitric oxide and glutathione is required for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent defense signaling in *Arabidopsis thaliana*. New Phytol. 208:860–872
Kubienová L, Kopečný D, Tylichová M, Briozzo P, Skopalová J, Šebela M, Navrátil M, Tâche R, Luhová L, Barroso JB, Petřivalský M (2013) Structural and functional characterization of a plant *S*-nitrosoglutathione reductase from *Solanum lycopersicum*. Biochimie 95:889–902
Kulik A, Noirot E, Grandperret V, Bourque S, Fromentin J, Salloignon P, Truntzer C, Dobrowolska G, Simon-Plas F, Wendehenne D (2015) Interplays between nitric oxide and reactive oxygen species in cryptogein signalling. Plant Cell Environ 38:331–348
Kwon E, Feechan A, Yun BW, Hwang BH, Pallas JA, Kang JG, Loake GJ (2012) AtGSNOR1 function is required for multiple developmental programs in Arabidopsis. Planta 236:887–900
Lamotte O, Courtois C, Barnavon L, Pugin A, Wendehenne D (2005) Nitric oxide in plants: the biosynthesis and cell signalling properties of a fascinating molecule. Planta 221:1–4
Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E (2008) Modulation of nitrosative stress by *S*-nitrosoglutathione reductase is critical for thermotolerance and plant growth in Arabidopsis. Plant Cell 20:786–802
Leshem YY, Pinchasov Y (2000) Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries *Fragaria ananassa* (Dutch.) and avocados *Persea americana* (Mill.). J Exp Bot 51:1471–1473

Leterrier M, Chaki M, Airaki M, Valderrama R, Palma JM, Barroso JB, Corpas FJ (2011) Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. Plant Signal Behav 6:789–793

Leterrier M, Airaki M, Palma JM, Chaki M, Barroso JB, Corpas FJ (2012) Arsenic triggers the nitric oxide (NO) and S-nitrosoglutathione (GSNO) metabolism in Arabidopsis. Environ Pollut 166:136–143

Leterrier M, Barroso JB, Valderrama R, Begara-Morales JC, Sánchez-Calvo B, Chaki M, Luque F, Viñegla B, Palma JM, Corpas FJ (2016) Peroxisomal NADP-isocitrate dehydrogenase is required for Arabidopsis stomatal movement. Protoplasma. 253:403–415

Lindermayr C, Saalbac G, Durner J (2005) Proteomic identification of S-nitrosylated proteins in Arabidopsis. Plant Physiol 137:921–930

Liu S, Yang R, Pan Y, Ma M, Pan J, Zhao Y, Cheng Q, Wu M, Wang M, Zhang L (2015) Nitric oxide contributes to minerals absorption, proton pumps and hormone equilibrium under cadmium excess in Trifolium repens L. plants. Ecotoxicol Environ Saf 119:35–46

Lozano-Juste J, Leon J (2010) Enhanced abscisic acid-mediated responses in nia1nia2noa1-2 triple mutant impaired in NIA/NR- and AtNOA1-dependent nitric oxide biosynthesis in Arabidopsis. Plant Physiol 152:891–903

Lu Y, Li N, Sun J, Hou P, Jing X, Zhu H, Deng S, Han Y, Huang X, Ma X, Zhao N, Zhang Y, Shen X, Chen S (2013) Exogenous hydrogen peroxide, nitric oxide and calcium mediate root ion fluxes in two non-secretor mangrove species subjected to NaCl stress. Tree Physiol 33:81–95

Manai J, Gouia H, Corpas FJ (2014a) Redox and nitric oxide homeostasis are affected in tomato (*Solanum lycopersicum*) roots under salinity-induced oxidative stress. J Plant Physiol 171:1028–1035

Manai J, Kalai T, Gouia H, Corpas FJ (2014b) Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (*Solanum lycopersicum*) plants. J Soil Sci Plant Nutr 14:433–446

Manjunatha G, Lokesh V, Neelwarne B (2010) Nitric oxide in fruit ripening: trends and opportunities. Biotechnol Adv 28:489–499

McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytol 172:221–228

Meilhoc E, Boscari A, Bruand C, Puppo A, Brouquisse R (2011) Nitric oxide in Legume-Rhizobium symbiosis. Plant Sci 181:573–581

Molassiotis A, Tanou G, Diamantidis G (2010) NO says more than 'YES' to salt tolerance: salt priming and systemic nitric oxide signaling in plants. Plant Signal Behav 5:209–212

Molina-Favero C, Creus CM, Simontacchi M, Puntarulo S, Lamattina L (2008) Aerobic nitric oxide production by Azospirillum brasilense Sp245 and its influence on root architecture in tomato. Mol Plant Microbe Interact 21:1001–1009

Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signaling in plants. New Phytol 159:11–35

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176

Ortega-Galisteo AP, Rodríguez-Serrano M, Pazmiño DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. J Exp Bot 63:2089–2103

Pagnussat GC, Simontacchi M, Puntarulo S, Lamattina L (2002) Nitric oxide is required for root organogenesis. Plant Physiol 129:954–956

Peng D, Wang X, Li Z, Zhang Y, Peng Y, Li Y, He X, Zhang X, Ma X, Huang L, Yan Y (2015) NO is involved in spermidine-induced drought tolerance in white clover via activation of antioxidant enzymes and genes. Protoplasma. doi:10.1007/s00709-015-0880-8

Pfeiffer S, Mayer B, Hemmens B (1999) Nitric oxide: chemical puzzles posed by a biological messenger. Angew Chem Int Ed 38:1714–1731

Piterková J, Luhová L, Mieslerová B, Lebeda A, Petřivalský M (2013) Nitric oxide and reactive oxygen species regulate the accumulation of heat shock proteins in tomato leaves in response to heat shock and pathogen infection. Plant Sci 207:57–65

Prado AM, Porterfield DM, Feijó JA (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 131:2707–2714

Prado AM, Colaço R, Moreno N, Silva AC, Feijó JA (2008) Targeting of pollen tubes to ovules is dependent on nitric oxide (NO) signaling. Mol Plant 1:703–714

Prats E, Mur LA, Sanderson R, Carver TL (2005) Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis* f. sp. hordei. Mol Plant Pathol 6:65–78

Puyaubert J, Baudouin E (2014) New clues for a cold case: nitric oxide response to low temperature. Plant Cell Environ 37:2623–2630

Rockel P, Strube F, Rockel AJ, Wildt JWM, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot 53:103–110

Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, Corpas FJ, Gómez M, del Río LA, Sandalio LM (2006) Cadmium effect on oxidative metabolism of pea (Pisum sativum L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. Plant Cell Environ 29:1532–1544

Romero-Puertas MC, Laxa M, Mattè A, Zaninotto F, Finkemeier I, Jones AM, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) *S*-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell 19:4120–4130

Romero-Puertas MC, Campostrini N, Mattè A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M (2008) Proteomic analysis of *S*-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. Proteomics 8:1459–1469

Rubbo H, Radi R (2008) Protein and lipid nitration: role in redox signaling and injury. Biochim Biophys Acta 1780:1318–1324

Sainz M, Pérez-Rontomé C, Ramos J, Mulet JM, James EK, Bhattacharjee U, Petrich JW, Becana M (2013) Plant hemoglobins may be maintained in functional form by reduced flavins in the nuclei, and confer differential tolerance to nitro-oxidative stress. Plant J 76:875–887

Sainz M, Calvo-Begueria L, Pérez-Rontomé C, Wienkoop S, Abián J, Staudinger C, Bartesaghi S, Radi R, Becana M (2015) Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism. Plant J 81:723–735

Sakamoto A, Ueda M, Morikawa H (2002) Arabidopsis glutathione-dependent formaldehyde dehydrogenase is an *S*-nitrosoglutathione reductase. FEBS Lett 515:20–24

Sánchez-Calvo B, Barroso JB, Corpas FJ (2013) Hypothesis: nitro-fatty acids play a role in plant metabolism. Plant Sci 199–200:1–6

Santisree P, Bhatnagar-Mathur P, Sharma KK (2015) NO to drought-multifunctional role of nitric oxide in plant drought: do we have all the answers? Plant Sci 239:44–55

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O (2015) Nitric oxide (NO) and phytohormones crosstalk during early plant development. J Exp Bot 66:2857–2868

Scheler C, Durner J, Astier J (2013) Nitric oxide and reactive oxygen species in plant biotic interactions. Curr Opin Plant Biol 16:534–539

Sehrawat A, Deswal R (2014) *S*-nitrosylation analysis in *Brassica juncea* apoplast highlights the importance of nitric oxide in cold-stress signalling. J Proteome Res 13:2599–25619

Serpa V, Vernal J, Lamattina L, Grotewold E, Cassia R, Terenzi H (2007) Inhibition of AtMYB2 DNA-binding by nitric oxide involves cysteine S-nitrosylation. Biochem Biophys Res Commun 361:1048–1053

Shi YF, Wang DL, Wang C, Culler AH, Kreiser MA, Suresh J, Cohen JD, Pan J, Baker B, Liu JZ (2015) Loss of GSNOR1 function leads to compromised auxin signaling and polar auxin transport. Mol Plant 8:1350–1365

Signorelli S, Möller MN, Coitiño EL, Denicola A (2011) Nitrogen dioxide solubility and permeation in lipid membranes. Arch Biochem Biophys 512:190–196

Signorelli S, Corpas FJ, Borsani O, Barroso JB, Monza J (2013) Water stress induces a differential and spatially distributed nitro-oxidative stress response in roots and leaves of *Lotus japonicus*. Plant Sci 201–202:137–146

Simontacchi M, Jasid S, Puntarulo S (2004) Nitric oxide generation during early germination of Sorghum seeds. Plant Sci 167:839–847

Singh HP, Kaur S, Batish DR, Sharma VP, Sharma N, Kohli RK (2009) Nitric oxide alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa* (rice). Nitric Oxide 20:289–297

Singh VP, Srivastava PK, Prasad SM (2013) Nitric oxide alleviates arsenic-induced toxic effects in ridged Luffa seedlings. Plant Physiol Biochem 71:155–163

Szabó C, Ischiropoulos H, Radi R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 6:662–680

Szuba A, Kasprowicz-Maluśki A, Wojtaszek P (2015) Nitration of plant apoplastic proteins from cell suspension cultures. J Proteomics 120:158–168

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009a) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Tanou G, Molassiotis A, Diamantidis G (2009b) Hydrogen peroxide- and nitric oxide-induced systemic antioxidant prime-like activity under NaCl-stress and stress-free conditions in citrus plants. J Plant Physiol 166:1904–1913

Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V, Molassiotis A (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. Plant J 72:585–599

Tavares CP, Vernal J, Delena RA, Lamattina L, Cassia R, Terenzi H (2014) S-nitrosylation influences the structure and DNA binding activity of AtMYB30 transcription factor from *Arabidopsis thaliana*. Biochim Biophys Acta 1844:810–817

Terrile MC, París R, Calderón-Villalobos LI, Iglesias MJ, Lamattina L, Estelle M, Casalongué CA (2012) Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. Plant J 70:492–500

Trostchansky A, Bonilla L, González-Perilli L, Rubbo H (2013) Nitro-fatty acids: formation, redox signaling, and therapeutic potential. Antioxid Redox Signal 19:1257–1265

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Floh EI, Scherer GF (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Valderrama R, Corpas FJ, Carreras A, Fernández-Ocaña A, Chaki M, Luque F, Gómez-Rodríguez MV, Colmenero-Varea P, Del Río LA, Barroso JB (2007) Nitrosative stress in plants. FEBS Lett 581:453–461

Vanin AF, Malenkova IV, Serezhenkov VA (1997) Iron catalyzes both decomposition and synthesis of *S*-nitrosothiols: optical and electron paramagnetic resonance studies. Nitric Oxide 1:191–203

Vanzo E, Ghirardo A, Merl-Pham J, Lindermayr C, Heller W, Hauck SM, Durner J, Schnitzler JP (2014) S-nitroso-proteome in poplar leaves in response to acute ozone stress. PLoS One 9(9), e106886

Wang H, Xian M (2011) Chemical methods to detect *S*-nitrosation. Curr Opin Chem Biol 15:32–37

Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK (2015) Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. Proc Natl Acad Sci U S A 112:613–618

Wendehenne D, Gao QM, Kachroo A, Kachroo P (2014) Free radical-mediated systemic immunity in plants. Curr Opin Plant Biol 20:127–134

Wimalasekera R, Tebartz F, Scherer GFE (2011a) Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. Plant Sci 181:593–603

Wimalasekera R, Villar C, Begum T, Scherer GF (2011b) COPPER AMINE OXIDASE1 (CuAO1) of *Arabidopsis thaliana contributes* to abscisic acid- and polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction. Mol Plant 4:663–678

Wojtaszek P (2000) Nitric oxide in plants. To NO or not to NO. Phytochemistry 54:1–4
World Health Organization (2007) Health risks of heavy metals from long-range transboundary air pollution, p 130. Germany, ISBN: 978 92 890 7179 6
Wünsche H, Baldwin IT, Wu J (2011) S-Nitrosoglutathione reductase (GSNOR) mediates the biosynthesis of jasmonic acid and ethylene induced by feeding of the insect herbivore *Manduca sexta* and is important for jasmonate-elicited responses in *Nicotiana attenuata*. J Exp Bot 62:4605–4616
Xu S, Guerra D, Lee U, Vierling E (2013) S-nitrosoglutathione reductases are low-copy number, cysteine-rich proteins in plants that control multiple developmental and defense responses in Arabidopsis. Front Plant Sci 4:430
Yamasaki H, Sakihama Y, Takahashi S (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trends Plant Sci 4:128–129
Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167:1604–1615
Yu M, Yun BW, Spoel SH, Loake GJ (2012) A sleigh ride through the SNO: regulation of plant immune function by protein S-nitrosylation. Curr Opin Plant Biol 15:424–430
Yun BW, Feechan A, Yin M, Saidi NB, Le Bihan T, Yu M, Moore JW, Kang JG, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478:264–268
Zafra A, Rodríguez-García MI, Alché JD (2010) Cellular localization of ROS and NO in olive reproductive tissues during flower development. BMC Plant Biol 10:36
Zhang YY, Wang LL, Liu YL, Zhang Q, Wei QP, Zhang WH (2006) Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na+/H+ antiport in the tonoplast. Planta 224:545–555
Zhao J (2007) Interplay among nitric oxide and reactive oxygen species: a complex network determining cell survival or death. Plant Signal Behav 2:544–547
Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu Rev Plant Biol 61:535–559
Zhou J, Jia F, Shao S, Zhang H, Li G, Xia X, Zhou Y, Yu J, Shi K (2015) Involvement of nitric oxide in the jasmonate-dependent basal defense against root-knot nematode in tomato plants. Front Plant Sci 6:193

Extracellular ATP: An Essential Apoplastic Messenger in Plants

Ting Liu and Zhonglin Shang

Contents

Abstract Adenosine triphosphate (ATP) plays major roles in cell metabolism as an energy supplier and as a substrate for enzymatic reactions. While ATP is well known for its role as an intracellular energy carrier, recent studies have found that ATP exists not only in the cytoplasm, but also in the extracellular matrix. Cytoplasmic ATP can be secreted into the apoplast through wound leakage, secretory vesicles, or transporters in the plasma membrane. As a signaling molecule, extracellular ATP (eATP) regulates plant metabolism, growth and development, and responses to biotic and abiotic stimuli. eATP binds to receptors in the plasma membrane, where it triggers the generation of second messengers, including Ca^{2+}, NO, and reactive oxygen species. These second messengers induce expression of a series of functional genes that promote changes in the cellular structure and

T. Liu • Z. Shang (✉)
College of Life Sciences, Hebei Normal University, Shijiazhuang 050016, Hebei, P. R. China
e-mail: shangzhonglin@hebtu.edu.cn

Progress in Botany (2017) 78: 121–144, DOI 10.1007/124_2016_9,
Published online: 28 May 2016

physiological function of plant cells. Here, we discuss the progress in research on the function and signaling properties of this novel apoplastic messenger.

1 Introduction

As an energy-carrying molecule, adenosine triphosphate (ATP) plays key roles in energy metabolism and as a substrate in signal transduction pathways. While ATP had historically been considered an intracellular molecule, its unexpected detection in the extracellular matrix (ECM) of animal and human cells (Drury and Szent-Györgyi 1929) led to extensive investigation of its existence, downstream signaling pathways, and physiological functions. It is now known that extracellular ATP (eATP), which can be found in the blood and tissue fluid, participates in a range of functions, including neuron transmission, blood pressure regulation, cell differentiation, smooth muscle contraction, exocrine and endocrine secretion, inflammation, platelet aggregation, pain, and cardiac function modulation. eATP is secreted from the cell through secretory vesicles generated in the Golgi apparatus or through transporters in the plasma membrane (PM). Deregulation of secretion can lead to abnormal levels of eATP, which in turn causes cell death, uncontrolled cell division, and aberrant cell differentiation, and can result in serious illness in animals and humans (reviewed by Ralevic and Burnstock 1998; Burnstock 2006, 2007; Burnstock et al. 2013; Idzko et al. 2014; Kennedy 2015; Cavaliere et al. 2015; Ferrari et al. 2015).

ATP influences cellular processes by binding to purinergic receptors in the PMs of target cells. There are two classes of purinergic receptors for ATP: P2X receptors and P2Y receptors. P2X receptors are ligand-gated ion channels and P2Y receptors are heterotrimeric G-protein-coupled receptors. The binding of ATP to these receptors in target cells triggers signaling cascades that lead to transmembrane signal transduction and the generation of second messengers. These second messengers include cytosolic Ca^{2+}, nitrogen monoxide (NO), reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), inositol triphosphate (IP_3), and diacetyl glycerol (DAG). A series of biochemical reactions occurs downstream of second messengers, including the phosphorylation and dephosphorylation of structural and catalytic proteins, as well as the activation of transcription factors and the genes they promote. These reactions induce changes in cell metabolism and development, which can eventually lead to changes in the structure and function of the entire body (reviewed by Ralevic and Burnstock 1998; Burnstock 2006, 2007; Burnstock et al. 2013; Idzko et al. 2014; Kennedy 2015; Cavaliere et al. 2015; Ferrari et al. 2015).

In the 1970s, plant biologists first investigated the effects of exogenous ATP on plant metabolism and the responses of plants to stimuli. These early studies found that addition of ATP promotes fast closing of venus fly trap leaves (Jaffe 1973), endonuclease synthesis (Udvardy and Farkas 1973), and stomatal opening (Raghavendra 1981; Nejidat et al. 1983). At the time, these effects were attributed ATP absorption and increased intracellular energy supply. Lüttge et al. (1974) reported that ATP promoted K^+ absorption of cut oat leaves by chelating inhibitory divalent cations (e.g., Ca^{2+} and Mg^{2+}). The possibility that ATP could act as a messenger in the extracellular space was realized only decades later, in the 1990s. Due in large part to the ideas and methodologies established in research on the roles of eATP in animals, research on eATP in plants has made some advances during the past two decades. ATP is now known to be prevalent in the apoplast of various plant species. In addition, eATP level is correlated with the viability and growth rate of plant cells, suggesting it is a key regulator of cellular metabolism. Results of pharmacological experiments revealed that cytoplasmic ATP is secreted via presumed ATP transporters, by secretory vesicles originating in the Golgi apparatus, or during the stress response caused by a wound to the PM. eATP signal transduction has been actively investigated in the last decade. Work from these studies has shown that binding of eATP to its receptors stimulates transmembrane signaling events, including the generation of second messengers. Unsurprisingly, most of the second messengers involved in eATP signaling in animal cells are also involved in eATP-induced physiological responses in plant cells. These messengers directly or indirectly regulate metabolic reactions and gene expression, resulting in changes in structure, function, growth, and development of plant cells under different conditions (reviewed by Roux and Steinebrunner 2007; Clark and Roux 2009, 2011; Tanaka et al. 2010a, 2014; Chivasa and Slabas 2012; Cao et al. 2014). So far, research on eATP has concentrated on three fields: its physiological function, its secretion mechanisms, and its signal transduction. These three fields will be the focus of this review.

2 The Physiological Function of eATP

Researchers have manipulated ATP levels in experimental systems as a means by which to investigate the roles of eATP in plant cell growth, development, and stress responses. A simple way to elevate eATP level is by adding ATP into an experimental system. Generally, to ensure eATP acts as a signaling molecule rather than as an energy molecule, the effects of weakly hydrolyzable ATP analogs must be investigated after determining the effects of added ATP. Three widely used ATP analogs are ATPγS (adenosine 5′-*O*-(3-thio) triphosphate), Bz-ATP (3′-*O*-(4-benzoyl) benzoyl adenosine 5′-triphosphate), and 2me-ATP (2-methylthio-adenosine 5′-triphosphate). Since these analogs can only weakly be hydrolyzed by ATPase, they are unlikely to participate in energy-consuming or substrate phosphorylation reactions. Therefore, if the effects of ATP analogs are similar to those of exogenous

ATP, it can be concluded that ATP participates in these reactions as a signaling molecule. Moreover, to verify the specificity of ATP in the physiological functions of plant cells, the effects of other nucleotides, including ADP (adenosine di-phosphate), AMP (adenosine mono-phosphate), adenosine, GTP (guanosine-5′-triphosphate), CTP (cytidine triphosphate), and TTP (thymidine triphosphate) should also be investigated.

ATP hydrolysis enzymes such as apyrase (ATP-diphosphatase) and glucose hexokinase can be used to decrease eATP levels. In early experiments, these enzymes were added into the bath solution of cultured plant cells or smeared onto the surface of plant organs. The effects of these enzymes were presumed to result from eATP depletion-induced physiological reactions. In later studies, levels of extracellular apyrase have been manipulated through transgenic regulation. Phenotypic differences between wild-type and apyrase null mutants or overexpression lines were thought to result from changes in eATP levels. However, Schiller et al. (2012) found that an apyrase in *Arabidopsis thaliana*, AtAPY1, localized to the Golgi apparatus rather than to the ECM. Activity assays revealed that AtAPY1 prefers to use GDP and UDP as substrates, rather than ATP. These results indicated that not all apyrases localize to the ECM and participate in eATP turnover-related physiological responses. Therefore, the effects of transgenic modification of apyrases must be very carefully evaluated before concluding the result from eATP degradation.

Despite the challenges associated with manipulating ATP levels, findings from a large body of research have led to the conclusion that eATP is involved in the regulation of the following processes as a multifunctional messenger:

2.1 Cell Viability

Chivasa et al. (2005) reported that eATP is necessary for maintaining the viability of plant cells. They found that suspension-cultured *A. thaliana* cells released ATP into the bathing medium. Addition of apyrase and glucose–hexokinase led to reduced levels of ATP in the medium and to the death of cultured cells, indicating that deprivation of eATP compromised cell viability. Likewise, addition of AMP-PCP (β,γ-methylene adenosine 5′-triphosphate), a weakly hydrolyzable ATP analog that competitively inhibits binding of eATP to its receptor, also led to death of cultured cells, providing further evidence that eATP signaling is required for cell viability. When AMP-PCP or ATP hydrolyzing enzymes were smeared onto the surface of growing leaves of *A. thaliana*, maize, tobacco, and soybean, the tissues within the treated zone collapsed, while tissues outside of the treated zone retained a healthy appearance (Fig. 1). These results suggest that blockade or impairment of eATP signaling leads to cell death, and led the authors to conclude that eATP could be involved in maintaining key metabolic processes in plant cells.

Fig. 1 ATP hydrolysis enzymes and analogs lead to death of *Arabidopsis* tissues. Apyrase, glucose–hexokinase (Glc-Hk), or AMP-PCP was smeared on localized area (*top row*) or entire leaves (*bottom row*) of *Arabidopsis thaliana* leaves. Leaf cells being treated eventually died (Chivasa et al. 2005)

To verify the physiological function of eATP depletion-induced cell death, Chivasa et al. (2005) investigated the effect of fumonisin B1 (FB1), a programmed cell death-eliciting mycotoxin, on eATP level and cell viability of *A. thaliana*. FB1 treatment remarkably decreased eATP level and led to severe injury or death of plant cells. Exogenous ATP markedly rescued plant cells from FB1-induced cell death (Fig. 2). These results suggest that this mycotoxin causes cell death partially

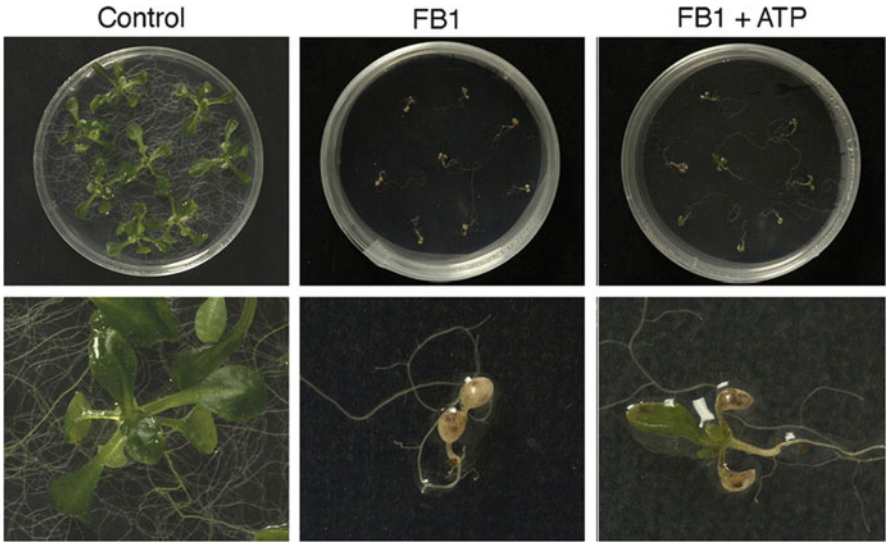

Fig. 2 ATP rescues *Arabidopsis* plants from FB1-induced death. *Arabidopsis thaliana* plants were transferred and being cultured on nutrient agar containing 1 μM FB1 or 1 μM FB1 mixed with 1 mM ATP for 5 days. *Top row*, retardation of growth in all treatments containing FB1. *Bottom row*, close-up photographs of representative plants from corresponding plates in the top row (Chivasa et al. 2005)

through promoting eATP degradation, and that conversely, exogenous ATP stimulates protective reactions, thereby rescuing plant cells.

Findings from another research group were at odds with a protective role for ATP, revealing instead that eATP induces programmed cell death (PCD). Sun et al. (2012a, b) reported that eATP triggered PCD of cultured *Populus euphratica* cells in a dose-dependent manner: high concentrations ($\geq$0.5 mM) of ATP induced PCD while low concentrations of eATP did not. Specifically, high levels of ATP led to an increase in DNA fragmentation, chromatin condensation, and caspase activity, which are all typical features of PCD. It is not excluded that high apoplastic eATP concentrations of about 0.5 mM have physiological functions, because cytoplasmic ATP concentration is 1–2 mM and ATP can be released from a wound after injury. The disparate conclusions from Sun et al. (2012a, b) and Chivasa et al. (2005) were not due to differences in ATP levels in the culture medium, as both studies used similar concentrations (around 1 mM). Instead, their results may indicate that different plant species respond to eATP in distinct manners. Sun et al. (2012a, b) suggest a physiological function of eATP-induced PCD in which biotic or abiotic stresses stimulate ATP release, leading to transient or sustained increases in eATP level. Then, eATP-induced PCD may eliminate injured or infected cells to prevent further injury of adjacent cells, and may be an important step in the plant response to stress.

2.2 Vegetative Growth

A large body of work supports a role for eATP in promoting vegetative growth. Thomas et al. (1999) reported that exogenous apyrase promotes growth of *Arabidopsis* cells. In their experiments, apoplastic ATP, which can be degraded by apyrase to release phosphate, was regarded as a source of inorganic phosphate (Pi), a nutrient important for plant growth. Lew and Dearnaley (2000) reported that exogenous ATP, ADP, and GTP promoted root hair growth and depolarized the PM potential of growing root hairs. In addition, Kim et al. (2006) reported that eATP localization and concentration are positively correlated with cell expansion. Specifically, eATP concentration is higher in the walls of elongating cells such as root epidermal cells in the elongation zone and division zone (Fig. 3). In growing root hairs, a tip to base eATP gradient was detected and is thought to be the basis for root hair elongation, since this gradient is less prominent or missing in the cell walls of non-growing root hairs. Finally, Tonón et al. (2010) reported that etiolated *Arabidopsis* seedlings growing in reduced medium containing glutathione (GSH) or dithiothreitol (DTT) show decreased hypocotyl elongation rate. Addition of ATP effectively restores the normal growth of GSH-suppressed hypocotyl elongation. Additional data suggests that eATP-stimulated ROS generation plays a basic role in maintaining plant cell growth rate.

Although the above findings demonstrate that eATP positively regulates plant growth, still other findings suggest that eATP suppresses plant cell growth. Wu et al. (2007) reported that the expression levels of two *A. thaliana* apyrase genes, *APY1* and *APY2*, are correlated with growth rate. Expression of *APY1* and *APY2* was markedly higher in rapidly growing tissue and cell types such as root tip, root cap, columella cells, and the root–hypocotyl junction than in other tissues and cells. Expression of apyrases significantly affected growth rate of seedlings both in light and in darkness. In *apy1*/*apy2* double-knockout mutants, the growth rates of the primary roots of seedlings growing in light and the hypocotyls of etiolated seedlings growing in darkness were both markedly lower than those in wild-type plants. Furthermore, Wolf et al. (2007) found that in *A. thaliana apy1*/*apy2* double-knockout mutants, root and shoot meristems were abnormal and far less functional, and the morphological features of cotyledons were different from wild-type plants. Some *apy1*/*apy2* double-knockout mutants were seedling lethal or had developmental defects in cell division and growth. Riewe et al. (2008) reported that apyrase located in the apoplast of potato cells plays essential roles in tuber generation and development. Suppression of apyrase expression led to increased tuber number and decreased tuber size. In addition, tubers of the apyrase null mutants had more longitudinal shapes than wild-type tubers. Decreased apyrase expression in the tubers also changed their gene expression patterns. Specifically, increased expression was observed in genes encoding cell wall proteins related to cell elongation, carbon and energy provision for starch biosynthesis, and mitochondrial ATP/ADP translocators, whereas decreased expression was observed in key enzymes related to starch synthesis. These data indicate that sufficient apyrase molecules are needed

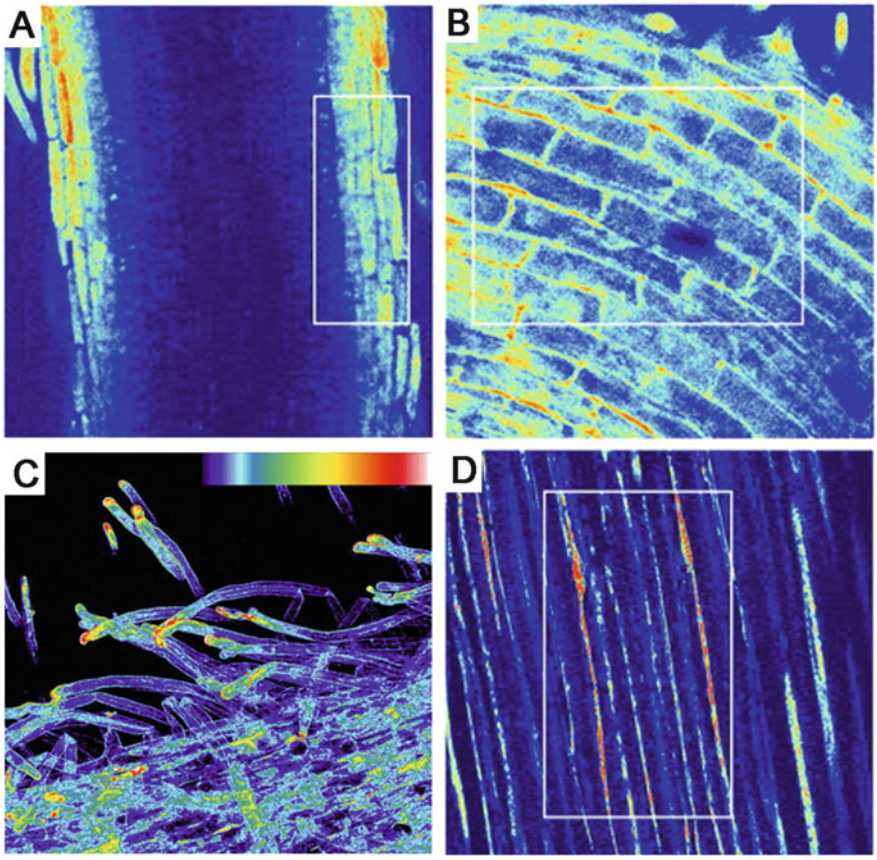

Fig. 3 The distribution of eATP on the root surface of *Medicago truncatula*. CBD (cell wall binding domain peptide)-luciferase reporter was used to visualize eATP. Intensity-coded (inset range in **c**, where white areas show the strongest ATP concentration and black is the background) images showing ATP to exist in interstitial spaces at growing regions of the root [including meristematic region (**a**), elongation region (**b**), root hairs (**b**), and etiolated hypocotyl (**d**)] (Kim et al. 2006)

for normal growth of vegetative organs and reproductive cells. Since the main function of apoplast apyrase is to hydrolyze ATP, it can be concluded that eATP accumulation may not always be beneficial for cell growth, and that eATP hydrolysis may be necessary for maintaining stable growth and development.

Consistent with this idea, Clark et al. (2010) demonstrated that levels of eATP affect its physiological functions. They reported that eATP is involved in growth of cotton fibers. When cotton fibers enter their rapid growth phase, there is a significant increase in the expression of two extracellular apyrases in cotton: *GhAPY1* and *GhAPY2*. In a cultured ovule system, growing fibers released ATP into culture medium. Inhibition of apyrase activity by apyrase inhibitor or apyrase antibodies led to increased eATP level in the medium and inhibited fiber growth. Although

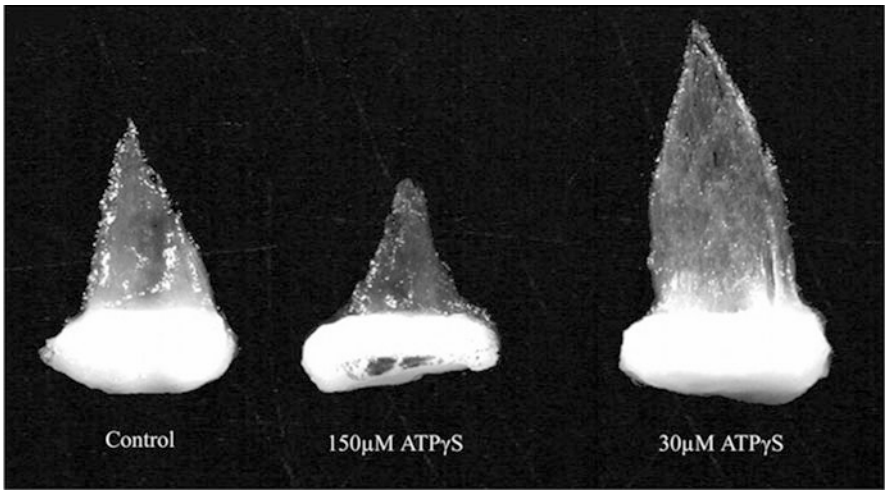

Fig. 4 High concentration (150 μM) of ATPγS decreased cotton fiber growth while low concentration (30 μM) of ATPγS increased it (Clark et al. 2010)

addition of ATPγS at a low concentration (30 μM) promoted fiber growth, addition at a high concentration (150 μM) suppressed fiber growth (Fig. 4). These data suggest that eATP released by ovule cells is required for cotton fiber growth. However, since sustained fiber growth requires steady, low eATP levels, the timely hydrolysis of ATP by apyrase is necessary in order to prevent eATP over accumulation.

2.3 Pollen Germination and Pollen Tube Growth

Steinebrunner et al. (2003) found that pollen germination (PG) and pollen tube growth (PTG) of *A. thaliana* were suppressed by apyrase inhibitors, and completely blocked by double-knockout mutagenesis of *APY1* and *APY2*. However, complementation with either one of these apyrases rescued PG in the double knockouts. These results indicate that apyrases are required for PG and PTG. Moreover, they suggested that quenching of an eATP signal may be one possible reason for apyrase suppressed PG and PTG. Furthermore, Reichler et al. (2009) reported that addition of ATPγS markedly inhibited PG and PTG of wild-type *A. thaliana*. Nevertheless, the dynamics and function of endogenous eATP in pollen grain cell wall are still uncertain. Whether there is any positive regulatory role of eATP in PG and PTG needs to be clarified.

2.4 Stomatal Movement

Thirty years ago, it was reported that exogenous ATP promoted stomatal opening in *Commelina benghalensis* (Raghavendra 1981) and *Commelina communis* (Nejidat et al. 1983). In those studies, ATP-promoted stomatal opening was thought to result from increased energy supply. However, recent research revealed that eATP may function as a signaling molecule, rather than as an energy carrier, to promote stomatal movement. Clark et al. (2011) found such a role for ATP in *A. thaliana* by showing that both exogenous ATP and ATPγS promote stomatal movement. Interestingly, both stimuli that promote stomatal opening (light) and stimuli that promote stomatal closing (ABA) lead to eATP increase and apyrase accumulation in the outer space of guard cells, indicating a role for eATP as a signal transducer in these processes. It was also reported that ATP, ADP, GTP, and weakly hydrolyzable ATP analogs (ATPγS, Bz-ATP, and 2meATP) promoted stomatal opening of *A. thaliana* (Hao et al. 2012) and *Vicia faba* (Wang et al. 2014) in light and darkness, further proving the involvement of purine signals in stomatal movement.

2.5 Root Gravitropism

Tang et al. (2003) reported that addition of 1–2 mM ATP strongly blocked gravitropic growth of *Arabidopsis* root, leading to horizontal growth of roots that normally grow vertically. Higher concentration of ATP (3 mM) even led to root curling. Later investigation of the mechanisms of eATP-inhibited root gravitropism revealed that eATP disturbs auxin distribution in root cells by inhibiting auxin export from root tip cells (Liu et al. 2012). Root growth orientation is sensitive to environmental stimuli, including gravity and light. The inhibitory effect of ATP on root gravitropism can be defined as eATP-regulated root growth reorientation. The physiological significance of this process remains to be clarified.

2.6 Nodulation

Etzler et al. (1999) and Day et al. (2000) reported that eATP levels affect root nodulation of leguminous plants. Nod factors released by rhizobia promote expression and secretion of apyrase. Apyrase binds with nod factors, decomposes eATP, and promotes nodulation of *Dolichos biflorus* and *Glycine soja* roots. Consistent with this, function-blocking antibodies against apyrase markedly inhibit nodulation. McAlvin and Stacey (2005) also found that overexpression of apyrase genes in *Lotus japonica* led to a remarkable increase in root nodules, while suppression of apyrase expression resulted in decreased nodulation. These results indicate that

eATP may be involved in the communication between plant cell and outer microorganisms.

2.7 Stress Responses

Several studies have shown that eATP leads to reduced plant resistance to antibiotics or herbicides (Thomas et al. 2000; Windsor et al. 2003; Chivasa et al. 2009). Specifically, Thomas et al. (2000) reported that ATP markedly increased the sensitivity of yeast and *Arabidopsis* cells to cycloheximide. Likewise, apyrase decreased eATP level and enhanced resistance of these cells to cycloheximide. Windsor et al. (2003) also reported that overexpression of apyrase in *Arabidopsis* markedly promoted the resistance of plants to herbicides. Cells of apyrase overexpression lines excreted more herbicides than wild-type, which weakened the effects of herbicides on plant cells. Results from Chivasa et al. (2009) showed that exogenous glucose–hexokinase or AMP-PCP led to death of tobacco leaf cells, though leaf cells grown in darkness were far less sensitive to glucose–hexokinase and AMP-PCP than leaf cells grown in light. eATP depletion resulted in increased expression of pathogenesis-related genes and enhanced resistance to tobacco mosaic virus. Under low-light conditions, AMP-PCP treatment induced expression of three pathogenesis-related genes: *PR-1*, *PR-2*, and *PR-5*. In tobacco leaves pretreated with glucose–hexokinase or AMP-PCP, growth of inoculated tobacco mosaic virus was significantly suppressed. Glucose–hexokinase or AMP-PCP pretreatment also promoted leaf cells' resistance to the bacteria *Pseudomonas syringae* pv. *tabaci*. These results indicate that eATP effectively suppresses plant pathogen resistance (PR). Negative regulation of PR responses by eATP may have important physiological roles. Fast-growing young tissues, which can respond to pathogens rapidly and effectively, have high eATP levels. Chivasa et al. (2009) suggest that these eATP may switch off unnecessary defensive responses to let cells grow and develop intensively. In older senescent cells, which are unable to respond efficiently to pathogens, eATP levels decline and expression of genes related to PR will be switched on, preparing for possible attacks.

Most recently, eATP was reported to be involved in cold tolerance of *P. euphratica* (Deng et al. 2015). Cold stress stimulated the release of ATP. Addition of low concentrations of ATP promoted cold tolerance, while addition of high concentrations of ATP inhibited it. Furthermore, low temperature upregulated the expression of the apyrase *PeAPY2* in callus cells. Ectopic expression of *PeAPY2* in *A. thaliana* strongly enhanced the cold tolerance of transgenic seedlings. While cold stimulation led to injury of the PM and leakage of intracellular solutes in wild-type plants, metabolite leakage was reduced in *PeAPY2* transgenic plants. Transgenic plants also showed increased vesicular trafficking (e.g., endocytosis and exocytosis) during cold treatment and recovery in normal temperature. Exogenous ATP at low concentrations also accelerated vesicular trafficking, while ATP at high concentrations suppressed it. Based on these results,

it was concluded that cold stress-induced eATP elevation may stimulate vesicular trafficking and efficient PM repair. However, continuous accumulation of eATP hurts cell viability, so apyrase is needed to drop eATP concentration to a safe level. Under cold stress, an accurate regulatory mechanism controlling ATP release and turnover is required for modulating cellular responses related to cold tolerance.

Kim et al. (2009) found that eATP had a positive effect on hypertonic stress responses of *Arabidopsis* seedlings. ATP release and apyrase accumulation in the apoplast were stimulated in high concentration NaCl, $Mg(NO_3)_2$, or $MgCl_2$ solutions. Accumulated eATP enhanced cell resistance to hypertonic stress. Apyrase-mediated eATP degradation, which occurs after eATP triggers downstream signal transduction cascades, may also be a necessary step in hypertonic tolerance. Addition of AMP-PCP to competitively block eATP signaling resulted in hypertonic stress and cell death.

eATP is also involved in salt tolerance of suspension-cultured *P. euphratica* cells (Sun et al. 2012a, b). Addition of NaCl induced ATP release and stimulation of salt-tolerance responses. Depletion of ATP by exogenous ATP hydrolase or blockade of eATP signaling by PPADS (pyridoxalphosphate-6-azophenyl-2′, 4′-disulfonic acid) and suramin resulted in a significant decrease in the viability of NaCl treated cells. These results indicate that eATP is needed for maintaining cell viability under salt stress.

The physiological roles of eATP in stress tolerance have been discussed by Choi et al. (2014b). When plant or animal cells are damaged by physical, chemical, or biotic stimuli, intracellular molecules known as damage-associated molecular patterns (DAMPs) are released through the wound to initiate defense responses. In animals, eATP has been considered a DAMP. Since in plants, eATP is released to extracellular spaces when cells are injured by biotic or abiotic stresses, and triggers signal cascades to induce resistant responses, it is reasonable to consider it a DAMP in plants.

Different labs have demonstrated seemingly contradictory effects of eATP on plant growth, development, and stress resistance. Relatively low concentrations of eATP are necessary for plant growth, development, and stress resistance, while high concentrations of eATP lead to growth inhibition or even PCD. Choi et al. (2014b) suggested a model that illustrates the mechanism by which eATP may perform such seemingly contradictory effects. As shown in Fig. 5, eATP concentration is normally maintained at a certain level by the balance of ATP secretion and hydrolysis. This level is beneficial for plant cell growth and development. Small or moderate increases or decreases in eATP level will lead to defense responses, including inhibited plant cell growth rate and special metabolite accumulation. However, very low or very high eATP levels can lead to PCD. This model explains the effects of exogenous ATP or apyrase on plant cell growth, development, and stress resistance. Nevertheless, the mechanism by which low and high levels of eATP can stimulate similar responses remains to be verified.

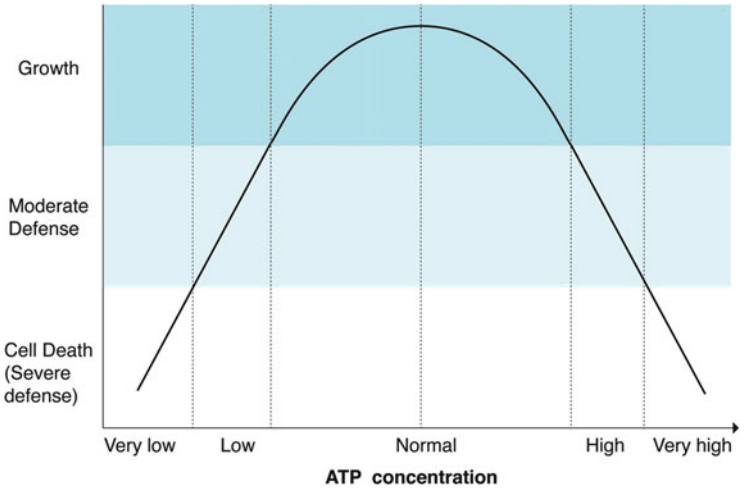

Fig. 5 A model of the effect of eATP concentration on plant growth and immunity. When eATP levels deviate from the optimal concentration for plant growth, ATP serves as a danger signal to inform cells of their abnormal status. Those concentrations that deviate slightly from optimal extracellular ATP levels appear to trigger plant defense responses. In contrast, extreme depletion or excess of extracellular ATP is correlated with cell death (Choi et al. 2014b)

3 The Existence and Secretion of eATP

Although application of exogenous ATP or apyrase shows remarkable effects in various experiments, the existence of endogenous eATP must be verified before it can be defined as an apoplastic messenger. To measure eATP level in the ECM, ATP was extracted and its concentration in extracted fluid was measured to estimate the eATP level in cell wall. Estimates of the resting eATP concentration in the ECM range from dozens of nM to dozens of μM (Thomas et al. 2000; Song et al. 2006; Wu et al. 2008; Clark et al. 2010; Weerasinghe et al. 2009). Nevertheless, methods which can in situ accurately measure the eATP level in the apoplast are still absent.

In most experiments, ATP concentration was calibrated by using the bioluminescent indicator luciferin. Luciferase catalyzes the oxidation of luciferin in an ATP-dependent manner, generating a fluorescent signal that can be detected by illuminance-detecting instruments. Illuminant intensity is correlated to ATP concentration. To detect eATP localization and dynamics in situ, Kim et al. (2006) fused firefly luciferase with a peptide containing cellulose binding domain (CBD). The CBD peptide ensures that the fused proteins can be anchored to the cell wall. The localization and density of bioluminescence reflects the localization and relative concentration of ATP in the apoplast. Using this indicator, they visualized eATP in the cell wall of *A. thaliana*, alfalfa (*Medicago truncatula*), wheat (*Triticum aestivum*), and lotus (*Lotus japonicus*). Development of this method prompted investigation of eATP localization, secretion, turnover, and dynamics in a number of plant systems.

Most recently, Vanegas et al. (2015) developed a self-referencing electrochemical biosensor for direct ATP flux measurement. They coated a graphene-functionalized platinum microelectrode with a laponite sol gel containing two enzymes: glycerol kinase and glycerol-3-phosphate oxidase. By detecting electropotential dynamics driven by enzyme-catalyzed ATP degradation, the dynamics of ATP concentration in solution could be sensitively detected. This electrochemical biosensor has been used to rapidly and sensitively detect touch- and wound-stimulated ATP efflux from maize (*Zea mays*) root cells and light-induced ATP release from developing *Ceratopteris* spores. This biochemical biosensor has proven to be a useful tool for non-invasive measurement of eATP secretion from living cells.

In animal cells, ATP is released through secretory vesicles, specific transporters, or PM wound. Studies using the methods described above revealed that similar mechanisms of secretion might also exist in plants. Secretory vesicles originating from the Golgi apparatus are major resources of eATP. Kim et al. (2006) reported that eATP concentration is correlated with extracellular Ca^{2+}. The high eATP level at the tip of root hairs was decreased by the Ca^{2+} chelator EGTA or the Ca^{2+} channel blocker (Gd^{3+}), and increased by addition of exogenous Ca^{2+}, indicating that Ca^{2+} influx is necessary for eATP secretion. Wu et al. (2008) also found that EGTA and the Ca^{2+} channel blocker La^{3+} strongly inhibited ATP release from cultured hairy roots of *Salvia miltiorrhiza*. Since cytosolic Ca^{2+} is an essential regulator of secretory vesicle transport and fusion with PM, these results suggest that eATP may be secreted through vesicles. Further support for the role of these vesicles in eATP secretion comes from the finding that Brefeldin A, a toxin that inhibits vesicle transport, significantly decreased eATP accumulation in root hair tip (Kim et al. 2006).

ATP Binding Cassette (ABC) transporters play crucial roles in ATP secretion in animal cells. Although similar transporters have been detected in plant cells (Crouzet et al. 2006), their role in eATP secretion remains uncertain. Thomas et al. (2000) reported that overexpression of some members of the plant ABC transporter family resulted in accelerated ATP secretion in yeast and *A. thaliana*.

In animals, mechanical touch and stress stimulate ATP release (Bodin and Burnstock 2001). Similar mechanisms have been found in plants. Touch stimulation and osmotic stress increased eATP level in *Arabidopsis* seedlings (Jeter et al. 2004). Mechanical touch also stimulated ATP release from *Arabidopsis* root tip cells (Weerasinghe et al. 2009). Kim et al. (2006) reported that treatment with a chitin mixture, to mimic a pathogen attack, promoted ATP secretion. In addition, Wu et al. (2008) reported that polysaccharide elicitors in yeast extract promoted ATP release from cultured hairy roots of *S. miltiorrhiza*. Hypertonic stress also stimulated ATP secretion and promoted a cell resistance response (Kim et al. 2009). Osmotic stress (high concentration of sorbitol), ABA, and glutamate also induced ATP release from *A. thaliana* roots (Dark et al. 2011). Finally, wounding-induced eATP accumulation played crucial roles in inducing protective responses (Song et al. 2006). In stress-induced ATP release, ATP efflux may occur primarily through

PM wound. These eATP molecules may be required for triggering cytoplasmic reactions to enhance tolerance of plant cells to related stresses.

4 The Signal Transduction of eATP

In animal systems, eATP participates in cell signaling in two ways: (1) as a substrate to regulate protein activity through phosphorylation, and (2) as a primary messenger to stimulate signaling cascades through binding of eATP receptors in the PM. eATP may play similar roles in plant systems. Using proteomic analysis, Chivasa et al. (2002) identified phosphorylated proteins in the ECM of plant cells. Although there is no direct evidence that eATP participates in protein phosphorylation outside the cell, the possibility cannot be excluded.

Research concepts and methods used widely in animal eATP signaling research have been adopted in most studies of plant eATP signal transduction. Although these methods may be applicable in plant research based on the similarities between plant and animal cells, the distinct characteristics of plant cells may require additional specific research ideas and methods.

4.1 eATP Receptors

In early studies, animal eATP receptor antagonists or inhibitors were widely used in research on plant eATP signaling. Three such commonly used antagonists, PPADS, suramin, and reactive blue (RB), effectively inhibited or blocked eATP-induced physiological effects in most plant studies in which they were used (Demidchik et al. 2003; Chivasa et al. 2005; Song et al. 2006; Sun et al. 2012a). However, genomic sequence surveys revealed that the animal P2-like receptor, which is sensitive to these antagonists, exists only in green algae (however, it is proved to be exist intracellularly rather than in PM) (Fountain et al. 2008) and not in higher plants. The target of these antagonists in higher plant cells needs to be verified before any conclusions can be drawn about the roles of eATP in plant cells.

In animal cells, eATP binding with P2X receptors, which are ligand-gated ion channels, triggers Ca^{2+} influx into cytoplasm. eATP-triggered Ca^{2+} influx has been detected in several plant species. Furthermore, Ca^{2+} influx channel was identified using electrophysiological methods (Demidchik et al. 2009; Wang et al. 2014). Based on these data, it is reasonable to propose that eATP receptor-like Ca^{2+} channels may exist in the PM of plant cells.

Recently, an eATP receptor was identified in *A. thaliana* (Choi et al. 2014a). By screening ethyl methanesulfonate (EMS)-mutagenized seedlings, 2 mutants that were unresponsive to ATP stimulation were identified. The mutants, named *dorn1*, had impairments in ATP-induced $[Ca^{2+}]_{cyt}$ increase, MAPK activation, and gene expression, all of which are typical ATP-inducible responses in wild-type plants.

DORN1 encodes a lectin receptor kinase (LecRK-1.9) that consists of three domains: (1) extracellular legume-type lectin domain, (2) single transmembrane domain, and (3) intracellular serine/threonine kinase domain. T-DNA insertion mutagenesis revealed that point mutation of each domain caused complete impairment of eATP-induced cellular responses, indicating that all three domains are necessary for eATP signaling (Choi et al. 2014b). Reduced *LecRK-1.9* gene expression resulted in suppressed eATP responses, while its overexpression resulted in strengthened ATP responses. Likewise, most wounding- or eATP-induced gene expression was suppressed in *dorn1* and significantly promoted in *DORN1* overexpression lines. These findings indicate that ATP released upon wounding binds to DORN1, stimulating signaling that triggers functional gene expression. Since DORN1 possesses protein kinase activity, it was later named P2K receptor to distinguish it from P2X and P2Y receptors in animal cells. Ectopic expression of the *Arabidopsis LecRK1.9* (*DORN1*) gene in tobacco and potato plants significantly enhanced their resistance to *Phytophthora infestans* (Bouwmeester et al. 2013), indicating the involvement of P2K-related ATP signaling in pathogen attack responses.

4.2 Transmembrane Signal Transduction and Generation of Second Messengers

4.2.1 Heterotrimeric G Protein

Data suggest that heterotrimeric G proteins in PM may be involved in eATP signaling in plant systems. Weerasinghe et al. (2009) reported that heterotrimeric G protein is involved in the eATP-regulated obstacle-avoidance response of *A. thaliana* roots. Roots of Gα/Gβ double null mutants do not respond to touch stimulation and change root growth direction more slowly than wild-type roots. In addition, the refractory period after touch-induced ATP release is significantly attenuated in the Gα/Gβ double null mutant compared to wild-type. Based on these results, the authors suggest that touch-induced ATP release and the fine-tuning of eATP level that follows, both of which are controlled primarily by heterotrimeric G proteins, may be key signaling events in the obstacle-avoidance response. Tanaka et al. (2010b) suggest Gα and Gβ subunits participate in ATP-stimulated Ca^{2+} increase to different degrees. They found that in Gα null mutants, eATP-stimulated Ca^{2+} increase was similar to that of wild-type, while in Gβ null mutants, eATP-stimulated Ca^{2+} increase was enhanced, indicating that Gβ may be a negative regulator of eATP-induced cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) increase. Hao et al. (2012) reported that eATP promoted stomatal opening and generation of secondary messengers (Ca^{2+}, ROS) in *A. thaliana*, but that these processes were impaired in two Gα null mutants: *gpa1-1* and *gpa1-2*. These findings indicate that heterotrimeric G protein may participate in eATP signaling

in guard cells. The role of heterotrimeric G protein in transmembrane signal transduction of eATP needs further verification.

4.2.2 Ca^{2+}

Ca^{2+} was the first agent studied for its relation to plant eATP signaling, and remains the most widely investigated. Exogenous ATP stimulates transient Ca^{2+} elevation or Ca^{2+} oscillation and is thought to be involved in eATP-regulated physiological functions in plant cells.

Pharmacological manipulation of Ca^{2+} channels has revealed distinct sources of eATP-induced Ca^{2+}. Exogenous ATP induces a transient two-peak elevation curve of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in *A. thaliana* root cells (Demidchik et al. 2003; Tanaka et al. 2010b). eATP-induced Ca^{2+} dynamics were inhibited by a Ca^{2+} chelator (EGTA) and Ca^{2+} channel blockers (Demidchik et al. 2003; Jeter et al. 2004; Hao et al. 2012). However, while ATP-induced gene expression was strongly inhibited by the Ca^{2+} channel blockers La^{3+} and Gd^{3+}, it was unaffected by the Ca^{2+} release inhibitor U73122, indicating that Ca^{2+} influx, rather than Ca^{2+} release, may be the main source of eATP-induced Ca^{2+} (Jeter et al. 2004). A key mediator of eATP-induced Ca^{2+} influx is a voltage-dependent Ca^{2+} channel in PM that is activated by hyperpolarization of the PM potential (Demidchik et al. 2009; Wang et al. 2014). Tanaka et al. (2010b) suggested that in the ATP-induced two-peak Ca^{2+} increase, the first peak may result from Ca^{2+} influx through Gd^{3+} sensitive channels in the PM, and the second peak may result from both Ca^{2+} influx from ECM and Ca^{2+} release from intracellular Ca^{2+} stores.

A surprising result from Tanaka et al. (2010b) is that eATP induces a 4–6 peak $[Ca^{2+}]_{cyt}$ oscillation when the apyrase inhibitor NGXT191 is present in the apoplast of *Arabidopsis* cells. This oscillation can be blocked by Gd^{3+} or U73122, suggesting it is mediated by both Ca^{2+} influx and Ca^{2+} release. eATP did not induce $[Ca^{2+}]_{cyt}$ oscillations in the absence of NGXT191, indicating that suppression of apyrase activity is necessary for its occurrence. Brefeldin A also blocked eATP-induced $[Ca^{2+}]_{cyt}$ oscillations, indicating that when apyrase is inhibited by NGXT191, increased eATP levels may stimulate further ATP release via secretory vesicles. Released ATP may stimulate Ca^{2+} influx and release, eventually initiating $[Ca^{2+}]_{cyt}$ oscillation. The authors propose that the ATP-induced ATP release and $[Ca^{2+}]_{cyt}$ oscillation under conditions of apyrase suppression may be critical for several physiological responses. Since decreased apyrase gene expression results in significant changes in plant cell growth and responses to stimuli, it can be deduced that constantly accumulated eATP may regulate physiological reactions through cytosolic Ca^{2+} oscillations.

4.2.3 Reactive Oxygen Species

In plant cells, ROS concentration in the cytoplasm is correlated with eATP level. More specifically, exogenous ATP induced accumulation of ROS (including superoxide anions, hydroxyl radicals, and H_2O_2), whereas exogenous apyrase induced a decrease in ROS (Kim et al. 2006; Song et al. 2006; Demidchik et al. 2009; Tonón et al. 2010; Hao et al. 2012; Sun et al. 2012b; Wang et al. 2014; Lim et al. 2014).

NADPH oxidase in the PM is a key component of eATP-induced ROS generation. The NADPH oxidase inhibitor diphenylene iodonium (DPI) effectively inhibited eATP-induced ROS accumulation. In addition, eATP-induced ROS generation was impaired in the null mutants of NADPH oxidase, *rhd-2*, and *atrbohD/F* (Song et al. 2006; Demidchik et al. 2009; Tonón et al. 2010; Hao et al. 2012; Wang et al. 2014). It has also been revealed that heterotrimeric G protein in PM (Hao et al. 2012), cytoplasmic Ca^{2+}, and calmodulin (Song et al. 2006) may be involved in eATP-stimulated NADPH oxidase activation.

eATP-induced ROS is involved in a number of functions related to the regulation of plant cell growth, development, and stress tolerance. These include root hair growth (Kim et al. 2006), defense response (Kim et al. 2006; Song et al. 2006), stomatal movement (Hao et al. 2012; Wang et al. 2014), cell wall construction (Lim et al. 2014), hypocotyl growth of etiolated seedlings (Tonón et al. 2010), and PCD (Sun et al. 2012a, b). Though little is known about ROS-regulated downstream responses, ROS may stimulate ion transport (Demidchik et al. 2009; Hao et al. 2012; Wang et al. 2014) and expression of several defense genes (Song et al. 2006).

4.2.4 Nitrogen Monoxide

NO has been detected in eATP-treated plant cells and is an important component of eATP signaling (Foresi et al. 2007; Wu and Wu 2008; Reichler et al. 2009; Tonón et al. 2010; Clark et al. 2011). NO is involved in eATP-regulated cell growth (Foresi et al. 2007; Wu and Wu 2008), pollen germination (Reichler et al. 2009), hypocotyl growth (Tonón et al. 2010), and stomatal movement (Clark et al. 2011).

Wu and Wu (2008) reported that in *S. miltiorrhiza* hairy root cells, NO production could be detected 30 min after ATP treatment. NO level was increased by low doses of ATP (10–100 μM) and decreased by higher doses of ATP (>100 μM). Results of pharmacological experiments suggest that NO is generated from reactions catalyzed by NO synthase and/or nitrate reductase (Wu and Wu 2008; Tonón et al. 2010), which rely on Ca^{2+} and calmodulin. The key role of these enzymes in eATP-triggered signaling and physiological responses is supported by findings in null mutants of nitrate reductase (*nia1nia2*) or NO synthase, in which eATP-stimulated NO accumulation and physiological reactions were impaired (Reichler et al. 2009; Clark et al. 2011).

4.3 eATP-Regulated Gene Expression

Investigation of gene expression after eATP or apyrase treatment is necessary to clearly understand the role and working mechanism of eATP. eATP-regulated gene expression was investigated in several plant species using cDNA microarray or proteomic analysis methods.

Jeter et al. (2004) reported that wound, exogenous eATP, and osmotic stress all led to increased expression of MAPK signaling components. eATP-induced expression of ethylene biosynthetic enzymes and several ethylene responsive factors (ERF). So, eATP may trigger downstream reactions related to stress tolerance through ethylene signaling and MAPK cascades.

Chivasa et al. (2010) found that ATP upregulated or downregulated many functional genes in tobacco cells. The downregulated proteins include photosynthetic proteins, mitochondrial ATP synthetic proteins, redox proteins, and some defense proteins. Consistent with their downregulation by ATP, expression of defense proteins increased remarkably when eATP signaling was blocked by AMP-PCP. These changes in the expression levels of defense proteins led to a corresponding increase in the resistance of tobacco leaf cells to pathogens after AMP-PCP treatment and a corresponding decrease after ATP treatment. In a 2011 study, Chivasa et al. (2011) pretreated suspension-cultured *Arabidopsis* cells with FB1 to deplete endogenous eATP, then added exogenous ATP into culture media and analyzed changes in protein levels in cells. This analysis led to identification of 26 proteins controlled by eATP, including molecular chaperones, cellular redox enzymes, glycolytic enzymes, and cellular protein degradation machinery components. Although the mechanism of action of FB1 is unclear, FB1 treatment led to significant downregulation of 8 ATP synthase β-subunits, while subsequent ATP treatment restored the expression level of these proteins. As mentioned above, FB1 caused cell death of *Arabidopsis* leaves, but addition of ATP rescued cells from FB1 injury. In ATP synthase β-subunit knockout mutants, FB1-induced cell death was effectively blocked, indicating ATP synthase β-subunit might be a target of FB1. Based on these data, they suggest that FB1 inhibits ATP synthase by suppressing its expression or by directly binding and inhibiting its activity. The consequent disruption of oxidative phosphorylation may be the main reason for FB1-induced cell death. Exogenous ATP restored the expression of these proteins, rescued cellular energy metabolism, and increased cell viability. These findings may explain why eATP-treated cells are resistant to FB1 treatment.

Lim et al. (2014) reported that decreased apyrase expression led to an increase in eATP levels and suppression of plant cell growth rate. In apyrase knockdown lines, upregulation of genes related to systemic acquired resistance, defense response to fungus or bacteria, regulation of H_2O_2 metabolism, regulation of hypersensitive response, and some signal transducer genes (e.g., MAP kinase) was observed, and downregulation of genes related to metal ion and nitrate transport, root hair differentiation, UDP-glycosyltransferase, and sterol biosynthetic process was observed. Further study is necessary to verify the relationship between changes in

gene expression and physiological functions, such as decreased growth rate of root cells and increased resistance to biotic attack.

4.4 Crosstalk Between eATP and Plant Hormones

Plant hormones, including auxin, ethylene, and salicylic acid, are involved in eATP-regulated physiological functions. eATP may regulate some physiological processes through crosstalk with these hormones.

Auxin is involved in eATP-regulated plant cell growth and development. Tang et al. (2003) reported that eATP inhibited root elongation and disturbed root gravitropic growth of *Arabidopsis*. In addition, ATP inhibited basipetal auxin transport in *Arabidopsis* and maize roots, resulting in auxin accumulation in root tip cells. Furthermore, inhibition of apyrase activity significantly changed auxin level and distribution by suppressing auxin polar transport in root and hypocotyl of *Arabidopsis*. This led to abnormal growth behavior of these organs. In root tip of a null mutant of apyrase, asymmetric auxin distribution induced by gravity stimulation was impaired, and the root tip did not show gravitropic growth (Liu et al. 2012).

Ethylene, a key component in stress responses, is also involved in cellular responses to eATP. Wound, eATP, and osmotic stress promoted expression of ACS6, which is a key enzyme for biosynthesis of the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid). ATP also induced expression of several ERFs. These proteins are well-known participants in biotic or abiotic stress-induced plant defense responses. Based on these findings, it was thought that ethylene synthesis and ethylene-stimulated signal transduction could be involved in eATP-induced downstream reactions (Jeter et al. 2004). Consistent with this idea, ATP stimulated ethylene accumulation in cultured ovule cells. ACC strengthened cotton fiber growth stimulated by low doses of eATP, while the ethylene antagonist aminovinylglycine strongly blocked this effect (Clark et al. 2010).

Salicylic acid (SA) is also involved in eATP-induced intracellular responses. SA effectively depleted eATP and induced pathogen resistance responses in *Arabidopsis*. Exogenous ATP decreased intracellular SA level and suppressed pathogen resistance (Chivasa et al. 2009). These findings suggest that eATP may suppress pathogenesis-related responses by inhibiting intracellular SA signaling.

5 Conclusions and Perspectives

Based on current research findings, it can be concluded that ATP exists in the apoplast of plants, where it functions as an essential extracellular messenger to promote regulation of cell metabolism, organ growth and development, and plant resistance to biotic and abiotic stresses. The downstream signal transduction pathways mediated by eATP have been intensively studied in the hopes that they would

reveal insights into the mechanisms of eATP-regulated physiological functions. So far, our knowledge of eATP is still insufficient to clearly understand its novel role as a signaling molecule. Future studies will need to undertake the difficult task of verifying the physiological significance of eATP. We need more research to answer the remaining questions of what underlies the purpose of ATP secretion in plant cells, and why ATP, rather than other metabolites, is secreted as a messenger. To clarify the mechanisms of eATP-regulated physiological responses, the characteristics of eATP receptors and transmembrane signal transducers need to be determined. We need to contribute to current knowledge about eATP-induced intracellular messengers by identifying their targets and downstream biochemical reactions in eATP-regulated physiological responses. We are also in urgent need of determining the reasons for why low and high eATP concentrations lead to the same physiological reactions. To accomplish these tasks, creative ideas and novel research methods are urgently required.

Acknowledgement We thank National Natural Science Foundation of China (Grant No. 31370319) and the Natural Science Foundation of Hebei Province (Grant No. C2014205079) for supporting this work.

References

Bodin P, Burnstock G (2001) Purinergic signalling: ATP release. Neurochem Res 26:959–969

Bouwmeester K, Han M, Blanco-Portales R, Song W, Weide R, Guo L, van der Vossen E, Govers F (2013) The *Arabidopsis* lectin receptor kinase LecRK-1.9 enhances resistance to *Phytophthora infestans* in *Solanaceous* plants. Plant Biotechnol J 12:10–16

Burnstock G (2006) Historical review: ATP as a neurotransmitter. Trends Pharmacol Sci 27:166–176

Burnstock G (2007) Purine and pyrimidine receptors. Cell Mol Life Sci 64:1471–1483

Burnstock G, Arnett T, Orriss I (2013) Purinergic signalling in the musculoskeletal system. Purinergic Signal 9:541–572

Cao Y, Tanaka K, Nguyen C, Stacey G (2014) Extracellular ATP is a central signaling molecule in plant stress responses. Curr Opin Plant Biol 20:82–87

Cavaliere F, Donno C, Ambrosi N (2015) Purinergic signaling: a common pathway for neural and mesenchymal stem cell maintenance and differentiation. Front Cell Neurosci 9:211

Chivasa S, Slabas AR (2012) Plant extracellular ATP signalling: new insight from proteomics. Mol Biosyst 8:445–452

Chivasa S, Ndimba B, Simon W, Robertson D, Yu X, Knox J, Bolwell P, Slabas A (2002) Proteomic analysis of the *Arabidopsis thaliana* cell wall. Electrophoresis 23:1754–1765

Chivasa S, Ndimba BK, Simon WJ, Lindsey K, Slabas A (2005) Extracellular ATP functions as an endogenous external metabolite regulating plant cell viability. Plant Cell 17:3019–3034

Chivasa S, Simon WJ, Murphy AM, Lindsey K, Carr J, Slabas A (2010) The effects of extracellular adenosine 5′-triphosphate on the tobacco proteome. Proteomics 10:235–244

Chivasa S, Tome D, Hamilton J, Slabas A (2011) Proteomic analysis of extracellular ATP-regulated proteins identifies ATP synthase beta-subunit as a novel plant cell death regulator. Mol Cell Proteomics 10:M110.003905

Chivasa S, Murphy A, Hamilton J, Lindsey K, Carr J, Slabas A (2009) Extracellular ATP is a regulator of pathogen defence in plants. Plant J 60:436–448

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee S, Stacey G (2014a) Identification of a plant receptor for extracellular ATP. Science 343:290–294

Choi J, Tanaka K, Liang Y, Cao Y, Lee S, Stacey G (2014b) Extracellular ATP, a danger signal, is recognized by DORN1 in *Arabidopsis*. Biochem J 463:429–437

Clark G, Roux SJ (2009) Extracellular nucleotides: ancient signaling molecules. Plant Sci 177:239–244

Clark G, Roux S (2011) Apyrases, extracellular ATP and the regulation of growth. Curr Opin Plant Biol 14:700–706

Clark G, Torres J, Finlayson S, Guan X, Handley LJ, Kays J, Chen Z, Roux S (2010) Apyrase (NTPDase) and extracellular nucleotides regulate cotton fiber elongation in cultured ovules. Plant Physiol 152:1073–1083

Clark G, Fraley D, Steinebrunner I, Cervantes A, Onyirimba J, Liu A, Torres J, Tang W, Kim J, Roux S (2011) Extracellular nucleotides and apyrases regulate stomatal aperture in *Arabidopsis*. Plant Physiol 156:1740–1753

Crouzet J, Trombik T, Fraysse AS, Boutry M (2006) Organization and function of the plant pleiotropic drug resistance ABC transporter family. FEBS Lett 580:1123–1130

Dark A, Demidchik V, Richards S, Shabala S, Davies J (2011) Release of extracellular purines from plant roots and effect on ion fluxes. Plant Signal Behav 6:1855–1857

Day R, McAlvin C, Loh J, Denny R, Wood T, Young N, Stacey G (2000) Differential expression of two soybean apyrases, one of which is an early nodulin. Mol Plant Microbe Interact 13:1053–1070

Demidchik V, Nichols C, Oliynyk M, Dark A, Glover B, Davies J (2003) Is ATP a signalling agent in plants? Plant Physiol 133:456–461

Demidchik V, Shang Z, Shin R, Thompson E, Rubio L, Laohavisit A, Mortimer J, Chivasa S, Slabas A, Glover B, Schachtman D, Shabala S, Davies J (2009) Plant extracellular ATP signaling by plasma membrane NADPH oxidase and Ca^{2+} channels. Plant J 58:903–913

Deng S, Sun J, Zhao R, Ding M, Zhang Y, Sun Y, Wang W, Tan Y, Liu D, Ma X, Hou P, Wang M, Lu C, Shen X, Chen S (2015) *Populus euphratica* APYRASE2 enhances cold tolerance by modulating vesicular trafficking and extracellular ATP in *Arabidopsis* plants. Plant Physiol 169:530–548

Drury AN, Szent-Györgyi A (1929) The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J Physiol 68:213–237

Etzler M, Kalsi G, Ewing N, Roberts N, Day R, Murphy J (1999) A nod factor binding lectin with apyrase activity from legume roots. Proc Natl Acad Sci U S A 96:5856–5861

Ferrari D, Vitiello L, Idzko M, la Sala A (2015) Purinergic signaling in atherosclerosis. Trends Mol Med 21:184–192

Foresi NP, Laxalt AM, Tonón CV, Casalongué C, Lamattina L (2007) Extracellular ATP induces nitric oxide production in tomato cell suspensions. Plant Physiol 145:589–592

Fountain S, Cao L, Yound M, North R (2008) Permeation properties of a P2X receptor in the green algae *Ostreococcus tauri*. J Biol Chem 283:15122–15126

Hao L, Wang W, Chen C, Wang Y, Liu T, Li X, Shang Z (2012) Extracellular ATP promotes stomatal opening of *Arabidopsis thaliana* through heterotrimeric G protein α subunit and reactive oxygen species. Mol Plant 5:852–864

Idzko M, Ferrari D, Riegel A, Eltzschig H (2014) Extracellular nucleotide and nucleoside signaling in vascular and blood disease. Blood 124:1029–1037

Jaffe M (1973) The role of ATP in mechanically stimulated rapid closure of the venus's-flytrap. Plant Physiol 51:17–18

Jeter C, Tang W, Henaff E, Butterfield T, Roux S (2004) Evidence of a novel cell signalling role for extracellular adenosine triphosphates and diphosphates in *Arabidopsis*. Plant Cell 16:2652–2664

Kennedy C (2015) ATP as a cotransmitter in the autonomic nervous system. Auton Neurosci 191:2–15

Kim SY, Sivaguru M, Stacey G (2006) Extracellular ATP in plants. Visualization, localization, and analysis of physiological significance in growth and signaling. Plant Physiol 142:984–992

Kim S, Yang S, Kim T, Han J, Suh J (2009) Hypertonic stress increased extracellular ATP levels and the expression of stress responsive genes in *Arabidopsis thaliana* seedlings. Biosci Biotechnol Biochem 73:1252–1256

Lew RR, Dearnaley J (2000) Extracellular nucleotide effects on electrical properties of growing *Arabidopsis thaliana* root hairs. Plant Sci 153:1–6

Lim M, Wu J, Yao J, Gallardo I, Dugger J, Webb L, Huang J, Salmi M, Song J, Clark G, Roux S (2014) Apyrase suppression raises extracellular ATP levels and induces gene expression and cell wall changes characteristic of stress responses. Plant Physiol 164:2054–2067

Liu X, Wu J, Clark G, Lundy S, Lim M, Arnold D, Chan J, Tang W, Muday G, Gardner G, Roux S (2012) Role for apyrases in polar auxin transport in *Arabidopsis*. Plant Physiol 160:1985–1995

Lüttge U, Schöch EV, Ball E (1974) Can externally applied ATP supply energy to active ion uptake mechanisms of intact plant cells? Aust J Plant Physiol 1:211–220

McAlvin C, Stacey G (2005) Transgenic expression of the soybean apyrase in *Lotus japonicus* enhances nodulation. Plant Physiol 137:1456–1462

Nejidat A, Itai C, Roth-Bejerano N (1983) Stomatal response to ATP mediated by phytochrome. Physiol Plant 57:367–370

Raghavendra A (1981) Energy supply for stomatal opening in epidermal strips of *Commelina benghalensis*. Plant Physiol 67:385–387

Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50:415–492

Reichler S, Torres J, Rivera AL, Cintolesi V, Clark G, Roux S (2009) Intersection of two signaling pathways: extracellular nucleotides regulate pollen germination and pollen tube growth via nitric oxide. J Exp Bot 60:2129–2138

Riewe D, Grosman L, Fernie A, Wucke C, Geigenberger P (2008) The potato-specific apyrase is apoplastically localized and has influence on gene expression, growth, and development. Plant Physiol 147:1092–1109

Roux SJ, Steinebrunner I (2007) Extracellular ATP: an unexpected role as a signaler in plants. Trends Plant Sci 12:522–527

Schiller M, Massalski C, Kurth T, Steinebrunner I (2012) The *Arabidopsis* apyrase AtAPY1 is localized in the Golgi instead of the extracellular space. BMC Plant Biol 12:123

Song C, Steinebrunner I, Wang X, Stout S, Roux S (2006) Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in *Arabidopsis*. Plant Physiol 140:1222–1232

Steinebrunner I, Wu J, Sun Y, Corbett A, Roux S (2003) Disruption of apyrases inhibits pollen germination in *Arabidopsis*. Plant Physiol 131:1638–1647

Sun J, Zhang C, Deng S, Lu C, Shen X, Zhou X, Zheng X, Hu Z, Chen S (2012a) An ATP signalling pathway in plant cells: extracellular ATP triggers programmed cell death in *Populus euphraticapce*. Plant Cell Environ 35:893–916

Sun J, Zhang X, Deng S, Zhang C, Wang M, Ding M, Zhao R, Shen X, Zhou X, Lu C, Chen S (2012b) Extracellular ATP signaling is mediated by H_2O_2 and cytosolic Ca^{2+} in the salt response of *Populus euphratica* cells. PLoS One 12, e53136

Tanaka K, Gilroy S, Jones AM, Stacey G (2010a) Extracellular ATP signaling in plants. Trends Cell Biol 20:601–608

Tanaka K, Swanson S, Gilroy S, Stacey G (2010b) Extracellular nucleotides elicit cytosolic free calcium oscillations in *Arabidopsis*. Plant Physiol 154:705–719

Tanaka K, Choi J, Cao Y, Gary S (2014) Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. Front Plant Sci 5, Article 446

Tang W, Brady S, Sun Y, Muday G, Roux S (2003) Extracellular ATP inhibits root gravitropism at concentrations that inhibit polar auxin transport. Plant Physiol 131:147–154

Thomas C, Sun Y, Naus K, Lloyd A, Roux S (1999) Apyrase functions in plant phosphate nutrition and mobilizes phosphate from extracellular ATP. Plant Physiol 119:543–551

Thomas C, Rajagopal A, Windosr B, Dudler R, Lloyd A, Roux S (2000) A role for ectophosphatase in xenobiotic resistance. Plant Cell 12:519–533

Tonón C, Terrile MC, Iglesias MJ, Lamattina L, Casalongué C (2010) Extracellular ATP, nitric oxide and superoxide act coordinately to regulate hypocotyl growth in etiolated *Arabidopsis* seedlings. J Plant Physiol 167:540–546

Udvardy J, Farkas GL (1973) ATP stimulates the formation of nucleases in excised *Avena* leaves. Z Pflanzenphysiol 69:394–401

Vanegas D, Clark G, Cannon A, Roux S, Chaturvedi P, McLamore E (2015) A self-referencing biosensor for real-time monitoring of physiological ATP transport in plant systems. Biosens Bioelectron 74:37–44

Wang F, Jia J, Wang Y, Wang W, Chen Y, Liu T, Shang Z (2014) Hyperpolization-activated Ca^{2+} channels in guard cell plasma membrane are involved in extracellular ATP-promoted stomatal opening in *Vicia faba*. J Plant Physiol 171:1241–1247

Weerasinghe R, Swanson S, Okada S, Garrett M, Kim S, Stacey G, Boucher R, Gilroy S, Jones A (2009) Touch induces ATP release in *Arabidopsis* roots that is modulated by the heterotrimeric G-protein complex. FEBS Lett 583:2521–2526

Windsor B, Roux SJ, Lloyd A (2003) Multiherbicide tolerance conferred by AtPgp1 and apyrase in *Arabidopsis*: a novel mechanism of herbicide resistance. Nat Biotechnol 21:428–433

Wolf C, Hennig M, Romanovicz D, Steinebrunner I (2007) Developmental defects and seedling lethality in apyrase *AtAPY1* and *AtAPY2* double knockout mutants. Plant Mol Biol 64:657–672

Wu S, Wu J (2008) Extracellular ATP-induced NO production and its dependence on membrane Ca^{2+} flux in *Salvia miltiorrhiza* hairy roots. J Exp Bot 59:4007–4016

Wu J, Steinebrunner I, Sun Y, Butterfield T, Torres J, Arnold D, Gonzalez A, Jacob F, Reichler S, Roux S (2007) Apyrases (NTPDases) play key role in growth control in *Arabidopsis*. Plant Physiol 144:961–975

Wu S, Liu Y, Wu J (2008) The signaling role of extracellular ATP and its dependence on Ca^{2+} flux in elicitation of *Salvia miltiorrhiza* hairy root cultures. Plant Cell Physiol 49:617–624

Exploration of Sweet Immunity to Enhance Abiotic Stress Tolerance in Plants: Lessons from CAM

Nathalie Ceusters, Wim Van den Ende, and Johan Ceusters

Contents

Abstract The concept of 'sweet immunity' or 'sugar-enhanced defence' is based on the accumulating evidence that sweet, endogenous saccharides might act as signalling molecules that are activated by exposure to stress and hence initiate signal amplification and lead to more rapid and robust activation of defence, immunity and stress tolerance. Sugars such as glucose, fructose and sucrose have acquired important regulatory functions in evolution and are becoming more and more recognized as signalling molecules in plants controlling gene expression related to plant metabolism, stress resistance and development. This offers opportunities for 'sweet priming', defined as a physiological process that prepares plants for a faster and/or stronger defence response to future stress conditions, but does not impose the costs associated with full implementation of an induced defence

N. Ceusters • J. Ceusters (✉)
Faculty of Engineering Technology, Department of Microbial and Molecular Systems, Bioengineering Technology TC, KU Leuven, Technology Campus Geel, Kleinhoefstraat 4, 2440 Geel, Belgium
e-mail: johan.ceusters@kuleuven.be

W. Van den Ende
Faculty of Sciences, Department of Biology, Laboratory of Molecular Plant Biology, KU Leuven, Kasteelpark Arenberg 31, 3001 Leuven, Belgium

Progress in Botany (2017) 78: 145–166, DOI 10.1007/124_2016_1,
Published online: 2 June 2016

response. Future possibilities to substitute toxic agrochemicals with biodegradable sugar-(like) compounds in agricultural and horticultural practice requires a thorough understanding of how sugars can play a crucial role in perceiving, anticipating and counteracting abiotic stresses. In this review, the physiological responses of crassulacean acid metabolism (CAM) plants to different conditions of abiotic stress will be discussed with particular attention to sucrose dynamics. CAM plants are ideally suited to different abiotic stress conditions and carbohydrate cycling and availability are of paramount importance for plant growth, photosynthesis and homeostasis. By evaluating the plethora of effects sugars can exert on plant metabolism, growth and development the possibilities for sugars as potential priming agents to enhance abiotic stress tolerance will be explored.

1 Introduction

1.1 Crassulacean Acid Metabolism

Crassulacean acid metabolism (CAM) is one of three metabolic pathways present in the photosynthetic tissues of vascular plants with optimized water-use efficiency (WUE) by taking up CO_2 at night when evapotranspiration rates are low. When stomata are open at night CO_2 is sequestered via the enzyme phosphoenolpyruvate carboxylase (PEPC) with the 3-C substrate, phosphoenolpyruvate (PEP) provided from the glycolytic breakdown of carbohydrate accumulated during the previous day. Depending on the species and environmental conditions this carbohydrate source constitutes vacuolar sugars (i.e. glucose, fructose and/or sucrose), chloroplastic starch or a combination of both. The final 4-C product, malic acid, is stored in a large central vacuole, and is subsequently broken down by malic enzyme (ME) or PEPcarboxykinase to release CO_2 that is fixed by Rubisco during the following day behind closed stomata, thereby conserving water (Borland et al. 2011). This photosynthetic specialization is a striking example of convergent evolution of plants in response to selective pressures imposed by the daytime limitation of CO_2 availability brought about by water-conserving stomatal closure in arid terrestrial habitats, and is estimated to be present in approximately 7 % of vascular plant species (Winter and Smith 1996; Cushman 2001; Cushman and Borland 2002). An ubiquitous feature of the majority of CAM plants is the integration of circadian and metabolite control over nocturnal C_4 and daytime C_3 carboxylation processes, hereby providing plasticity for optimizing carbon gain and water use by extending or curtailing the period of net CO_2 uptake over any 24-h period (Borland et al. 1999; Ceusters et al. 2010; Haydon et al. 2011). Traditionally,

diel CAM has been defined within a four-phase framework to describe the photosynthetic performance (Osmond 1981). Enormous variations in the patterns of diel CAM photosynthesis are commonplace, which underpins the ecological diversity of CAM species and contributes to the potential for high biomass production in water-limited habitats (Yang et al. 2015). Depending on the species concerned, its developmental state and prevailing environmental conditions, a variety of CO_2 assimilation, acid flux and stomatal behaviour characteristics may be observed outside the conventional framework (Winter and Smith 1996; Dodd et al. 2002).

1.2 Sweet Immunity

Since vascular plants are sessile organisms they cannot escape from their continuously changing environment and as such they need to develop several adaptation mechanisms to defend themselves and to ensure their existence. Plants habitat a variety of environments with certain limitations (rainforest, desert, arctic conditions) to which organisms need to adapt. In order to survive, plants exhibit different morphological (e.g. reduction of surface area, reduction in air flow and reflectivity) and physiological (e.g. succulence, CAM, dormancy and modification of seeds) adaptations. Plant innate immunity involves two kinds of responses: (1) a response to slowly emerging pathogen/microbe-associated molecular patterns (PAMP or MAMP, respectively) or damage-associated molecular patterns (DAMPs) through transmembrane pattern recognition receptors leading to PAMP- or DAMP-triggered immunity and (2) a response inside the cell via the activation of resistance genes, known as effector-triggered immunity (Jones and Dangl 2006; Chisholm et al. 2006). Widely accepted players in this plant innate immunity are plant cell-wall-derived and pathogen-derived oligosaccharides. Sugars play multiple roles in all aspects of plant life and are well-known to activate various pathogenesis related (PR) genes (Johnson and Ryan 1990; Herbers et al. 1996a, b; Van den Ende 2013). Evidence is accumulating that sweet, endogenous saccharides might fulfil crucial roles in perceiving, mediating and counteracting both biotic and abiotic stresses. Next to their role as carbon and energy source, sugars such as glucose, fructose and sucrose have acquired important regulatory functions in evolution and are becoming more and more recognized as signalling molecules in plants controlling gene expression related to plant metabolism, stress resistance and development (Pego et al. 2000; Rolland et al. 2006; Ramon et al. 2008; Bolouri-Moghaddam et al. 2010; Trouvelot et al. 2014). This novel concept termed 'sweet immunity' or 'sugar-enhanced defence' consists of the principle that after sugars have been detected by sensors at the plasma membrane (as DAMPs that are released from affected cells) or a specific intracellular location, the information is passed on through signal transduction and amplifying cascades, ultimately resulting in appropriate responses, varying from changes in gene expression to altered enzyme activities. This offers opportunities for 'sweet priming', by immersing seeds or foliar spraying (Trouvelot et al. 2014; Paparella et al. 2015). Priming can be defined

as a physiological process that prepares plants for a faster and/or stronger defence response to future biotic or abiotic stresses (Conrath 2011; Bolouri-Moghaddam and Van den Ende 2012). The achieved condition of readiness due to priming allows for accelerated induced resistance once an attack occurs, but does not impose the costs associated with full implementation of an induced defence response.

Fructans and raffinose family oligosaccharides (RFOs) are water-soluble sucrose-derived carbohydrates, which play a role in abiotic stress responses through membrane stabilization and antioxidant effects (Demel et al. 1998; Van den Ende 2013). Together with glucose, fructose and sucrose, cell-wall-derived oligosaccharides, some rare and non-reducing sugars, and plant hormones have been described (or suggested) to fulfil a potential role in sweet immunity processes.

Glucose signalling by hexokinase (HXK) is the best characterized pathway (Moore et al. 2003; Sheen 2014), but the dual role of HXK as a metabolic enzyme and sensor complicates the separation of metabolic and signalling effects (Kunz et al. 2015). Data indicate that only hexoses and glucose analogues that can be phosphorylated by HXK are effective to elicit responses (Jang et al. 1997).

Sugar sensing through cell-wall-derived oligogalacturonides (OGs) represents a HXK-independent pathway. These oligosaccharides have been indicated as DAMPs (Ferrari et al. 2013) and elicit a wide range of defence responses in several plant species, with wall-associated kinase 1 (WAK1) as the actual receptor (Brutus et al. 2010). Through sensing of extracellular OGs, which are thought to be released from the plant cell wall during pathogen attack or abiotic stresses, the WAK signalling pathway controls the expression of many defence genes and alters cellular sugar metabolism.

Also some rare sugars such as psicose and D-allose and the sugar like 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, a fructose analogue) can stimulate the plant immune system and upregulate defence-related genes by acting as signalling molecules through HXK-dependent or independent pathways (Birch et al. 1993; Moore and Sheen 1999; Ramon et al. 2008; Kano et al. 2011).

Glucose, fructose and sucrose-specific signalling pathways have now been described in plants and in addition to the total amounts of these small water-soluble carbohydrates, it seems that the relative proportions of these sugars are more important (Rolland et al. 2002; Bolouri-Moghaddam and Van den Ende 2013a; Van den Ende and El-Esawe 2014). Thus, invertases and other sucrose splitting enzymes (e.g. sucrose synthase or Susy), which are involved in carbohydrate partitioning and the regulation of sucrose:hexose ratios, can have a drastic impact on these different signalling pathways (Ruan 2014).

Trehalose is another well-known non-reducing sugar and potential signalling metabolite in plant responses to both biotic and abiotic stresses. It is also involved in the regulation of stomatal conductance and WUE (Fernandez et al. 2010; Gao et al. 2013a, b; Lunn et al. 2014; Tayeh et al. 2014). Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is linked to important alterations in carbohydrate metabolism (e.g. altered sucrose-to-starch ratio) and it is well established that Tre6P levels in plant tissues change in parallel with endogenous

or imposed fluctuations in sucrose content. Suggested is a bi-directional network, in which Tre6P is a signal of sucrose availability and that the Tre6P:sucrose ratio is a critical parameter for the plant and forms part of a homeostatic mechanism to maintain sucrose levels within a range that is appropriate for the cell type and developmental stage of the plant (Yadav et al. 2014). Transgenic plants transformed with the gene for trehalose-6-phosphate synthase (TPS1) exhibited an improved drought tolerance with a better photosynthetic efficiency and a higher dry weight accumulation (Holmstrom et al. 1996). Among the potential Tre6P targets are members of the sucrose-non-fermenting-1-related kinase1 (SnRK1) family, which are characterized as central regulatory instances linking sugar metabolism to signalling networks and energy metabolism and inhibit plant growth and development during metabolic stress to maintain energy homeostasis (Tsai and Gazzarrini 2014; Nägele and Weckwerth 2014).

Furthermore, it has been documented that especially the longer water-soluble sucrose-derived oligo- and polysaccharides, such as fructans and RFOs, are involved in antioxidant systems where they might function as effective candidates for capturing reactive oxygen species (ROS) in tissues exposed to a wide range of environmental stresses (Van den Ende and Valluru 2009; Peshev et al. 2013). In an environment of molecular oxygen (O_2), all living cells are confronted with the reactivity and toxicity of active and partially reduced forms of oxygen. In plants, ROS are continuously produced during mitochondrial respiration and photosynthesis and the incongruity between the generation of toxic ROS and the availability of their scavengers-antioxidants results in oxidative stress (Asada 1999; Moller 2001; Couée et al. 2006; Bolouri-Moghaddam et al. 2010). Due to their extremely high reactivity and short lifetime, hydroxyl radicals form a special case, because cells have not been able to develop enzymes to detoxify these most dangerous ROS. Thus, scavenging of hydroxyl radicals can only occur by an accumulation of higher levels of organic compounds, such as sucrose, fructans or RFOs (Peukert et al. 2014; Matros et al. 2015).

Plant responses to environmental challenges, pathogens and abiotic stresses such as drought and salt stress, are also mediated by several plant hormones. Besides their well-documented physiological roles in plant growth and development, some phytohormones, such as salicylic acid (systemic acquired resistance), and jasmonate/ethylene (induced systemic resistance) are known to function as signalling molecules for stimulation of plant innate immunity to activate defence responses (Pieterse et al. 2009; Dicke and van Loon 2014). Genetic and molecular studies of sugar signalling mutants in *Arabidopsis* have revealed a tight interaction of sugar responses to abscisic acid (ABA) and ethylene signalling, in opposite ways (Finkelstein and Gibson 2001; León and Sheen 2003; Gibson 2004). Another group of plant hormones that interact in complex networks to balance the response to developmental and environmental cues and play a key role in plant defence are the cytokinins (Giron et al. 2013). The role for brassinosteroids (BRs) and gibberellins (GAs) is also particularly important in view of the 'trade-off' between growth and defence responses as reviewed by De Bruyne et al. (2014).

1.3 CAM and Sweet Immunity

In CAM plants, where carbohydrate cycling and availability are of paramount importance for plant photosynthesis, homeostasis and production, a central role for sucrose might be expected. Data show that CAM species which predominantly use starch to fuel nocturnal carboxylation still keep a similar, although to a lesser extent, diel cycle of sucrose constituting daytime increase and nocturnal degradation (Ceusters et al. 2010). Also a switch from starch to sucrose as the major carbohydrate source was noticed in a CAM bromeliad under different abiotic stresses such as drought stress and extremely low levels of irradiance (Ceusters et al. 2009a, b, 2011). After recovery from drought, carbohydrate was used predominantly for provision of substrate for nocturnal water-saving carboxylation whilst net carbon export (i.e. to sinks) was limited. This conservation of resources might be sucrose-mediated and adaptive with regard to responding more efficiently to a return of water stress. As such, leaf carbohydrate status and sugar transporters might serve as central checkpoints in CAM plants dividing carbohydrate towards competing forces of nocturnal carboxylation and export for growth (Antony and Borland 2009; Ceusters et al. 2010). The precise mechanisms are still unknown but sucrose might be involved. In addition the circadian clock has been identified as a central oscillator underlying the biophysical oscillator which mediates vacuolar malate transport dynamics in CAM (Lüttge 2000; Boxall et al. 2005). As such the molecular clock influences carboxylation, carbohydrate partitioning and successful adaptation under changing environments. The sweet immunity concept has very recently been situated in the plant circadian network. The circadian oscillator is particularly sensitive to sucrose-mediated signalling, which can affect circadian rhythms by altering the expression pattern of clock-regulated genes. Moreover, the expression of the clock genes themselves is also responsive to sugars; in particular, the clock's core central oscillators are stimulated by sucrose (Bolouri-Moghaddam and Van den Ende 2013a, b).

All these observations in CAM plants clearly highlight a potential central role for sucrose in perceiving, anticipating and counteracting abiotic stresses and pave the way for further investigation of the underlying mechanisms of the novel concept of sweet immunity in CAM plants.

2 Abiotic Stress Tolerance of CAM Plants

In our continuously changing world, plants are subjected to a plethora of stresses which can be divided into two main categories being: (1) biotic stress, as a result of damage suffered by plants due to other living organisms and (2) abiotic or environmental stress, as a result of the negative impact of non-living items that influence the environment beyond its normal range of variation. Since plants are sessile organisms, they have to develop mechanisms to cope with these stressors. In

this review the emphasis is on the aspect of abiotic stress. Stress can be defined as the response of a plant to a stressor such as an environmental condition or a stimulus and is followed by a particular pattern of physiological responses (Duque et al. 2013). Seasonal and circadian environmental variables are regular and predictable factors which do not cause stress due to the flexibility of normal metabolism. Apart from these regular perturbations, plants are typically exposed to different conditions of abiotic stress such as drought, salinity, light stress, extreme temperatures, nutrient stress and exposure to heavy metals during their entire life cycle (Clarkson et al. 2000; Sairam and Tyagi 2004; Jaleel et al. 2009; Osakabe and Osakabe 2012; Islam et al. 2015; Ordoñez-Salanueva et al. 2015). These are all unpredictable fluctuations imposed on regular metabolic patterns causing injury, disease or aberrant physiology. The complexity of it all lies in the fact that plants are not subjected to just one deviating environmental factor, but a complex of various interacting environmental factors contributes at varying degrees to overall stress. The precise underlying mechanisms to perceive and overcome these abiotic stresses remain to be established but involve the activation of multiple responses involving complex gene interactions and crosstalk with many molecular pathways (Debnath et al. 2011).

Water deficit and soil salinity are global issues, which menace the survival of agricultural crops and food production and can be categorized as most common stresses. Both stressors have an adverse effect on plant productivity and growth due to dehydration and osmotic imbalance of the cell. Drought is a weather-related phenomenon that can be defined as an abiotic stressor since it causes reduction of the available water in the soil and due to the atmospheric conditions it also causes continuous loss of water by transpiration or evaporation. In general, water deficit affects various physiological and biochemical processes and mostly leads to stomatal closure and limitation of gas exchange together with diminished leaf water potentials, turgor loss and decrease in cell enlargement and growth. Continuous and severe drought can potentially lead to an arrest of photosynthesis, gross disruption of metabolism and eventually to the cessation of enzyme catalysed reactions which may finally result in death of the plant (Smirnoff 1993; Jaleel et al. 2008). Due to their nocturnal carboxylation, obligate CAM plants are very well adapted to this abiotic stressor of water scarcity. The same holds true for facultative CAM species which are able to switch from C_3 to CAM under conditions of stress. The overall high WUE provides CAM with a great benefit in water-limited terrestrial environments by reducing stomatal opening during daytime (i.e. restricting gas exchange during Phases II and IV), as lower nocturnal air temperatures result in a reduced transpiration, allowing water to be conserved and drought stress to be avoided (Kluge and Ting 1978; Winter et al. 2005). More recently, carbohydrate plasticity has also been proposed to underpin drought acclimation of CAM plants, as a gradual progression has been noticed from starch to sucrose as transitory carbohydrate source to replenish the PEP building blocks to sustain nocturnal CO_2 uptake under progressing drought in *Aechmea* 'Maya' (Ceusters et al. 2009a, b). Ubiquitous among CAM plants is the remarkable adaptation known as CAM-idling to survive severe drought stress for longer periods. During this physiological response,

stomata remain closed during day and night, restricting water loss and exogenous CO_2 uptake, whereby small diel fluctuations of organic acids are observed mainly through recycling of internally produced CO_2 without any measurable gas exchange (Ting 1985). In addition, several CAM plants possess other structural and functional ways of dealing with water storage: short-term storage of water is mediated by diel cycles of osmotic solutes (i.e. organic acids), medium-term water storage can be achieved by forming external water reservoirs (phytotelmata) and water can also be stored for the longer term due to special non-green and non-photosynthetical water storage tissues, i.e. hydrenchymas (Lüttge 2004; Frank 2005).

Salts are natural elements of soils and water but their excess accumulation in soils adversely affects plant growth and development and can even become toxic. The major causes of salinization (i.e. the process of increasing salt content) are high levels of salt in irrigation water, landscape features that allow salts to become mobile, climatic trends that favour accumulation, human activities with improper irrigation systems, land clearing and deforestation (Yadav et al. 2011). Salt decreases the osmotic potential of the soil solution, can cause severe ion toxicity and might result in nutrient imbalances and deficiencies. Salinity and drought are two abiotic stressors that work synergistically since an excess of sodium ions due to salinization leads to mal-textured soil, hindered porosity and aeration which subsequently intensifies the effects of physiological water deficit. In general most CAM plants are not halophytic but highly salt sensitive (Nobel 1983; Nobel and Berry 1985). However, some species such as the facultative CAM plant *Mesembryanthemum crystallinum* are capable of adjusting to and surviving in highly saline conditions. Glycophytic CAM species inhabiting saline ecosystems are effectively functional salt excluders at the root level or mainly stress avoiders displaying either morphological root system dynamics in response to periodic stress or complete escape from the saline substrate by retreating to epiphytic niches (Nobel et al. 1984; Lüttge 2004).

Due to their sessile nature, plants need to be extremely adaptable to light. In order to execute the appropriate physiological and developmental processes plants need to monitor light quality, quantity, periodicity and direction. In response to a changing photoperiod or high light intensities, some facultative species such as *Clusia minor* and *Kalanchoë blossfeldiana* are able to upregulate CAM expression (Borland et al. 1993; Brulfert et al. 1973, 1988). CAM habitats are generally characterized by high solar radiations, e.g. exposed terrestrial or epiphytic habitats where photosynthetic active radiation (PAR) is not limiting photosynthesis (Lüttge 2002). Rather overenergization of the photosynthetic apparatus in CAM plants might form a greater threat. It has often been argued that high internal partial pressures of CO_2 (pCO_2) built up in Phase III protects CAM plants from overenergization by using most of the excitation energy for photosynthesis. However photorespiration, photoinhibition and oxidative stress have been noted during the light period including Phase III (Spalding et al. 1979; Lüttge 2010). To deal with those problems, CAM plants possess an entire complement of energy dissipation methods such as non-photochemical quenching via zeaxanthin and the futile

xanthophyll cycle of epoxidation and de-epoxidation (Castillo 1996; Miszalski et al. 1998; Broetto et al. 2002; Lu et al. 2003). However, under light limiting conditions as sometimes experienced by greenhouse cultured CAM ornamentals in northern hemisphere regions, CAM has recently been shown to cause overacidification of the cytosol probably brought about by a stunted decarboxylation, resulting in chlorenchyma cell death (Ceusters et al. 2011). Besides acting as the energy source of photosynthesis, light also affects expression and performance of CAM via different signalling pathways (Lüttge 2004). The precise effects of light quality on the performance of CAM are still to be established but blue light has recently been identified as a key determinant in regulating stomatal responses, organic acid mobilization from the vacuole and daytime decarboxylation. Red and/or green light, on the other hand, seems inevitable to align the diel turnover of storage carbohydrates (i.e. starch and sucrose) with malic acid dynamics (Ceusters et al. 2014).

The performance of CAM is widely assumed to be favoured by relatively low night and high day temperatures (Brandon 1967). The major effects result from interactions with individual enzymes, membranes, respiratory activity and stomatal aperture and from modulating the impact of other factors. Many studies have demonstrated that the carboxylating enzymes (PEPC and malate dehydrogenase) are favoured by lower temperatures while the decarboxylating enzymes, e.g. ME, are stimulated by higher temperatures (Buchanan-Bollig et al. 1984; Lüttge 2004, 2006; Guo and Lee 2006; Chen et al. 2008). Another influence of temperature is on the cell membranes, whereby temperature directly affects fluidity and hence permeability which is very important for organic acid compartmentation (Friemert et al. 1988). However, to optimize vacuolar transport of metabolites under different growth temperatures, CAM plants have been found to dispose of a certain degree of flexibility to phenotypically adapt their tonoplast fluidity (Kliemchen et al. 1993; Behzadipour et al. 1998). In some CAM plants stomata are highly sensitive to air humidity whereby temperature effects on air humidity may largely determine relationships between temperature and stomatal opening and hence influence nocturnal CO_2 uptake (Friemert et al. 1986). Partitioning of assimilates between various plant organs might also be affected by temperature as well as the ratio of sucrose over starch which increases due to a higher respiration when the temperature is elevated (Wolf et al. 1991; Geigenberger et al. 1998). More specifically, in *Phalaenopsis* translocation of sucrose was affected under high temperatures, since in warm-treated plants foliar spraying of sucrose to the source leaves increased sugar contents in the leaves but not in the inflorescence (Chen et al. 1994).

Since metal compartmentalization is a key component of metal tolerance, the morphological and physiological features of CAM plants like thick cuticles, low surface-to-volume ratios and large vacuoles offer an advantage to these plants to adapt to increasing levels of environmental pollution and toxic products from anthropogenic activities. Kholodova et al. (2011) demonstrated that the facultative halophyte *M. crystallinum* is able to adapt to excess copper and zinc and complete its life cycle by a shift from C_3- to CAM-type photosynthesis. When subjected to heavy metals, a complex suite of changes related to water status was initiated

including a decrease in root sap exudation, a heavy-metal induced proline accumulation and a decrease in leaf water content followed by a water deficit in leaf tissues. Information about the immediate changes in the roots was rapidly spread across the plant leading to a decrease in transpiration rate, which might be involved in the differential expression of aquaporin genes as well as the acceleration of the shift to CAM photosynthesis and the subsequent intensification of this process. *Sedum alfredii* Hance, which belongs to the Crassulaceae family, has been described as a zinc (Zn)/cadmium (Cd) co-hyperaccumulator which is also highly tolerant to copper (Cu) and lead (Pb) toxicity (Yang et al. 2006; Tian et al. 2011; Gao et al. 2013a, b). Heavy-metal stress (such as cadmium and nickel) can also affect the distribution of assimilates between organs as noticed in the C_3 plant *Oryza sativa* where a significant increase in carbohydrates such as starch, total soluble sugars and sucrose in the shoots accompanied heavy-metal exposure. Moreover, heavy-metal treatment of seedlings caused an inhibition of the transport of carbohydrate reserves from the seeds to the developing shoots and roots (Moya et al. 1993).

It is obvious that in general CAM photosynthesis can be considered as an additional source of physiological plasticity for plants living in continuously changing environments to ensure metabolic recovery and maintenance of homeostasis during and after periods of stress. Flexibility in the duration and intensity of the different phases of gas exchange and the availability and use of different transitory storage carbohydrate pools (e.g. glucose, fructose, sucrose or starch) are key determinants to secure CO_2 sequestration and biomass production in a changing world.

3 Pleiotropic Effects of Sugars

Sugars play multiple roles in all aspects of plant life: they are necessary for the generation of energy and metabolic intermediates, ribose and deoxy-ribose sugars form part of the structure of DNA and RNA, polysaccharides are major structural elements of plant cell walls and glycosylation is required for proper functioning of many proteins and lipids (Yu 1999). Besides their typical roles as carbon and energy sources, sugars such as glucose, fructose and sucrose are becoming more and more recognized as signalling molecules in plants with profound effects on plant metabolism, growth and development and stress responses.

3.1 Sugars and Plant Metabolism

Metabolism can be defined as the physical and chemical processes occurring within each living organism necessary for the maintenance of life, whereby some substances are degraded to yield energy for vital processes while other substances are

synthesized. Sugars are essential components of many metabolic processes, since sugar starvation generally triggers sequential changes in the following cellular events: (1) arrest of cell growth, (2) rapid consumption of cellular carbohydrate content and decrease in respiration rate, (3) degradation of lipids and proteins, (4) increase in accumulation of inorganic phosphate (Pi), phosphorylcholine and free amino acids and (5) decline in glycolytic enzymatic activities (Yu 1999). These effects are believed to be mediated via modulation of gene expression. Depending on the developmental stage of the plant and the environmental conditions, soluble sugars can exert different effects on gene expression and metabolism. In cold acclimated plants overall functioning and stability of the photosynthetic apparatus seems to be related to increased raffinose and sucrose levels (Tarkowski and Van den Ende 2015 and references therein). On the contrary, it has also been demonstrated that increased sucrose and glucose concentrations can suppress photosynthetic gene expression (Sheen 1994; Nie et al. 1995). For example, in the process of senescence, sugars are involved by causing repression of photosynthetic gene expression and activity (Pourtau et al. 2006). In addition, different experiments with exogenous supply of sucrose have indicated a significant infringe of photosynthesis together with a strong decrease in the abundance, activation state and activity of Rubisco and PEPC (Yoon et al. 2009; Moreira Lobo et al. 2015).

Generally transcript levels of thousands of genes correlate with changes in sugar levels (Kunz et al. 2015). Expression of these sugar-sensitive plant genes can be regulated at the transcriptional level or being mediated by mRNA stability. The abundance/activity of enzymes related with biosynthesis, utilization and storage of reserves (including starch, lipid and proteins) is mainly favoured by sugars. On the contrary, abundance/activity of enzymes involved in photosynthesis and reserve mobilization is repressed by high sugar contents (Koch 1996). A compelling amount of scattered information indicates that sucrose signals may control a vast array of metabolic processes. The concentration of sucrose in plant tissues correlates directly with light intensity and is inversely related to temperature. The effects of cold on the induction of fructan synthesis can be attributed to an increasing cell concentration of sucrose due to lower carbon utilization (Martínez-Noël et al. 2009). Within higher plants, sucrose is not just only the major form in which carbohydrate is transported, but it also fulfils a regulatory and integrative function (Farrar et al. 2000). When the level of sucrose exceeds a certain threshold it appears to act as a signalling molecule that initiates or activates starch and fructan synthesis by upregulation of diverse genes, by post-translational redox modifications and by enhancement of the expression of certain transporters related to carbohydrate uptake (Tognetti et al. 2013; Van den Ende and El-Esawe 2014). Sucrose also seems to have an effect on the photosynthetic capacity, although it is not always clear whether sucrose plays a signalling function or if it exerts a feedback effect as an end product. By upregulation of the gene that encodes for UDP-glucose pyrophosphorylase (UGPase), sucrose also controls indirectly its own synthesis. UDP-glucose (UDPG) is a key precursor to numerous polysaccharides in plants and it is utilized in the synthesis of sucrose and cell wall polysaccharides and also in the production of diverse carbohydrates (Ciereszko et al. 2004). The

systemic distribution of photosynthate (assimilate partitioning) is also controlled by sucrose at the level of phloem translocation by a sucrose-specific response pathway (Chiou and Bush 1998). Other metabolic processes that are demonstrated to be controlled by sucrose include: chlorophyll and non-photosynthetic pigment synthesis, anthocyanin and flavonoid biosynthesis, nitrogen assimilation and transport as well as carbon:nitrogen balance and stimulation of the amino acid biosynthetic pathways (Tognetti et al. 2013).

3.2 Sugars and Plant Growth and Development

In plants, growth and development are modulated through complex regulatory systems that promote growth (HXK glucose sensing, the Tre6P signal and the Target of Rapamycin (TOR) kinase system) or systems that are inhibitory for growth (SnRK1 and C/S1 bZIP transcription factor network) (Smeekens et al. 2010). These sophisticated nutrient signalling networks allow plants to continuously monitor their sugar status to optimize utilization of available sugars for growth and development. The activities of these components are regulated by plant's sugar status, for example, high hexose levels promote TOR activity and on the contrary overall sugar starvation will lead to activation of SnRK1. TOR is a central stimulator of growth and development and is mainly active in rapidly proliferating tissues and also regulates ribosome biogenesis and protein synthesis. On the contrary, SnRK1 is active under low energy conditions, represses biosynthetic processes and plant growth, affects the transition from vegetative to generative status and represses ribosomal protein gene expression (Lastdrager et al. 2014). Also the balance of source–sink activities is subjected to plant's sugar status. Source activities like photosynthesis, nutrient mobilization and export are upregulated under low overall sugar conditions and sink activities like growth and storage are upregulated when metabolizable hexoses are abundantly available. Sink–source allocation and intracellular distribution of different sugars and sugar derived metabolites is directed by spatial and temporal expression of sugar metabolizing enzymes and sugar transporters. Consequently, also sugar levels are both temporally and spatially regulated. The developmental stage of the plant determines the response to sugars and different sugars may have different regulatory roles in physiological processes. Therefore sugars can be considered as morphogens since they are signalling molecules that act directly on cells to produce specific cellular responses depending on their local concentration. In the process of *de novo* protein synthesis, which is of pivotal importance for plant growth and development, sugars also play a central role in the regulation of mRNA translation by providing energy and carbon building blocks for RNA and protein biosynthesis. In this process of sugar-mediated plant growth regulation, sugar sensing is of paramount importance, but is also integrated with other regulatory pathways like light and phytohormone signalling (like ABA and ethylene). Processes like embryogenesis, seed germination, early seedling development, tuberization and the regulation of α-amylase

activity are known to be regulated via crosstalk between sugars and phytohormones (Gibson 2004; Eveland and Jackson 2011).

Of all the sugars involved in this sugar-mediated plant growth regulation, sucrose and glucose can be considered as dominant regulators with a plethora of direct and indirect effects on the growth and development of plants. It is very difficult to discriminate between sucrose and glucose-specific effects upon exogenous application of sucrose on rapidly growing tissues, such as apical meristems, that typically contain very high invertase activities. The expression of cyclins, which are important regulators of the cell cycle and promote the generation of new cells, is also induced by glucose and sucrose as well as ribosome synthesis (Wang and Ruan 2013). Central to plant growth and development is auxin, which promotes cell proliferation and inhibits cell expansion. Sugars induce auxin levels by aid of phytochrome-interacting factors (PIF), which are important growth regulators (Sairanen et al. 2012).

Another essential growth signalling molecule is Tre6P, which possibly exerts an inhibitory function on the SnRK1 complex through induction of accumulation by sucrose. In plants the levels of sucrose and Tre6P are correlated and both play a role in the process of sugar sensing, whereby high sucrose and Tre6P signal a cellular sugar abundance status. Besides influencing plant growth sucrose has also been appointed as modifier of plant and leaf morphology. According to Horacio and Martinez-Noel (2013) sucrose can influence the time interval between the appearance of successive leaves and negatively impact leaf extension in monocots. In addition hexoses such as glucose and fructose have also been found to influence allometric relations between leaf length and leaf width in ornamental bromeliads (Ceusters et al. 2008; Croonenborghs et al. 2009).

Many other developmental processes, including gravitropic response, formation of underground storage organs, flowering, regulation of the circadian clock and senescence, also appear to be regulated by sugar signalling. Regarding the process of senescence a clear separation can be made between the effects of hexoses, where glucose and fructose cause induction of senescence while sucrose retards this process of ageing (Willemoës et al. 1988; Quirino et al. 2000).

3.3 Sugars and Stress Responses

Abiotic stress conditions in natural environments mostly imply a combination of different stresses which can be synergistically or antagonistically modified. As mentioned above, abiotic stress affects different cellular processes including growth, photosynthesis, carbon partitioning, carbohydrate and lipid metabolism, osmotic homeostasis, protein synthesis and gene expression. To cope with these situations of environmental stress, plants have evolved strategies consisting of a mixture of stress avoidance and tolerance mechanisms with soluble sugars (sucrose and hexoses) as key players in stress perception via specific signalling cascades. Under stress conditions a dynamic metabolism of soluble sugars is observed

including both catabolic and synthetic reactions. However, not all soluble sugars play similar roles in events associated with metabolism of stressed plants (Rosa et al. 2009). Accumulation of different sugars (glucose, fructose, sucrose and fructans) is a common process when plants experience abiotic stresses such as drought, salinity and low temperatures (Tarkowski and Van den Ende 2015). The major functions of these compatible solutes are osmotic adjustment, carbon storage and radical scavenging. Chloroplast and photosystem stabilization have been proposed for raffinose and sucrose during cold acclimation (Knaupp et al. 2011).

Furthermore, the sucrose controlled C/S1-bZIP transcription factor network is a powerful regulator of metabolism and provides the plant with extensive regulatory potential. This network, which also regulates genes involved in amino acid metabolism, reprograms plant growth in response to energy deprivation and environmental stresses and depends on the cellular sucrose level. The abundant presence of sucrose represses this regulatory network by arresting translation of the C/S1-bZIP mRNAs via a ribosome stalling mechanism (Lastdrager et al. 2014).

From all these data it can be concluded that sugars can act like hormones controlling various genes (Gupta and Kaur 2005). These sugar-regulated genes encode for important components since their function ranges from involvement in plant metabolism to light perception and cell cycle control. Stress also induces large alternations in source–sink relations due to a significant decrease in the efficiency of photosynthesis in source tissues which leads to a reduction of the supply of soluble sugars to sink tissues. As mentioned above, environmental stress factors may alter the types of carbohydrates that are synthesized and exported by the source tissues, which might act as a sugar signalling system for acclimation responses in the sink tissues (Ceusters et al. 2009a, b). An important parameter in plant's responses to abiotic stress is the sucrose to hexose ratio (e.g. the cellular sugar state). As such sucrose splitting enzymes (vacuolar, cell wall and neutral invertases and SuSy) can have a strong impact on the sugar-sensitive signalling pathways by influencing this ratio (Koch 2004). For example, invertases are induced by abiotic stress and can be regarded as central signal integrators and modulators to locally increase respiratory sink activity (Ruan 2014). These signalling pathways are known to control various plant physiological processes, probably also including innate immunity. All these differential effects allow the adaptation of carbon metabolism to changing environmental conditions and offer the plant sugar-mediated strategies to cope with different abiotic stresses.

4 Conclusions and Future Perspectives

As elucidated in this review the sweet immunity concept offers potential priming possibilities for an improved stress resistance. To delve into this exciting matter CAM plants constitute the ideal candidates of investigation for several reasons. CAM species which predominantly use starch to fuel nocturnal carboxylation still

keep a diel cycle of sucrose constituting daytime increase and nocturnal degradation (Ceusters et al. 2010). In addition a switch from starch to sucrose as major transient carbohydrate source has been noticed under several stress conditions such as low light and drought stress (Ceusters et al. 2009a, b, 2011). Moreover, calculated carbon budgets according to Borland (1996) indicated that nocturnal water-saving carboxylation was favoured whilst net carbon export to sinks was limited after recovery from drought in the CAM bromeliad *Aechmea* 'Maya'. In addition, recent progress revealed a prominent role for the circadian clock in CAM plants, where timing is of crucial importance, including the diversion of carbohydrates and successful adaptation under changing environments (Boxall et al. 2005). The circadian clock is particularly sensitive to sucrose-mediated signalling, which can affect circadian rhythms by altering the expression pattern of clock-regulated genes and of the clock genes themselves.

In the concept of sweet immunity, it is proposed that sugar signalling pathways are involved in a highly complicated network including many signalling molecules and various cross-talks. In this intricate network, various metabolites have been described to fulfil potential roles as signalling molecules contributing to effective immune responses. It is indicated that the relative proportions of small water-soluble carbohydrates, such as the sucrose to hexose ratio, are involved in many cell processes and those slight changes can have a drastic impact. In this aspect sugars act as signalling molecules that are activated by exposure to stress and thereby initiate signal amplification and lead to more rapid and robust activation of defence, immunity and stress tolerance. These considerations underlie the idea of sugar molecules as potential priming agents to enhance plants defence response and open up new avenues of research such as exploring the possibilities of using biodegradable sugar-(like) compounds as alternatives to toxic agrochemicals which also yield high levels of crop biomass by just adjusting sweet, endogenous saccharides.

References

Ana Sofia Duque, André Martinho de Almeida, Anabela Bernardes da Silva, Jorge Marques da Silva, Ana Paula Farinha, Dulce Santos, Pedro Fevereiro and Susana de Sousa Araújo (2013) Abiotic stress responses in plants: unraveling the complexity of genes and networks to survive. In: Vahdati K (ed) Abiotic stress - plant responses and applications in agriculture. InTech, Rijeka, Croatia. doi:10.5772/52779

Antony E, Borland AM (2009) The role and regulation of sugar transporters in plants with crassulacean acid metabolism. Prog Bot 70:127–143

Asada K (1999) The water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Behzadipour M, Ratajczak R, Faist K, Pawlitschek P, Tremolieres A, Kluge M (1998) Phenotypic adaptation of tonoplast fluidity to growth temperature in the CAM plant Kalanchoe daigremontiana Ham. et Per. is accompanied by changes in the membrane phospholipid and protein composition. J Membr Biol 166:61–70

Birch ANE, Roberson WM, Geoghegan IE, MC-Gavin WJ, Alpheyt JW, Porter EA (1993) DMDP – a plant-derived sugar analogue with systemic activity against plant parasitic nematodes. Nematologica 39:521–535

Bolouri-Moghaddam MR, Van den Ende W (2012) Sugars and plant innate immunity. J Exp Bot 63:3989–3998

Bolouri-Moghaddam MR, Van den Ende W (2013a) Sweet immunity in the plant circadian regulatory network. J Exp Bot 64:1439–1449

Bolouri-Moghaddam MR, Van den Ende W (2013b) Sugars, the clock and transition to flowering. Frontier Plant Sci 4, 4

Bolouri-Moghaddam MR, Le Roy K, Xiang L, Rolland F, Van den Ende W (2010) Sugar signaling and antioxidant network connections in plant cells. FEBS J 277:2022–2037

Borland AM (1996) A model for the portioning of photosynthetically fixed carbon during the C_3-CAM transition in *Sedum telephium*. New Phytol 134:433–444

Borland AM, Griffiths H, Broadmeadow MSJ, Fordham MC, Maxwell C (1993) Short-term changes in carbon-isotope discrimination in the C_3/CAM intermediate *Clusia minor* L. growing in Trinidad. Oecologia 95:444–453

Borland AM, Hartwell J, Jenkins GI, Wilkins MB, Nimmo HG (1999) Metabolite control overrides circadian regulation of phosphoenolpyruvate carboxylase kinase and CO_2 fixation in crassulacean acid metabolism. Plant Physiol 121:889–896

Borland AM, Zambrano VAB, Ceusters J, Shorrock K (2011) The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. New Phytol 191:619–633

Boxall SF, Foster JM, Bohnert HJ, Cushman JC, Nimmo HG, Hartwell J (2005) Conservation and divergence of circadian clock operation in a stress-inducible crassulacean acid metabolism species reveals clock compensation against stress. Plant Physiol 137:969–982

Brandon PC (1967) Temperature features of enzymes affecting crassulacean acid metabolism. Plant Physiol 42:977–984

Broetto F, Lüttge U, Ratajczak R (2002) Influence of light intensity and salt-treatment on mode of photosynthesis and enzymes of the antioxidative response system of *Mesembryanthemum crystallinum* L. Funct Plant Biol 29:13–23

Brulfert J, Guerrier D, Queiroz O (1973) Photoperiodism and enzyme activity: balance between inhibition and induction of the crassulacean acid metabolism. Plant Physiol 51:220–222

Brulfert J, Kluge M, Güclü S, Queiroz O (1988) Interaction of photoperiod and drought as CAM inducing factors in Kalanchoë blossfeldiana Poelln., Cv Tom Thumb. J Plant Physiol 133:222–227

Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proc Natl Acad Sci U S A 107:9452–9457

Buchanan-Bollig IC, Kluge M, Müller D (1984) Kinetic changes with temperature of phosphoenolpyruvate carboxylase from a CAM plant. Plant Cell Environ 7:63–70

Castillo FJ (1996) Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia 107:469–477

Ceusters J, Borland AM, Londers E, Verdoodt V, Godts C, De Proft M (2008) Diel shifts in carboxylation pathway and metabolite dynamics in the CAM bromeliad *Aechmea* 'Maya' in response to elevated CO_2. Ann Bot 3:389–397

Ceusters J, Borland AM, De Proft MP (2009a) Drought adaptation in plants with crassulacean acid metabolism involves the flexible use of different storage carbohydrate pools. Plant Signal Behav 4:212–214

Ceusters J, Borland AM, Londers E, Verdoodt V, Godts C, De Proft MP (2009b) Differential usage of storage carbohydrates in the CAM bromeliad *Aechmea* 'Maya' during acclimation to drought and recovery from dehydration. Physiol Plant 135:174–184

Ceusters J, Borland AM, Ceusters N, Verdoodt V, Godts C, De Proft P (2010) Seasonal influences on carbohydrate metabolism in the CAM bromeliad Aechmea 'Maya': consequences for carbohydrate partitioning and growth. Ann Bot 105:301–309

Ceusters J, Borland AM, Godts C, Londers E, Croonenborghs S, Van Goethem D, De Proft MP (2011) Crassulacean acid metabolism under severe light limitation: a matter of plasticity in the shadows? J Exp Bot 62:283–291

Ceusters J, Borland AM, Taybi T, Frans M, Godts C, De Proft MP (2014) Light quality modulates metabolic synchronization over the diel phases of crassulacean acid metabolism. J Exp Bot 65:3705–3714

Chen WS, Liu HY, Liu ZH, Yang L, Chen WH (1994) Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. Physiol Plant 90:391–395

Chen WH, Tseng YC, Liu YC, Chuo CM, Chen PT, Tseng KM, Yeh YC, Ger MJ, Wang HL (2008) Cool-night temperature induces spike emergence and affects photosynthetic efficiency and metabolizable carbohydrate and organic acid pools in *Phalaenopsis aphrodite*. Plant Cell Rep 27:1667–1675

Chiou TJ, Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci U S A 95:4784–4788

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Ciereszko I, Johansson H, Kleczkowski LA (2004) Interactive effects of phosphate deficiency, sucrose and light/dark conditions on gene expression of UDP-glucose pyrophosphorylase in *Arabidopsis*. J Plant Physiol 162:343–353

Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E (2000) Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. J Exp Bot 51:61–70

Conrath U (2011) Molecular aspects of defence priming. Trends Plant Sci 16:524–531

Couée I, Sulmon C, Gouesbet G, El Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57:449–459

Croonenborghs S, Ceusters J, Londers E, De Proft MP (2009) Effects of elevated CO_2 on growth and morphological characteristics of ornamental bromeliads. Sci Hortic 121:192–198

Cushman JC (2001) Crassulacean acid metabolism. A plastic photosynthetic adaptation to arid environments. Plant Physiol 127:1439–1448

Cushman JC, Borland AM (2002) Induction of crassulacean acid metabolism by water limitation. Plant Cell Environ 25:295–310

De Bruyne L, Hofte M, De Vleesschauwer D (2014) Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. Mol Plant 7:943–959

Debnath M, Pandey M, Bisen PS (2011) An OMICS approach to understand the plant abiotic stress. OMICS 15:739–762

Demel RE, Dorrepaal E, Ebskamp MJM, Smeekens S, de Kruijff B (1998) Fructans interact strongly with model membranes. Biochim Biophys Acta 1375:36–42

Dicke M, van Loon JJA (2014) Chemical ecology of phytohormones: how plants integrate responses to complex and dynamic environments. J Chem Ecol 40:653–656

Dodd AN, Borland AM, Haslam RP, Griffiths H, Maxwell K (2002) Crassulacean acid metabolism: plastic fantastic. J Exp Bot 53:569–580

Eveland AL, Jackson DP (2011) Sugars, signalling and plant development. J Exp Bot 63:3367–3377

Farrar J, Pollock C, Gallagher J (2000) Sucrose and the integration of metabolism in vascular plants. Plant Sci 154:1–11

Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C (2010) Trehalose and plant stress responses: friend or foe? Trends Plant Sci 15:409–417

Ferrari S, Savatin DV, Sicilia F, Gramegna G, Cervone F, De Lorenzo G (2013) Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. Front Plant Sci 4:49

Finkelstein RR, Gibson SI (2001) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? Curr Opin Plant Biol 5:26–32
Frank JH (2005) Phytotelmata. Encyclopedia of Entomology [Internet]. Springer Science + Business Media; 1718–20. Available from: http://dx.doi.org/10.1007/0-306-48380-7_3252
Friemert V, Kluge M, Smith JAC (1986) Net CO_2 output by CAM plants in the light: the roe of leaf conductance. Physiol Plant 68:353–358
Friemert V, Heininger D, Kluge M, Ziegler H (1988) Temperature effects on malic-acid efflux from the vacuoles and on the carboxylation pathways in crassulacean acid metabolism plants. Planta 174:453–461
Gao J, Sun L, Yang X, Liu JX (2013a) Transcriptomic analysis of cadmium stress response in the heavy metal hyperaccumulator *Sedum alfredii* Hance. PLoS One 8, e64643. doi:10.1371/journal.pone.0064643
Gao J, Wang N, Xu S-S, Li Y, Wang Y, Wang G-X (2013b) Exogenous application of trehalose induced H_2O_2 production and stomatal closure in *Vicia faba*. Biol Plant 57:380–384
Geigenberger P, Geiger M, Stitt M (1998) High-temperature perturbation of starch synthesis is attributable to inhibition of ADP-glucose pyrophosphorylase by decreased levels of glycerate-3-phosphate in growing potato tubers. Plant Physiol 117:1307–1316
Gibson SI (2004) Sugar and phytohormone response pathways: navigating a signaling network. J Exp Bot 55:253–264
Giron D, Frago E, Glevarec G, Pieterse CMJ, Dicke M (2013) Cytokinins as key regulators in plant-microbe-insect interactions: connecting plant growth and defence. Funct Ecol 27:S599–S609
Guo WJ, Lee N (2006) Effect of leaf and plant age, and day/night temperature on net CO_2 uptake in *Phalaenopsis amabilis* var. *formosa*. J Am Soc Hortic Sci 131(3):320–326
Gupta AK, Kaur N (2005) Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J Biosci 30:761–776
Haydon MJ, Bell LJ, Webb AAR (2011) Interactions between plant circadian clocks and solute transport. J Exp Bot 62:2333–2348
Herbers K, Meuwly P, Frommer W, Métraux JP, Sonnewald U (1996a) Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. Plant Cell 8:793–803
Herbers K, Meuwly P, Métraux JP, Sonnewald U (1996b) Salicyclic acid independent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage. FEBS Lett 397:239–244
Holmstrom KO, Mantyla E, Welin B, Mandal A, Palva ET, Tunnela OE, Londesborough J (1996) Drought tolerance in tobacco. Nature 379:683–684
Horacio P, Martinez-Noel G (2013) Sucrose signalling in plants: a world yet to be explored. Plant Signal Behav 8, e23316
Islam E, Khan MT, Irem S (2015) Biochemical mechanisms of signalling: perspectives in plants under arsenic stress. Ecotoxicol Environ Saf 114:126–133
Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R (2008) Alterations in morphological parameters and photosynthetic pigment responses in *Catharanthus roseus* under soil water deficits. Colloids Surf B Biointerfaces 62:298–303
Jaleel CA, Manivannan P, Wahid A, Farooq M, Somasundaram R, Panneerselvam R (2009) Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11:100–105
Jang JC, León P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell 9:5–19
Johnson R, Ryan CA (1990) Wound-inducible potato inhibitor II genes: enhancement of expression by sucrose. Plant Mol Biol 14:527–536
Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329
Kano A, Hosotani K, Gomi K et al (2011) D-Psicose induces upregulation of defence-related genes and resistance in rice against bacterial blight. J Plant Physiol 168:1852–1857

Kholodova V, Volkov K, Abdeyeva A, Kuznetsov V (2011) Water status in *Mesembryanthemum crystallinum* under heavy metal stress. Environ Exp Bot 71:382–389

Kliemchen A, Schomburg M, Galla HJ, Lüttge U, Kluge M (1993) Phenotypic changes in the fluidity of the tonoplast membrane of crassulacean acid metabolism plants in response to temperature and salinity stress. Planta 189:403–409

Kluge M, Ting IP (1978) Crassulacean acid metabolism. Analysis of an ecological adaptation. Springer, Berlin

Knaupp M, Mishra K, Nedbal L, Heyer AG (2011) Evidence for a role of raffinose in stabilizing photosystem II during freeze-thaw cycles. Planta 234:477–486

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol 47:509–540

Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr Opin Plant Biol 7:235–246

Kunz S, Gardeström P, Pesquet E, Kleczkowski LA (2015) Hexokinase 1 is required for glucose-induced repression of *bZIP63, At5g22920*, and *BT2* in *Arabidopsis*. Front Plant Sci 6:525

Lastdrager J, Hanson J, Smeekens S (2014) Sugar signals and the control of plant growth and development. J Exp Bot 65:799–807

León P, Sheen J (2003) Sugar and hormone connections. Trends Plant Sci 8:110–116

Lu C, Qiu N, Lu Q, Wang B, Kuang T (2003) PS II photochemistry, thermal energy dissipation, and the xanthophyll cycle in Kalanchoë daigremontiana exposed to a combination of water stress and high light. Physiol Plant 118:173–182

Lunn JE, Delorge I, Figueroa CM, Van Dijck P, Stitt M (2014) Trehalose metabolism in plants. Plant J 79:544–567

Lüttge U (2000) The tonoplast functioning as the master switch for circadian regulation of crassulacean acid metabolism. Planta 211:761–769

Lüttge U (2002) CO_2-concentrating: consequences in crassulacean acid metabolism. J Exp Bot 53:2131–2142

Lüttge U (2004) Ecophysiology of crassulacean acid metabolism (CAM). Ann Bot 93:629–652

Lüttge U (2006) Photosynthetic flexibility and ecological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. New Phytol 171:7–25

Lüttge U (2010) Photorespiration in Phase III of crassulacean acid metabolism: evolutionary and ecophysiological implications. Progr Bot 72:371–384

Martínez-Noël GMA, Tognetti JA, Salerno GL, Wiemken A, Pontis HG (2009) Protein phosphatase activity and sucrose-mediated induction of fructan synthesis in wheat. Planta 230:1071–1079

Matros A, Peshev D, Peukert M, Mock HP, Van den Ende W (2015) Sugars as hydroxyl radical scavengers: proof-of-concept by studying the fate of sucralose in Arabidopsis. Plant J 82:822–839

Miszalski Z, Slesak I, Niewiadomska E, Baczek R, Lüttge U, Ratajezak R (1998) Subcellular localization and stress responses of superoxide dismutase isoforms from leaves in the C_3-CAM intermediate halophyte *Mesembryanthemum crystallinum* L. Plant Cell Environ 21:169–179

Moller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591

Moore BD, Sheen J (1999) Plant sugar sensing and signaling – a complex reality. Trends Plant Sci 4:250

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Roles of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300:332–336

Moreira Lobo AK, de Oliveira M, Lima Neto MC, Caruso Machado E, Vasconcelos Ribeiro R, Gomes Silveira JA (2015) Exogenous sucrose supply changes sugar metabolism and reduces photosynthesis of sugarcane through the down-regulation of Rubisco abundance and activity. J Plant Physiol 179:113–121

Moya JL, Ros R, Picazo I (1993) Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynth Res 36:75–80
Nägele T, Weckwerth W (2014) Mathematical modeling reveals that metabolic feedback regulation of SnRK1 and hexokinase is sufficient to control sugar homeostasis from energy depletion to full recovery. Front Plant Sci 5:365
Nie G, Hendrix DL, Webber AN, Kimball BA, Long SP (1995) Increased accumulation of carbohydrates and decreased photo- synthetic gene transcript levels in wheat grown at an elevated CO, concentration in the field. Plant Physiol 108:975–983
Nobel PS (1983) Nutrient levels in Cacti – relation to nocturnal acid accumulation and growth. Am J Bot 70:1244–1253
Nobel PS, Berry WL (1985) Element responses of agaves. Am J Bot 72:686–694
Nobel PS, Lüttge U, Heuer S, Ball E (1984) Influence of applied NaCl on crassulacean acid metabolism and ionic levels in a cactus, *Cereus validus*. Plant Physiol 75:799–803
Ordoñez-Salanueva CA, Seal CE, Pritchard HW, Orozco-Segovia A, Canales-Martinez M, Flores-Ortiz CM (2015) Cardinal temperatures and thermal time in Polaskia Backeb (Cactaceae) species: effect of projected soil temperature increase and nurse interaction on germination timing. J Arid Environ 115:73–80
Osakabe K, Osakabe Y (2012) Plant light stress. In: Encyclopaedia of life sciences. Wiley, Chichester. http://www.els.net. doi:10.1002/9780470015902.a0001319.pub2
Osmond CB (1981) Crassulacean acid metabolism: a curiosity in context. Ann Rev Plant Physiol 29:379–414
Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A (2015) Seed priming: state of the arte and new perspectives. Plant Cell Rep 34:1281–1293
Pego JV, Kortstee AJ, Huijser C, Smeekens S (2000) Photosynthesis, sugars and the regulation of gene expression. J Exp Bot 51:407–416
Peshev D, Vergauwen R, Moglia A, Hideg E, Van den Ende W (2013) Towards understanding vacuolar antioxidant mechanisms: a role for fructans? J Exp Bot 64:1025–1038
Peukert M, Thiel J, Peshev D, Weschke W, Van den Ende W, Mock HP, Matros A (2014) Spatio-temporal dynamics of fructan metabolism in developing barley grains. Plant Cell 26:3728–3744
Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316
Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A (2006) Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in Arabidopsis. Planta 224:556–568
Quirino BF, Noh YS, Himelblau E, Amasino RM (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5:278–282
Ramon M, Rolland F, Sheen J (2008) Sugar sensing and signaling. Arabidopsis Book 6:e0117. doi:10.1199/tab.0117
Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. Plant Cell 14:S185–S205
Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709
Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M, Prado FE (2009) Soluble sugars – metabolism, sensing and abiotic stress. Plant Signal Behav 4(5):388–393. doi:10.4161/psb.4.5.8294
Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:33–67
Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci 86:407–421
Sairanen I, Novák O, Pencík A, Ikeda Y, Jones B, Sandberg G, Ljung K (2012) Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in *Arabidopsis*. Plant Cell 24:4907–4916

Sheen J (1994) Feedback control of gene expression. Photosynth Res 39:427–438
Sheen J (2014) Master regulators in plant glucose signaling networks. J Plant Biol 57:67–79
Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. Curr Opin Plant Biol 13:274–279
Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58
Spalding MH, Stumpf DK, Ku MSB, Burris RH, Edwards GE (1979) Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2-concentrations in *Sedum praealtum* DC. Funct Plant Biol 6:557–567
Tarkowski ŁP, Van den Ende W (2015) Cold tolerance triggered by soluble sugars: a multifaceted countermeasure. Front Plant Sci 6:203
Tayeh C, Randoux B, Dorothee V et al (2014) Exogenous trehalose induces defenses in wheat before and during a biotic stress caused by powdery mildew. Phytopathology 104:293–305
Tian S, Lu L, Labavitch J, Yang X, He Z, Hu H, Sarangi R, Newvill M, Commisso J, Brown P (2011) Cellular sequestration of cadmium in the hyperaccumulator plant species *Sedum alfredii*. Plant Physiol 157:1914–1925
Ting IP (1985) Crassulacean acid metabolism. Ann Rev Plant Physiol 36:595–622
Tognetti JA, Pontis HG, Martínez-Noël GM (2013) Sucrose signaling in plants: a world yet to be explored. Plant Signal Behav 8(3):e23316
Trouvelot S, Héloir MC, Poinssot B, Gauthier A, Paris F, Guillier C, Combier M, Trdá L, Daire X, Adrian M (2014) Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Front Plant Sci 5:592
Tsai AY-L, Gazzarrini S (2014) Trehalose-6-phosphate and SnRK1 kinases in plant development and signaling: the emerging picture. Front Plant Sci 5:119
Van den Ende W (2013) Multifunctional fructans and raffinose family oligosaccharides. Front Plant Sci 4:247
Van den Ende W, El-Esawe SK (2014) Sugar signaling pathways leading to fructan and anthocyanin accumulation: a dual function in abiotic and biotic stress responses? Environ Exp Bot 108:4–13
Van den Ende W, Valluru R (2009) Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? J Exp Bot 60:9–18
Wang L, Ruan Y-L (2013) Regulation of cell division and expansion by sugar and auxin signaling. Front Plant Sci 4:163
Willemoës JG, Beltrano J, Montaldi ER (1988) Diagravitropic growth promoted by high sucrose contents in *Paspalum vaginatum*, and its reversion by gibberellic acid. Can J Botany 66:2035–2037
Winter K, Smith JAC (1996) Crassulacean acid metabolism: current status and perspectives. In: Winter K, Smith JAC (eds) Crassulacean acid metabolism: biochemistry. Ecophysiology and evolution. Springer, Berlin, pp 230–246
Winter K, Aranda J, Holtum JAM (2005) Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. Funct Plant Biol 32:381–388
Wolf S, Marani A, Rudich J (1991) Effect of temperature on carbohydrate-metabolism in potato plants. J Exp Bot 42:619–625
Yadav S, Irfan M, Ahmad A, Hayat S (2011) Causes of salinity and plant manifestations to salt stress: a review. J Environ Biol 32:667–685
Yadav UP, Ivakov A, Feil R, Duan GY, Walther D, Giavalisco P, Piques M, Carillo P, Hubberten HM, Stitt M, Lunn EJ (2014) The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signaling by Tre6P. J Exp Bot 65:1051–1068
Yang X, Li T, Yang J, He Z, Lu L, Meng F (2006) Zinc compartmentation in root, transport into xylem, and absorption into leaf cells in the hyperaccumulating species of *Sedum alfredii* Hance. Planta 224:185–195
Yang X, Cushman J, Borland A, Edwards E, Wullschleger S, Tuskan G, Owen N, Griffiths H, Smith J, De Paoli H, Weston D, Cottingham R, Hartwell J, Davis S, Silveria K, Ming R,

Schlaugh K, Abraham P, Stewart R, Guo H, Albion R, Ha J, Lim S, Wone B, Yim W, Garcia T, Mayer J, Petereit J, Nair S, Casey E, Hettich R, Ceusters J, Ranjan P, Palla K, Yin H, Reyes-Garcia C, Andrade J, Freschi L, Dever L, Boxall S, Walker J, Davies J, Bupphada P, Kadu N, Winter K, Sage R, Aguilar C, Schmutz J, Jenkins J, Holtum J (2015) A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter drier world. New Phytol 207:491–504

Yoon YJ, Mobin M, Hahn EJ, Paek KY (2009) Impact of *in vitro* CO_2 enrichment and sugar deprivation on acclamatory responses of *Phalaenopsis* plantlets to *ex vitro* conditions. Environ Exp Bot 65:183–188

Yu SM (1999) Cellular and genetic responses of plants to sugar starvation. Plant Physiol 121:687–693

Physiology and Spatio-temporal Relations of Nutrient Acquisition by Roots and Root Symbionts

Alex J. Valentine, Aleysia Kleinert, Rochelle Thuynsma, Samson Chimphango, Joanna Dames, and Vagner A. Benedito

Abstract Among the various functions of roots, nutrient acquisition (via soil uptake or through symbiotic relationships) is one of the most essential for land plants. Soil from natural and agricultural ecosystems may impede plant nutrient acquisition, by many factors such as mineral availabilities either in excess or deficient supply, depletion of organic matter, extreme variations in water supply, and many other physical and chemical features. In order to survive, plants need to undergo developmental and physiological mechanisms to cope with these extreme soil situations. Here we review how plants control nutrient acquisition by dynamically changing root architecture for improved soil space exploration, as well as altering cellular-level function for enhanced nutrient uptake, via apoplastic acidification, exudation of enzymes and metabolites (organic acids, secondary metabolites) and constantly changing the composition of transporters at the plasma membrane. These changes start with environmental cues which induce cell signaling and involve hormones and coordinated regulatory genes networks that drive the

A.J. Valentine (✉) • A. Kleinert • R. Thuynsma
Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
e-mail: alexvalentine@mac.com

S. Chimphango
Department of Biology, Faculty of Science, University of Cape Town, Private Bag, Rondebosch, South Africa

J. Dames
Department of Microbiology and Biochemistry, Faculty of Science, Rhodes University, Grahamstown, South Africa

V.A. Benedito
Division of Plant and Soil Sciences, West Virginia University, 2090 Agricultural Sciences Building, P.O. Box 6108, Morgantown, WV 26506-6108, USA

Progress in Botany (2017) 78: 167–234, DOI 10.1007/124_2016_11,
Published online: 7 June 2016

root's developmental plasticity as well as the cell's biochemical dynamics. Mutualistic root symbioses, such as mycorrhizae and rhizobial-induced nodulation, are also important to provide essential nutrients to the plant, which are tightly regulated in order to only occur at plant's benefit. We also explore molecular mechanisms which roots have evolved to cope with nutritional, as well as other soil stresses, such as aluminium toxicity and heavy metals. Overall, understanding root dynamics under several environmental variables at different perspectives, from root architecture to biochemistry to genetic levels will allow us to better explore the spatial and temporal relations of roots with their mineral nutrient environment.

Contents

1 Introduction

Soil is a highly heterogeneous medium with complex properties, such as physical (texture, structure, density, porosity, temperature), chemical (moisture, pH, organic matter, aluminium and nutrient levels) as well as biological features (pathogenic,

beneficial and innocuous microorganisms; a macrofauna; inter- and intraspecies competitions, including allelopathic interactions). Over the course of plant evolution and since the conquest of land in the last 400 million years, roots have adapted to the local conditions and variability of the soil in which they evolved. More recently, in the last 10,000 years since the development of agriculture, farmers and breeders have introduced accessions and selected crop varieties adapted to extreme environments, including soils that are quite different from their original sites. Indeed, farming soils often are depleted of nutrients and organic matter, too saline or contaminated with heavy metals and organic compounds, too arid or flooded, too acidic and so forth. Thus, to succeed in these new environments, crops must use developmental and physiological mechanisms to cope with spatial and temporal variations of the soil in which they are placed.

Plant nutrient acquisition is one of the primary functions of roots. However, nutrients may now always be readily available to roots and may be distributed in varying concentrations across the planes of soil space and temporal availability (Gross et al. 1995; Ryel et al. 1996; Hodge et al. 2009). It is therefore not surprising that optimal nutrient acquisition by root systems is influenced by root modifications via altered anatomy and morphology as well as specialised symbiotic structures with soilborne microbes. Therefore, in order to appreciate the extent of root strategies for the acquisition of mineral nutrients across spatial and temporal plane, it is imperative to view the belowground root system as a modified soil-root interface, with functional, morphological and symbiotic modifications. This chapter will cover the spatio-temporal nutrient acquisition of the root system and its exquisite modifications, ranging from altered architectures to specialised cluster roots and symbiotic marvels such as mycorrhizal roots and nitrogen-fixing nodules.

2 Root Spatio-temporal Nutrient Acquisition

The effective acquisition of nutrients by the roots is governed by a number of factors including concentration and distribution of nutrients in the soil both in space and time (Gross et al. 1995; Ryel et al. 1996; Hodge et al. 2009). Nutrient concentration and distribution in the soil of both natural and cultivated areas tend to vary due to, amongst others, methods and type of fertiliser application (Jordan-Meille and Pellerin 2008); inputs and subsequent decomposition of leaf litter, dead roots and soil animals (Hodge 2003); intensity and frequency of fires on a landscape (van Wilgen and Le Maitre 1981; Brown and Mitchell 1986; Stock and Lewis 1986); progressive depletion of nutrient stocks from the soil associated with continuous uptake and sequestration of nutrients in living biomass over long periods of plant growth (Luo et al. 2004; Pregitzer and King 2005); a precipitation event that could stimulate nutrient mineralization rates (Davidson et al. 1993); and a snowmelt that leads to nutrient flush (Bilbrough and Caldwell 1997). Changes in the

length of the growing season associated with high temperatures (Beier 2004) could also increase net mineralization rates (Rustad et al. 2001; Schmidt et al. 2002) that could lead to greater nutrient availability in the soil. In order to capitalise on these variable nutrient availabilities, plants have developed root systems that vary in their architecture, morphology and physiology (Fransen et al. 1999; Zhu et al. 2005a, b; Trachsel et al. 2011; Hauck et al. 2015) for foraging and acquisition of nutrients from variable soil nutrients (Giehl and von Wirén 2014).

2.1 Root Architectural Plasticity

Many plants show architectural and morphological plasticity to variations in nutrient supply by altering root traits such as branching intensity, first-order branch density, root length, mean lateral root length, root-to-shoot ratio, specific root length (SRL), root tissue density and root diameter class (Lynch 1995; Fransen et al. 1999; Roumet et al. 2006; Richardson et al. 2009a, b; Chen et al. 2012a, b). Physiologically, plants can adjust their exudation of enzymes, organic acids, protons and hydroxyl ions (Jaillard et al. 2000; Gahoonia et al. 2006; Lambers et al. 2006) for the uptake of nutrients from the heterogeneous soil nutrient conditions. Furthermore, plants can increase their root nutrient transporters and uptake kinetics (Fransen et al. 1999; Vance 2001; Shemesh et al. 2010; Mommer et al. 2011) in response to the variations in nutrient availability. Root ontogenesis also plays a big role in modifying the physiological properties of a root (Pierret et al. 2007). There seem to be changes in nutrient uptake rates along individual roots where its physiological status changes with increasing distance from the tip (Clarkson 1996). The plastic root responses to variable supplies of nutrients from the soil were reviewed earlier by Hodge (2004, 2006).

The plant root architectural and morphological structures and the physiology of root nutrient uptake are also influenced by environmental factors such as gradients of nutrients and moisture (Reader et al. 1992; Nielsen et al. 1999; Hodge 2003; Zhu et al. 2005a, b; Pierret et al. 2007; Grift et al. 2011). For instance, Postma et al. (2014) showed that the lateral root branching density (LRBD) changes with variation in the ratio of nitrate to phosphorus in the soil. In general, greater LRBD and/or fine roots correlate positively with phosphorus acquisition due to its less mobility in the soil relative to nitrate, which has decreased uptake efficiency with increasing LRBD. Similarly, the distribution of roots in a soil profile is greatly affected by nutrient and moisture availability with deeper roots reported for plants in more arid areas (Reader et al. 1992; Jumpponen et al. 2002). Other soil characteristics such as soil type, soil bulk density and soil structure also influence the root traits on nutrient acquisition (Kuchenbuch and Ingram 2004; Wang and Smith 2004; Kuchenbuch et al. 2006). Moreover, the root architectural and morphological properties and physiological mechanisms of plants are phylogenetically constrained

in that some plant families are known to be superior on a particular mechanism relative to others (Zhu et al. 2005a, b; Pierret et al. 2007; Gahoonia et al. 2006; Grift et al. 2011; Chen et al. 2012a, b). For instance, Roumet et al. (2006) showed that annuals generally displayed low-density roots with high nitrogen concentration relative to perennials which have enhanced root persistence with thick dense root systems. The section below describes how plants use the root architectural and morphological properties and physiological mechanisms for nutrient acquisition from soil with variable nutrient availability in space and time.

2.2 Temporal Variation in Nutrient Acquisition

A larger body of literature was noted especially on plant responses to localised availability of nutrients relative to temporal variations of the nutrients (Pierret et al. 2007). Depending on plant species, Robinson (1996) indicated that plants are likely to respond to nutrient-rich patches in the soil by increasing root nutrient uptake rate or by increasing root growth and proliferation towards and within the nutrient-rich patch. However, a common root response to spatial variability in nutrient availability in literature is the localised proliferation of root growth around the nutrient-rich zone (Fig. 1) which is considered as foraging response (Robinson 1994, 1996; Hutchings et al. 2003; Hodge 2004; Bartelheimer et al. 2006) associated with changes in root growth and architecture for enhanced capturing of nutrients according to the demand of the plant (Nielsen et al. 1999; López-Bucio et al. 2003). This is often accompanied by a reduction in root growth outside the nutrient-rich area, thereby altering the root morphology (Drew 1975; Hodge et al. 1998). The change in root architecture and morphology to a dense fine-root biomass and high foraging capacity has also been reported for species growing in fertile environments (Priha et al. 1999; Curt and Prevosto 2003; Hynynen et al. 2010).

2.3 Spatial Variation in Nutrient Acquisition

The importance of root architecture in plant productivity was well documented previously in great details (see reviews Lynch 1995; Pierret et al. 2007; de Dorlodot et al. 2007; Hammer et al. 2011; Hodge et al. 2009; Kong et al. 2014), highlighting that it allows plant roots to explore and exploit nutrient-rich spatial patches in the soil. According to Lynch (1995), 'root architecture refers to the spatial configuration of root system, i.e. the explicit geometric deployment of root axes'. It includes aspects of root topology that describes the branching pattern of the root and root distribution which refers to the presence of roots at a particular place (Lynch 1995).

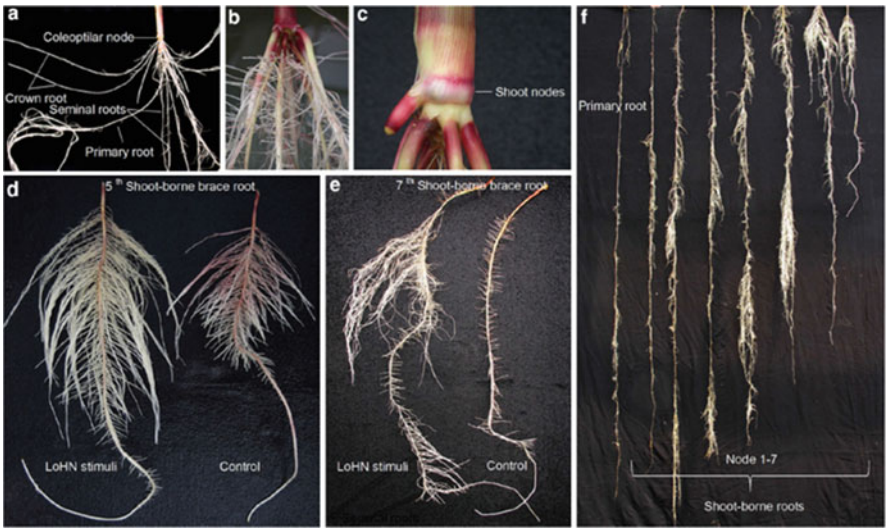

Fig. 1 Development of the maize root system from embryonic roots in seedlings to crown and brace roots initiated from shoot nodes in adult plants. (**a**) The root system of a 35-day-old maize plant with the major root types indicated. (**b**) The root system of a 49-day-old maize plant with its shoot-borne roots from the fifth node. (**c**) Roots emerging from shoot nodes on a 49-day-old maize plant. (**d**) Phenotypic responses of the shoot-borne roots from the fifth node of a 49-day-old maize plant to a nonuniform N environment (LoHN, local high nitrate supply; HN 4 mM, control 0.5 mM). (**e**) Phenotypic responses of the shoot-borne roots from the seventh node of a 70-day-old maize plant in response to nonuniform N environments. (**f**) All root types of a 70-day-old maize plant grown with a sufficient N supply. *Source*: From Yu et al. (2014)

Putting it simply, root architecture describes the shape and structure of a root system (de Dorlodot et al. 2007). Therefore, depending on nutrient and water availability in the soil, plants are able to deploy roots to areas with localised availability of soil resources by optimising their functional architecture (Hinsinger et al. 2009) through adjusting root angles and branching pattern towards the direction of the soil resource. Postma et al. (2014) reported that increased lateral root branching density increased nutrient acquisition particularly for less mobile elements such as phosphorus due to increased root surface area. In this regard, increase in root elongation rate is important for the root to grow and access the nutrient-enriched and unexploited patches in the soil (Silberbush and Barber 1983). Overall, the capacity of a plant to forage soil resources, thereby affecting plant's productivity, is attributed to the root system architecture (Lynch 1995). Similarly, root morphology, which describes the surface features of the root including root biomass, root length, lateral length, root hairs, root diameter and specific root length (SRL), is important for plant's ability to acquire nutrients in heterogeneous environment (Lynch 1995; Fransen et al. 1999; Jones et al. 2009). Increases in SRL due

to root morphological changes to longer thinner roots within the nutrient-rich zones have been frequently reported for rapid acquisition of nutrients from the localised place in the soil and are attributed to the increased root surface area and efficiency of nutrient capture (Eissenstat and Caldwell 1988; Bauhus and Messier 1999; Roumet et al. 2006).

Lateral roots are important not only for increasing root surface area and exploitation of large volume of soil, but they also determine the size and architecture of root systems on some plants (Mia et al. 1996). In a root, the proportion of fine lateral roots is shown by root diameter distribution which corresponds to plant's capacity for nutrient acquisition (Hodge et al. 1999; Robinson 2001). Information on root diameter distribution is important for understanding the role of roots in nutrient acquisition, long-distance transport pathways and anchorage and support for lateral roots (Blouin et al. 2007). Generally, most of the root biomass consists of large-diameter roots (Blouin et al. 2007). Lateral roots which generally correspond to fine roots account for most of the root length, but not necessarily of the root biomass, and have smaller diameter, and their emergence depends on the rate of primordia formation and the percentage of the primordia that develop into lateral roots (Postma et al. 2014). This area has received a lot of research interest in trying to unravel genes that are activated and the hormones involved during the process of lateral root development (Osmont et al. 2007; Lavenus et al. 2013). Noteworthy is that fine- and small-diameter roots may have higher construction and maintenance costs on a unit biomass basis than larger-diameter roots (Eissenstadt and Yanai 1997) due to high turnover rates. In an environment where light intensity is not limiting, plants can easily satisfy the demand for carbon associated with the increased sink due to the formation of lateral roots, thereby promoting the development of greater root length, lateral roots and nutrient acquisition.

Spatial variability of nutrients in the soils can occur both horizontally and vertically along a soil profile. Generally, relative nutrient availability decreases with soil depth (Jobbágy and Jackson 2001; Postma et al. 2014). Similarly, some root morphological characteristics that are associated with greater nutrient uptake efficiency such as root mass, root length and SLR (Sharratt and McWilliams 2005; Kuchenbuch et al. 2009; Buczko and Kuchenbuch 2013) decrease with soil depth, confirming earlier reports that root density is highest at the topsoil (Figs. 2 and 3) as reported by Köpke (1981) and Neukirchen et al. (1999). This negative correlation between soil depth and root traits associated with nutrient uptake could be attributed to changes in nutrient supply. However, some cultivated crops have evolved strategies for developing greater root length density in deeper soil (>90 cm) than others to overcome periods of low nutrient availability in the topsoil and also to acquire nutrients that might have been lost from the topsoil through leaching (e.g. nitrate) (Neukirchen et al. 1999). In some cropping systems, catch crops (also called cover crops) are recommended as one way of reducing nitrate losses by leaching and for improved N use efficiency during crop rotations (Meisinger et al. 1991; Thorup-Kristensen et al. 2003), and deep-rooted catch crops are

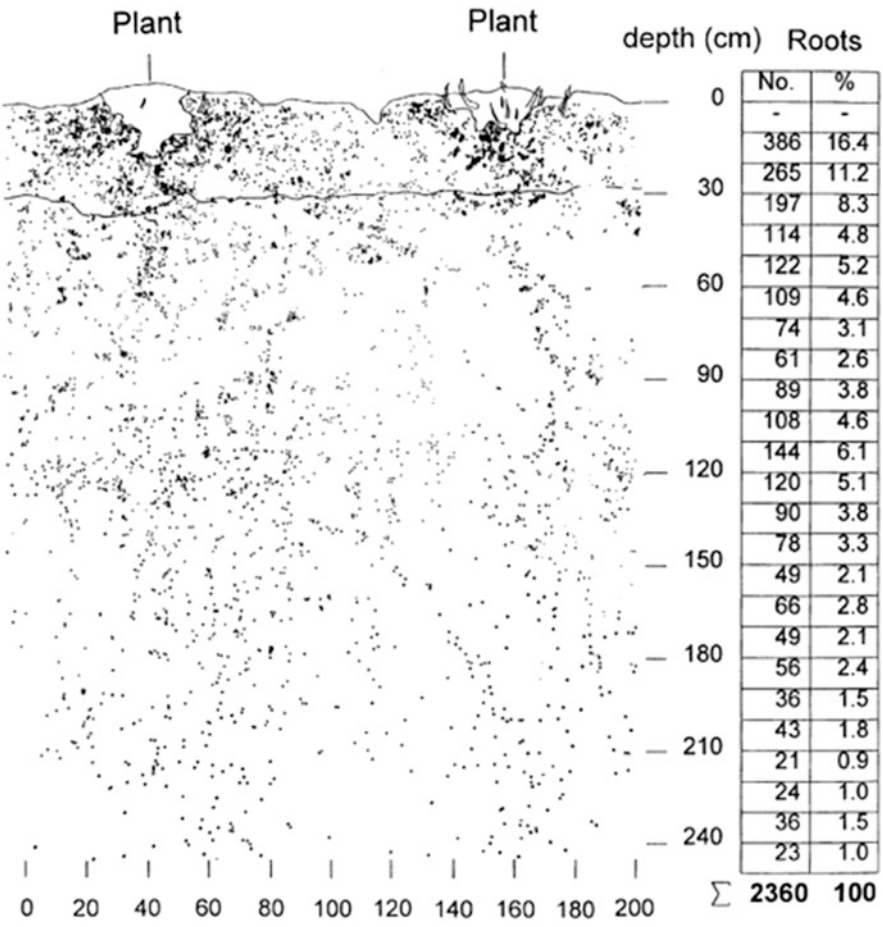

Fig. 2 Spatial distribution of the roots obtained with the trench profile method. *Source*: From Neukirchen et al. (1999)

particularly desired (Kristensen and Thorup-Kristensen 2004). In Denmark, for example, the use of catch crops is mandatory in autumn and winter seasons when there is high risk of nitrate leaching due to residual nitrate in the soils and high levels of precipitation (Wahlström et al. 2015).

Although root proliferation as a foraging response to nutrient-rich patches in the soil (Robinson 1994, 1996; Hutchings et al. 2003; Hodge 2004; Bartelheimer et al. 2006; Giehl and von Wirén 2014) is well established, similar root architecture and morphological and physiological traits for enhanced nutrient acquisition are also reported as plant adaptation mechanisms in low-phosphorus (P) soils. Marschner (1998) indicated that a large root system is either a constitutive or

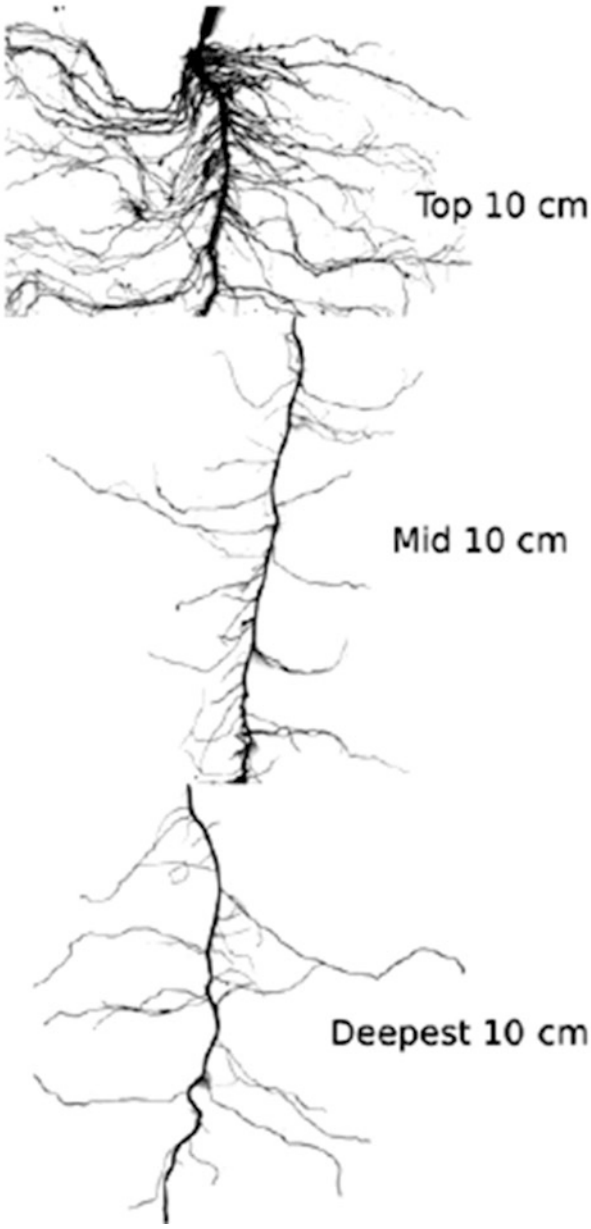

Fig. 3 Image showing how lateral root branching density (LRBD) may vary within a single root system. The image shows three root scans of 10-cm segments of a single primary root of a 28-day-old maize plant grown in a 20-L rhizotron box filled with a low-nutrient peat-basalt split mixture. The segments came from the basal part of the root (top = 0–10 cm), the middle part (mid = 40–50 cm) and the lowest part of the primary root that still carried laterals (deepest = 80–90 cm). The scan shows the much larger branching frequency on the top compared with the deeper segments. Differences in LRBD between the top and the rest of the primary root were highly significant based on 18 observations per location (three repetitions and six recombinant inbred lines from the same parents). *Source*: From Postma et al. (2014)

inducible trait that is very important for enhanced acquisition of P, a less mobile nutrient in the soil with low diffusion coefficient and fixed by some cations in the soil (Vance 2001; Gahoonia and Nielsen 2004; Hodge 2004; Lambers et al. 2006; Richardson et al. 2009a, b). The plasticity of roots and their ability to adapt to environmental changes are a unique characteristic of root systems (Williamson

et al. 2001). However, root architecture may also produce specialised roots, which are dedicated for specific nutrient acquisition, such as P.

3 Cluster Root Spatio-temporal Nutrient Acquisition

One of the primary effectors of root architectural changes is the limited availability of soil nutrients, specifically P (Lambers et al. 2006; Vance 2008). Phosphate is a scarcely available resource, rarely exceeding concentrations of 10 μM in the soil matrix (Watt and Evans 1999; Niu et al. 2013). By increasing the acquisition of P and conserving P use internally, plants can survive in severely P-limiting environments (Vance et al. 2003). Many physiological (decreased plant growth rate, increased root growth, prolific root hair production, exudation of organic acids and acid phosphatases) and biochemical (remobilizing internal P, alternative C metabolism and respiration that bypass P-requiring enzymes, increased P transporter expression) processes are involved in increasing and conserving P, with mycorrhizal associations being the most important plant adaptation for acquiring scarce P resources (Neumann et al. 2000; Vance et al. 2003; Lambers et al. 2006; Vance 2008; Niu et al. 2013). Some plant families however contain species that do not form this important symbiosis and instead produce specialised-complex cluster-like roots, including cluster and dauciform roots (Neumann and Martinoia 2002; Shane et al. 2005; Vance 2008). These specialised root structures are seen as the third major adaptation (after mycorrhizal and N_2-fixing symbiosis) for nutrient acquisition by plants in the terrestrial environment (Skene 2000; Neumann 2000; Neumann and Martinoia 2002; Lamont 2003; Shane et al. 2005; Vance 2008). Interestingly, most of the cluster root-forming species are able to form rhizobia symbiosis for N_2 fixation, but do not for mycorrhizal associations (Watt and Evans 1999) with the exception of *Casuarina*, which forms both (Lambers et al. 2008). The fast growth and turnover rate of cluster roots may confer an advantage in arid climates, where mycorrhizal associations would require more time to establish and be nutritionally and energetically more expensive (Neumann et al. 2000; Neumann and Martinoia 2002; Niu et al. 2013).

There are various morphologically distinct types of cluster roots in both monocotyledonous and dicotyledonous plants, all hypothesised to be functional in nutrient acquisition (Watt and Evans 1999; Lambers et al. 2006). Proteoid/cluster roots are described as densely clustered rootlets of defined length and were first identified in the Proteaceae family native to South Africa and Western Australia by Purnell (1960). It was originally thought that these rootlets were confined to this family, but subsequent investigations have identified them in species from Betulaceae, Moraceae, Casuarinaceae, Myricaceae, Cucurbitaceae, Elaeagnaceae and Fabaceae (Skene 1998; Lamont 2003; Lambers et al. 2006; Vance 2008). These species are often well adapted for growth in nutrient poor soils, using cluster roots to acquire

scarce nutrients. Cluster-like roots have also been described in Cyperaceae and Restionaceae (Lamont 2003; Lambers et al. 2006). Dauciform roots are formed by members of the Cyperaceae family and were first characterised by Selivanov and Utemova (1969). The mature root axis is carrot shaped and covered with dense clusters of long root hairs (approximately 2 mm) in contrast to the short rootlets with dense clusters found in cluster/proteoid roots (Lambers et al. 2006). Shane et al. (2006) showed that the development and function of these roots are analogous to cluster roots in Proteaceae and Fabaceae, respectively. Capillaroid roots are formed by members of the Restionaceae family and are described as root clumps with exceptionally long root hairs (Lambers et al. 2006). They are known for their sponge-like ability to hold soil water. Lambers et al. (2006) hypothesised that the physiology and function of capillaroid in monocotyledonous Restionaceae roots are analogous to proteoid/cluster roots in dicotyledonous plants.

Cluster roots mainly function for the acquisition of P from recalcitrant, acidic soils (Raghothama 1999; Watt and Evans 1999; Lynch and Brown 2001; Neumann et al. 1999, 2000; Neumann and Martinoia 2002). They have however also been shown to acquire Fe and Mn from alkaline soils and take up certain forms of organic N (Dinkelaker et al. 1995; Watt and Evans 1999). These densely clustered rootlets have been shown to mostly only proliferate in the top organic soil layer and can increase the absorptive area of the root system by up to 40 times (Shane et al. 2003). The exudation of large quantities of organic acids (prominently citrate and malate), protons and acid phosphatases functions to liberate P from complex metal cations and organic compounds, while increased P transporter expression leads to increased P uptake once P has been released (Watt and Evans 1999; Uhde-Stone et al. 2003; Shane et al. 2005; Lambers et al. 2006; Vance 2008). Cluster root induction, development, function and adaptations for enhanced P acquisition have most widely been studied in the agriculturally important legume, *Lupinus albus* (white lupin) (Johnson et al. 1996; Watt and Evans 1999; Vance et al. 2003; Liu et al. 2005; Lambers et al. 2006; Niu et al. 2013). White lupin is a fast-growing legume predominantly cultivated in the Mediterranean region and used as a food and forage legume.

3.1 Induction and Development of Cluster Roots

Cluster roots are mainly induced to form in the top organic layer of the soil, where P is most abundant (Watt and Evans 1999; Neumann and Martinoia 2002; Lamont 2003; Lambers et al. 2006). The induction of cluster roots by P deficiency is both internally and externally controlled, with the foliar application of P suppressing cluster root formation (Dinkelaker et al. 1995; Keertisinghe et al. 1998; Watt and Evans 1999). The availability of P is not the only controlling factor for cluster root induction. Various sugars have also been shown to contribute to the regulation of

cluster root formation (Uhde-Stone et al. 2003; Liu et al. 2005; Zhou et al. 2008). Interestingly plant species that form cluster roots in response to P usually do not form cluster roots in response to Fe and vice versa (Watt and Evans 1999). There are also factors that can stimulate cluster root formation, although not obligatory, and can include the presence of dissolved organic material and microbial factors as well as the pH of the soil (Lambers et al. 2006; Vance 2008). The formation, function and ultimate senescence of cluster root are tightly controlled in a spatio-temporal manner, allowing for maximum efficiency in the acquisition and recycling of nutrients, and are highly plant species specific.

Cluster roots are essentially a modified lateral root response to nutrient acquisition, but are developmentally distinct from lateral root formation (Lambers et al. 2006; Vance 2008). Lateral roots are initiated singularly opposite each protoxylem point in an alternating pattern from the pericycle of primary roots, near the metaxylem differentiation zone (Celenza et al. 1995; Fukaki and Tasaka 2009). In contrast, cluster roots are formed in multiples opposite each protoxylem point, in waves along the second- and third-order axis of lateral roots (Watt and Evans 1999; Pate and Watt 2001; Lamont 2003). The formation of root hairs is also markedly different in cluster roots when compared to lateral roots. Root hair production is tightly controlled in lateral root formation, with root hairs developing from a discrete number of trichoblasts. This tight regulation of trichoblasts is absent in cluster roots, and root hairs proliferate in large numbers (Dolan 2001; Müller and Schmidt 2004). Finally, cluster roots exhibit determinate growth, in contrast to the indeterminate growth of lateral roots, with fast turnover rates, distinct developmental stages of growth, function and finally senescence (Watt and Evans 1999; Neumann and Martinoia 2002). Meristems are not present in mature cluster roots, in contrast to lateral roots where a dense region of nuclei is found near the root tip. Juvenile cluster roots reaching mature length show a smaller region of dense nuclei near the root tip with this zone disappearing upon maturation (Zhou et al. 2008).

Cluster root development can be divided into four distinct, spatially and functionally separated developmental stages, (1) meristematic (pre-emergent), (2) juvenile, (3) mature and (4) senescent. Cluster roots can either develop to form single (*L. albus*) or complex clusters (*Banksia* spp.) (Pate and Watt 2001; Neumann and Martinoia 2002; Lamont 2003; Shane et al. 2005; Vance 2008). Complex clusters are formed when a rootlet within a cluster becomes the axis for a new rootlet; this can lead to mat-like root structures in the topsoil (Skene 1998). The vascular structure controls the number of rootlet rows as they develop from a singular xylem pole and is highly species specific (Watt and Evans 1999). In *Hakea* spp., six xylem poles give rise to three to seven longitudinal rows of rootlets (Lamont 1982), compared to *L. albus* where two xylem poles give rise to two to four rootlet rows (Johnson et al. 1996).

3.2 Plant Hormone Interactions During Cluster Root Development

3.2.1 Auxins

Lateral roots are known to be controlled by various plant hormonal signals, and this has also been shown to be true for cluster root formation (Watt and Evans 1999; Meng et al. 2013; Wang et al. 2014b, 2015b). The polar shoot-to-root transportation of auxin is well known for the induction of lateral root formation and was hypothesised to also be involved in cluster root formation (Watt and Evans 1999; Gilbert et al. 2000; Neumann et al. 2000; Fukaki and Tasaka 2009; Jung and McCouch 2013). A pulsed signal of polar auxin transport from shoots to roots was first suggested by Watt and Evans (1999). This communication would thus allow changes in shoot P status to be relayed to the root system. During P-induced cluster root formation, auxin synthesis and signalling genes are prolifically expressed (Vance et al. 2003; Yamagashi et al. 2011). Meng et al. (2013) and Wang et al. (2015b) showed that root derived auxins, and not polar auxin transport, may be more influential in cluster root development. The removal of shoot apices (main source of polar auxin) and application of auxin transport inhibitors to the shoot base did not affect cluster root formation in *L. albus* grown under P deficiency (Gilbert et al. 2000; Meng et al. 2013). The application of an auxin inhibitor to the root medium however did suppress cluster root formation. Wang et al. (2015b) confirmed temporal fluctuations in auxin transport during cluster root development (4–5 days), but found no difference in the amount of auxin translocated between shoots and roots in P-sufficient and P-deficient treatments. In pre-emergent cluster roots (sites for cluster root primordia formation), there was however an increase in auxin influx (*AUX1*) and efflux (*PIN1*) and biosynthesis (*YUCCA*) genes, with expression declining as clusters matured (Wang et al. 2015b). Thus root-derived auxins may play a more pivotal role in cluster root development than shoot-derived auxins.

3.2.2 Brassinosteroids and Cytokinins

Auxin can also interact synergistically with other plant hormones such as cytokinins and brassinosteroids to affect root architecture during P deficiency (Fukaki and Tasaka 2009; Jung and McCouch 2013). Brassinosteroid biosynthesis genes were predominantly expressed in the premature and juvenile cluster root stages, similar to the pattern observed for genes involved in auxin biosynthesis and transport (Wang et al. 2015b). External application of a brassinosteroid biosynthesis inhibitor (Brz) inhibited cluster root formation, lateral root elongation and induced root thickening. These changes are strikingly similar to changes observed in root architecture when plants were supplied with the ethylene precursors (ACC).

Interestingly exogenous application of active brassinosteroid (EBR) had no effect on cluster root formation (Wang et al. 2015b).

The role of cytokinins is traditionally characterised by shoot growth stimulation and root growth inhibition (Aloni et al. 2006). Cytokinin analogue kinetin also showed inhibitory effects on root architecture by inhibiting root elongation in P-deficient plants (Neumann et al. 2000; Wang et al. 2015b). Cytokinin receptors were shown to be most highly expressed in the pre-emergent stage of cluster root development, declining in mature cluster roots. Cytokinin degradation gene cytokinin oxidase/dehydrogenase (CKX) and corresponding ESTs were most highly expressed in mature cluster roots during P deficiency (Uhde-Stone et al. 2003; Vance et al. 2003; Wang et al. 2015b). Increased cytokinin concentrations in P-deficient vs P-sufficient *L. albus* plants were also observed by Neumann et al. (2000). Cytokinin concentrations were shown to be highest in juvenile cluster roots, perhaps due to the presence of large amounts of young root tips producing cytokinins. Cytokinin concentrations declined as cluster roots matured and could thus point to cytokinin being involved in lateral root inhibition during cluster root development (Neumann et al. 2000; Wang et al. 2015b).

A change in cytokinin concentration/signalling is however only a secondary response, primarily due to the crosstalk between auxin and P signalling cascades during P deficiency (Niu et al. 2013). Cytokinin biosynthesis has been shown to be rapidly regulated by auxins, while cytokinins can inhibit lateral root formation by modulating auxin transport and homeostasis (Nordström et al. 2004; Aloni et al. 2006; Laplaze et al. 2007). Root architecture changes during P deficiency, including cluster roots, could thus be partly controlled by auxin-cytokinin interactions at a metabolic level (Niu et al. 2013).

3.2.3 Ethylene

Ethylene production has been implicated in lateral root formation, with low concentrations exerting a stimulatory effect (Zhang et al. 2003; Fukaki and Tasaka 2009). It was thus hypothesised by Wang et al. (2015b) that ethylene could also have an effect on cluster root formation. By assessing the expression of ACC oxidase during cluster root developmental stages, they were able to show the diurnal nature of ethylene production during cluster root development. Pre-emergent cluster roots showed increased ACC expression when compared to juvenile cluster roots, with mature cluster roots showing the highest increase in expression. This is associated with the inhibition of lateral root elongation and subsequent proliferation of root hairs (Niu et al. 2013; Wang et al. 2015b). Ethylene has also been shown to interact with auxin biosynthesis and transport possibly cofunctioning during P-deficiency root responses (Osmont et al. 2007; Niu et al. 2013). These two hormones may act independently or through biosynthesis and response pathways to affect the same target genes (Stepanova et al. 2007).

Exogenously applied sucrose has also been shown to increase ethylene production. Plants supplied with sufficient P and sucrose and then treated with ethylene

precursor (ACC) and synthesis inhibitor ($CoCl_2$) showed complete repression of sucrose-induced cluster root formation (Wang et al. 2015b). Similarly, P-deficient plants also showed inhibition of P-deficiency-induced cluster root formation with the application of ACC and $CoCl_2$; however, interestingly ACC also affected the morphology of the rest of the root system. Root clusters were more densely spaced and restricted to the oldest first-order laterals (Wang et al. 2015b). Ethylene biosynthetic components have also been identified in the phloem sap of *L. albus*, which is in proximity of the pericycle, the initiation site for cluster root formation (Rodriguez-Medina et al. 2011).

3.2.4 Other Signals

Repression of cluster root production and citrate exudation with foliar P application and split-root systems (Keertisinghe et al. 1998; Shane et al. 2003) points to cluster root induction being a systemic response (Liu et al. 2005; Tesfaye et al. 2007; Zhou et al. 2008). Shoot P status could thus mediate the induction of cluster root formation, organic acid synthesis and acid phosphatase release, through various signals/signalling cascades (Liu et al. 2005; Müller et al. 2007; Zhou et al. 2008). As previously discussed some of these signals have been elucidated to be plant hormones including auxin, ethylene, cytokinins and brassinosteroids.

Systemic sugar sensing and signalling are a well-known mechanism used by plants to monitor metabolism, physiology, growth and ion transport to various biotic and abiotic stresses (Gibson 2005; Hammond and White 2008; Lei et al. 2011; Niu et al. 2013). Liu et al. (2005) showed that exogenously applied sugars/photosynthates mediate increased expression of P-deficiency-induced genes in *L. albus*, including *LaSAP1*, *LaPT1* and *LaMate*. This study also hypothesised that sugar signalling would be involved in cluster root induction and development. Similar work done in the model legume *Arabidopsis thaliana* showed sugar-responsive genes were regulated by P deficiency and similarly P-inducible/P-responsive genes were sugar inducible (Müller et al. 2005, 2007; Lei et al. 2011). These studies highlight the influence of P deficiency on genes involved in C metabolism (sugar sensing) and the influence of sugars on Pi (orthophosphate)-responsive genes.

A study conducted by Zhou et al. (2008) then showed that sucrose independent of P concentration in leaves stimulated cluster root formation. The effects of osmotic pressure and enriched energy source were excluded, by supplying plants with sorbitol and energy-supplying organic acids (malate and 2-oxo-glutaric acid), respectively, with no change in cluster root formation observed. The application of other hexose sugars, specifically the breakdown products of sucrose, fructose and glucose, was also investigated. These hexoses were shown to not significantly increase cluster root formation during P deficiency (Zhou et al. 2008). The supply of sucrose furthermore regulated the expression of *LaPT1*, *LaPEPC3* and *LaSAP* genes during P deficiency and cluster root formation (Zhou et al. 2008). Wang et al. (2015b) also showed that sucrose stimulated cluster root development in

P-deficient and P-sufficient *L. albus*, but saw no response to glucose or fructose. Exogenously supplied sucrose also increased P-status-induced lateral root density in *Arabidopsis*, but showed no effect on tap root length. This could point to sucrose being partially involved in lateral root formation during P deficiency (Jain et al. 2007; Karthikeyan et al. 2007). Sucrose could thus act as a direct shoot-derived signal for cluster root induction and development during P deficiency, while interacting with plant hormones to modulate root architecture during the P-deficiency response.

Cluster root induction and development are thus controlled by a wide range of interacting signalling cascades induced by P deficiency. This interplay between nutrient sensing, gene expression, plant hormones and sugars shows the complexity of regulating root architecture responses during nutrient stress. The specialised morphology and structure, adaptive physiology and specialised biochemistry of cluster roots allow them to thrive on P-deficient soils (Shane et al. 2005; Lambers et al. 2006). They are essentially a modified lateral root response to nutrient deficiency, sharing many similarities in both development and function. Cluster roots are however the product of the coordinated development of hundreds of lateral roots by reprogramming existing cellular structures for specific function (Lambers et al. 2006).

3.3 Functional Adaptations of Cluster Roots

Plant roots are known to exude a wide variety of organic compounds including carboxylates, sugars and phenolics (flavonoids and isoflavonoids) (Vance 2008). These organic exudates are functionally diverse and can effect plant growth, rhizosphere chemistry, plant-microbe interactions and nutrient acquisition (Ryan et al. 2001; Kochian et al. 2004; Vance 2008). As P deficiency is a main effector of root architecture changes, it is not surprising then that cluster roots have many adaptations for acquiring P from recalcitrant sources. Physiological adaptations such as increased absorptive area and soil exploration function to increase the effectiveness of adaptations in metabolism and biochemistry. These adaptations mostly involve normal root function and yet upregulated in response to P deficiency, showing increased exudation of organic compounds, enzymes and transporters for the recovery of P from both organic and inorganic sources (Shane et al. 2004, 2006; Vance 2008; George et al. 2011).

3.3.1 Carboxylate Exudation

Carboxylates (such as malate and citrate) are produced in all plant cells and are known to be exuded from plant roots during non-limiting and limiting nutrient conditions (Shane et al. 2004, 2006; Lambers et al. 2006; Vance 2008). The release of these carboxylates in a diurnal and exudative burst has however so far only been

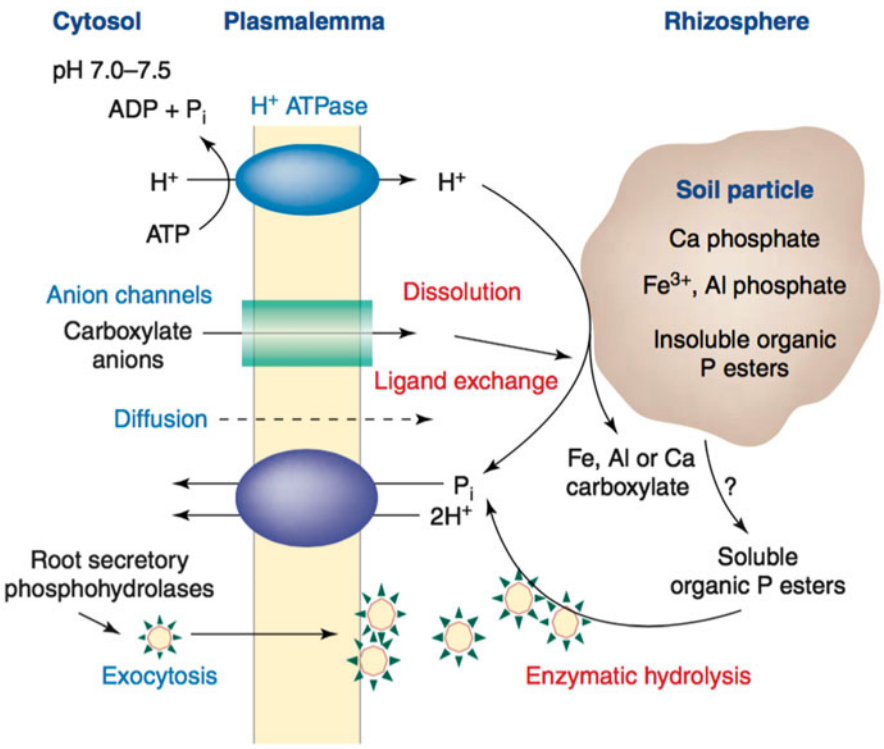

Fig. 4 Model for phosphorus-deficiency-induced metabolic changes related to intracellular accumulation and exudation of citrate. *Source*: From Neumann and Martinoia (2002)

described by Lambers et al. (2006) for cluster roots (Figs. 4 and 5). The combination of cluster root formation and carboxylate release confers a distinct advantage over carboxylate release alone. When several crop species, known for root carboxylate release, were grown under severe P limitation, those who used this combination (*L. albus* and *L. consentinii*) outperformed those that only exudated carboxylates (*L. angustifolius*, *T. aestivum*, *P. sativum*, *B. napus* and *C. arietinum*) (Lambers et al. 2006). Furthermore within the *Lupinus* genus, Bolland et al. (2000) and Hocking and Jeffrey (2004) showed that *L. albus* and *L. luteus* (cluster root formation and carboxylate exudation) outperformed *L. angustifolius* (carboxylate exudation only) when grown in field conditions. Cluster roots thus confer a distinct advantage during P-limiting conditions, and this is partly due to the coordinated release of carboxylates.

Carboxylates function by altering rhizosphere chemistry and releasing Pi from bound organic and inorganic sources (Lambers et al. 2002; George et al. 2011). This is achieved through dissolution of P-containing minerals, chelating Pi from metal cations (Al^{3+}, Ca^{2+} and Fe^{3+}), ligand exchange and desorption from soil particles (Lambers et al. 2002; George et al. 2011). Subsequent acidification of the rhizosphere via anion exudation and proton extrusion may also function to create a

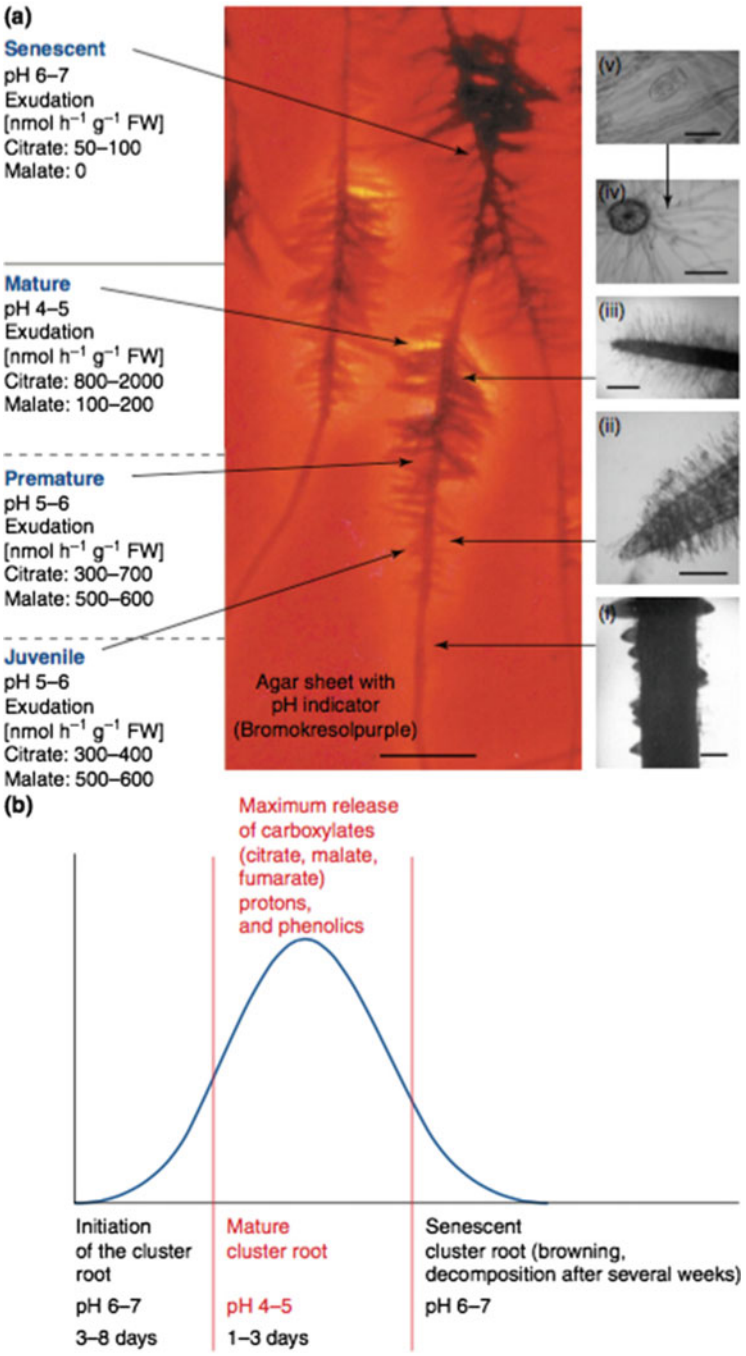

Fig. 5 Spatial and temporal variation in root morphology and root exudation during cluster root development. (**a**) Stages of cluster root development in *Lupinus albus*. Root-induced pH changes monitored by the application of agar sheets with pH indicator. (*i*) Juvenile, initial stage with emerging laterals; (*ii*) juvenile, root hair development on a lateral rootlet, predominant exudation

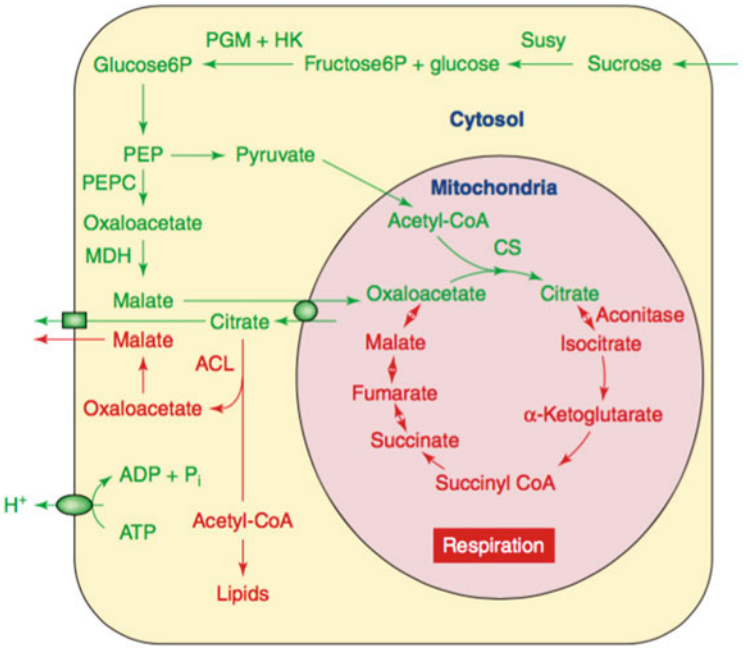

Fig. 6 Model for phosphorus-deficiency-induced metabolic changes related to intracellular accumulation and exudation of citrate in cluster roots of *Lupinus albus*. Stimulation of metabolic reactions and sequences marked in *green* and inhibition, marked in *red*. *Abbreviations*: *ACL* ATP-citrate lyase, *CS* citrate synthase, *HK* hexokinase, *MDH* malate dehydrogenase, *PEP* phospho*enol*pyruvate, *PEPC* phospho*enol*pyruvate carboxylase, *PGM* phosphoglucomutase, *Susy* sucrose synthase. *Source*: From Neumann and Martinoia (2002)

favourable environment for acid phosphatase (APase) activity (Jones 1998; Li et al. 2003; George et al. 2011). Citrate and malate have been shown to be the main forms of exuded organic acids from P-deficient cluster roots (Vance et al. 2003; Shane et al. 2005). During P-deficient conditions, cluster roots were shown to exude 20 to 40 times more citrate and malate when compared to roots supplied with sufficient P (Figs. 5 and 6) (Watt and Evans 1999).

The exudation of organic acids follows a distinct transient, spatial and temporal pattern of exudation during cluster root development (Watt and Evans 1999; Neumann et al. 2000; Neumann and Martinoia 2002) (Fig. 4). Early in cluster root development, little or no citrate exudation is seen, but this changes as the

Fig. 5 (continued) of malate; (*iii*) mature, lateral rootlets without further growth activity, completely covered with root hairs, burst of citrate exudation and release of H^+; (*iv*) transversal section; and (*v*) Protozoa (*Vorticella*) living on the surface of root hairs in mature root clusters. (**b**) Temporal changes during cluster root development and root exudation in Proteaceae and *Lupinus albus*. *Source*: From Neumann and Martinoia (2002)

cluster roots mature (Watt and Evans 1999). Between 10 and 12 days after cluster root initiation, an exudative burst occurs lasting 1–3 days. During this pulse, citrate production and exudation are kept at a maximum, returning to basal levels after the pulse and during cluster root senescence (Watt and Evans 1999; Neumann and Martinoia 2002). While malate exudation predominates at low levels during pre-emergent and juvenile developmental stages, citrate exudation is at a maximum during cluster root maturation. This may be a reflection of the increased effectiveness of tricarboxylic compared to dicarboxylic acids in chelating Pi from metal ions (Ryan et al. 2001; Richardson et al. 2007). Little or no organic acids are exuded during the senescent stage of cluster root growth, even though there is little difference in citrate tissue concentration between active mature and senescent cluster roots (Keertisinghe et al. 1998; Neumann et al. 1999; Watt and Evans 1999). This could point to citrate exudation-regulated transporters and not synthesis (Ryan et al. 2001; Zhang et al. 2004).

Citrate was proposed to be exuded via energetically passive anion channels in the plasma membrane (Fig. 4) by Neumann et al. (1999). This could be due to the large negative potential difference across the plasma membrane, caused by the high accumulation of trivalent citrate ions in the cytoplasm (Neumann and Römheld 1999; Neumann et al. 1999). Zhang et al. (2004) described two permeable channels located in the plasma membrane for anion transport in P-deficient cluster and non-cluster roots. These transporter channels were separately identified and designated as (1) an inwardly rectifying anion conductance (IRAC) and (2) an outwardly rectifying anion conductance (ORAC) channel. Membrane hyperpolarization and depolarization caused IRAC and ORAC activation, respectively. Both transporters however showed citrate ion transport, with IRAC showing preference for citrate, but also allowing transport of malate ions (Keertisinghe et al. 1998; Sas et al. 2001; Zhang et al. 2004). The IRAC channel is however proposed to be the main efflux channel for citrate due to its higher affinity for this anion. There was however no difference in IRAC and ORAC activity between P-deficient cluster and non-cluster roots, even though cluster roots are known for increased citrate exudation during P deficiency (Kania et al. 2003; Zhang et al. 2004). This may be due to (1) the large surface area of cluster roots, resulting in more fresh weight and thus citrate efflux (Zhang et al. 2004), (2) increased activity of plasma membrane H^+-ATPase in cluster roots causing cells to become increasingly hyperpolarised and acidifying cytoplasmic pH creating a greater driving force for citrate efflux through IRAC (Yan et al. 2002; Kania et al. 2003) and (3) higher tissue concentrations of both malate and citrate in cluster roots possibly leading to increased exudation through IRAC (Neumann et al. 1999; Kihara et al. 2003; Zhang et al. 2004).

3.3.2 Adaptive Metabolic Changes

Changes in carbon metabolism and gene expression are needed to drive the increased production and exudation of organic acids from cluster roots during P deficiency (Neumann et al. 2000; Massonneau et al. 2001; Uhde-Stone et al. 2003;

Shane et al. 2004). The C needed for organic acid exudation has been shown to be 60–65% shoot (from photosynthetic CO_2 fixation) and 35–40% root (dark, anaplerotic CO_2 fixation) derived (Johnson et al. 1994, 1996). Furthermore, changes in glycolytic enzymes, cytosolic sucrose synthase and organic acid metabolism, specifically PEPC (phosphoenolpyruvate carboxylase) and MDH and P-circumventing bypass reactions involving PK, all contribute to P conservation and organic acid exudation during P deficiency in cluster roots (Plaxton and Carswell 1999; Neumann and Martinoia 2002; Vance 2008). During P deficiency, root CO_2 fixation by PEPC also increases. Carbon lost due to organic acid exudation can thus be supplemented by root PEPC activity (Penaloza et al. 2005). Johnson et al. (1996) showed that 25% of labelled C exuded as citrate was fixed by PEPC in cluster roots and 34% of malate.

Plant-type PEPCs (PTPCs) are highly regulated, ubiquitous, multifunctional enzymes involved in organic acid synthesis, the supply of carbon skeletons for amino acid biosynthesis, generation of substrates for the TCA cycle and maintenance of cellular pH (Watt and Evans 1999; Uhde-Stone et al. 2003; Shane et al. 2013). This enzyme catalyses the irreversible β-carboxylation reaction of PEP (phosphoenolpyruvate), to release oxaloacetate (OAA) and Pi (Shane et al. 2013). Increased PEPC activity and expression in P-deficient cluster roots have also been associated with increased organic acid production and exudation (Fig. 6) (Johnson et al. 1996; Neumann et al. 2000; Vance et al. 2003; Uhde-Stone et al. 2003; Shane et al. 2004, 2013). PEPC activity however varies with cluster root developmental stage with the highest activity in mature clusters, although lower levels of activity are also present in juvenile clusters. Furthermore increased PEPC activity was observed at zones of possible citrate exudation specifically the meristem and cortex of cluster roots (Keertisinghe et al. 1998; Uhde-Stone et al. 2003).

In P-deficient *L. albus* cluster roots, three different isoforms of PEPC have been identified by Penaloza et al. (2005). These PEPC transcripts were designated as LaPEPC2, LaPEPC3 and LaPEPC4. LaPEPC2 is constitutively expressed in most plant tissues, whereas LaPEPC3 and LaPEPC4 are almost exclusively expressed in cluster roots and at very low levels in leaves (Penaloza et al. 2005). The abundance of these isoforms in cluster roots was mainly controlled by the Pi concentration of the medium (Kai et al. 2002). During P deficiency, LaPEPC3 and LaPEPC4 are highly upregulated in cluster roots. When P-sufficient conditions return, all PEPC transcripts are strongly downregulated. The LaPEPC3 isoform is however regulated well before Pi levels change and might be regulated by a different mechanism (Penaloza et al. 2005). PEPC is further also allosterically regulated by malate (inhibition); Glc-6-P (activation) and phosphorylation/dephosphorylation occur via PEPC protein kinase (PPCK) and protein phosphatase type 2A (PP2A), respectively. Phosphorylation acts as an activator of PEPC by decreasing malate inhibition, while increasing activation by Glc-6-P (O'Leary et al. 2011).

Malate is an essential organic acid in plant metabolism and has been shown to be used as an energy source in growing plant roots and cluster roots (Langlade et al. 2002). Malate dehydrogenase (MDH) is a crucial enzyme involved in many plant metabolic pathways and is widely distributed functioning in the glycolytic

pathway and reversibly catalysing the oxidation of malate to OAA (Uhde-Stone et al. 2003). Uhde-Stone et al. (2003) identified two MDH isoforms induced in P-deficient cluster roots of white lupin. These isoforms were designated LaMDH1 and LaMDH2 and showed high homology to cytosolic forms of MDH in *Z. mays*, *M. sativa* and *A. thaliana*. Expression of MDH was highest in P-deficient cluster roots, but was restricted to pre-emergent and juvenile developmental stages. During cluster root maturation, MDH expression was repressed. The OAA produced by upregulated PEPC expression and activity could now be used by citrate synthase for citrate instead of malate production (Neumann et al. 1999; Uhde-Stone et al. 2003).

The shift in organic acid exudation from malate in juvenile to citrate in mature clusters may further be affected by aconitase and ATP-citrate lyase (ACL) (Langlade et al. 2002). Aconitase is involved in citrate turnover in both the TCA cycle and cytosol (Neumann et al. 2000). Reduced activity of aconitase, coupled with increased PEPC activity, may account for increased citrate accumulation in P-deficient cluster roots (Neumann et al. 2000). ATP-citrate lyase catalyses the formation of OAA and acetyl-CoA from citrate by hydrolyzation and is mainly known for its action in lipid biosynthesis due to the production of acetyl-CoA. The activity of ACL was shown to be upregulated in juvenile cluster roots and decreased in mature cluster roots. The decreased activity of ACL in mature cluster roots would allow for citrate to be removed from the TCA cycle to not be converted to malate and must thus be exuded to maintain homeostasis (Langlade et al. 2002). This could point to ACL playing a key role in the shift from malate to citrate exudation. The other by-product of ACL function acetyl-CoA also functions in flavonoid synthesis (Weisskopf et al. 2006b). This enzyme could thus explain two important exudation shifts during cluster root development, the shift from malate to (1) increased citrate exudation and (2) decreased isoflavonoid exudation during cluster root maturation (Langlade et al. 2002; Weisskopf et al. 2006b).

Several other glycolytic enzymes are also affected by P deficiency in cluster roots and may serve as P-conserving bypass reactions (Vance 2008). Pyruvate kinase (PK) activity is limited during P deficiency, due to the requirement of P-containing compounds ADP and Pi. A P-conserving bypass reaction involving PEPC, MDH and NAD-malic enzyme functions to maintain TCA cycle intermediates without consuming ADP and producing Pi (Plaxton and Carswell 1999; Uhde-Stone et al. 2003; Morcuende et al. 2007). Reduced levels of cellular ADP and Pi can also affect respiratory pathways by inhibiting electron transport in the cytochrome pathway. Cellular metabolic integrity can however be maintained by the alternative oxidase (AOX) system, which bypasses energy and P requiring steps of respiration (Neumann et al. 2000; Vance et al. 2003; Shane et al. 2004; Hernandez et al. 2007; Morcuende et al. 2007). Another P-requiring step in glycolysis is ATP-dependent action of phosphofructokinase (PFK), which catalyses the phosphorylation of fructose 6-phosphate. The use of ATP can be circumvented by the use of the PPi-dependent PFK. White lupin cluster roots grown under P deficiency showed increased expression of PPi-dependent PFK, when compared to P-sufficient non-cluster roots, pointing to the importance of this bypass reaction in P conservation (Uhde-Stone et al. 2003). Sucrose synthase activity has also been shown to be

upregulated in both juvenile and mature cluster roots during P deficiency, with low activity during the senescent stage (Neumann et al. 2000). Sucrose synthase is a key enzyme in sucrose catabolism and is able to mobilise sucrose into multiple pathways involved in metabolic functions (Subbaiah et al. 2006).

3.3.3 Regulation of Pi Transporters

The transport of P from the soil once made available by cluster root function is mediated by P transporters specifically present in cluster and non-cluster roots during P deficiency (Smith et al. 2000). Due to the unfavourable nature of the P concentration gradient, P must be moved into the cell at the expense of ATP, coupled with the movement of protons (H^+) (Vance et al. 2003). There are two broad classes of P transporters, high and low affinity (Smith et al. 2000). High-affinity transporters are expressed mainly during P deficiency as a means for increasing P uptake from the soil. Low-affinity transporters are constitutively expressed, to allow for continuous P uptake (Smith et al. 2000).

Cluster roots show increased uptake of P when compared to non-cluster roots. Two phosphate transporters, LaPT1 and LaPT2, have been characterised in P-deficient white lupin cluster roots (Liu et al. 2001). Both LaPT1 and LaPT2 were identified as high-affinity P transporters and were seemingly unresponsive to Al, Fe, Mn and N stress. LaPT1 was not only functional in cluster roots but also in leaves and stems to a lesser extent. This could point to an internal P-mobilising function as well as increased soil P uptake during P deficiency. The induction of LaPT1 closely correlated with increased PEPC activity and organic acid exudation. The expression of LaPT2 was however localised to the root tissue and showed little response to P deficiency, showing more constitutive expression. This difference in transporter expression patterns may be due to differing physiological roles during P deficiency (Liu et al. 2001).

3.3.4 Proton Extrusion

Several authors have suggested a link between organic acid exudation and proton extrusion during P deficiency (Ligaba et al. 2004; Shen et al. 2005; Zhu et al. 2005b; Tomasi et al. 2009). Increased plasma membrane H^+-ATPase activity and protein expression were first observed in P-deficient white lupin cluster roots. Kania et al. (2001) and Yan et al. (2002) showed the involvement of a plasma membrane H^+-ATPase in organic acid release and proton extrusion. Similarly, Ligaba et al. (2004) found increased citrate exudation, linked to increased plasma membrane proton pump activity in blue lupin (*Lupinus pilosus*). This link between organic acid exudation and proton extrusion was also shown to be functional in aluminium-stressed soybean roots and P-deficient white lupin cluster roots. Changes in the activity of the plasma membrane H^+-ATPase were coupled with

citrate exudation and involved both transcriptional and post-translational enzyme modifications (Shen et al. 2005; Tomasi et al. 2009).

The anionic nature of the carboxylates being released via the plasma membrane (Ma et al. 2001; Zhang et al. 2004) may point to proton release functioning to energise the membrane and act as a charge compensator (Tomasi et al. 2009). Changes in the cation/anion influx ratio have also been linked to increased proton extrusion in P-deficient plants and may even be involved in plant-microbe interactions (Sas et al. 2001; Shen et al. 2005). The acidification of the rhizosphere, via proton extrusion, by juvenile and mature white lupin, P-deficient cluster roots, was shown to be associated with citrate exudation (Tomasi et al. 2009). The application of plasma membrane H^+-ATPase effectors fusicoccin (enhancer) and vanadate (repressor) to cluster roots also increased proton release (Zhu et al. 2005b; Tomasi et al. 2009). Interestingly fusicoccin stimulated citrate, but not malate release (Tomasi et al. 2009). Vanadate application completely repressed rhizosphere acidification by plasma membrane H^+-ATPase. The exudation of citrate was however repressed in a diurnal fashion and varied from 17 to 50% during an 11 h light period (Tomasi et al. 2009). This suggests that P-deficiency-induced proton extrusion may also be affected by time of day.

The concomitant release of organic ions and protons may thus serve several functions. Protons could participate in maintenance of the charge balance ratio, compensating for the negatively charged citrate anions being secreted as well as solubilising P from recalcitrant soils (Neumann et al. 1999; Zhu et al. 2005b). The low pH produced by proton extrusion may also function to provide the optimal pH range for the functioning of APases, glucanases and chitinases. Furthermore, they can also function to limit bacterial density and breakdown of carboxylates in the rhizosphere during cluster root maturation (Weisskopf et al. 2006a).

3.3.5 Acid Phosphatases

A large percentage (30–80%) of soil P is bound up in organic compounds as phosphate monoesters and unavailable for direct plant uptake (Fig. 4) (Tang et al. 2013). The predominant monoester, inositol hexaphosphate, comprises 20–50% of this total organic P fraction and must be hydrolyzed by acid phosphatases (APases) to release orthophosphate (Pi) for plant uptake (Richardson et al. 2009a, b; Tang et al. 2013). APases function in the acidic pH range to mobilise orthophosphate from a range of organic P-containing soil compounds (Lopez-Hernandez et al. 1998; Gilbert et al. 1999; Oehl et al. 2001; George et al. 2002). Extracellular APases function in the rhizosphere and are either secreted by roots or localised in the cell wall. Intracellular APases are mostly localised in the vacuole and function as soluble proteins (George et al. 2011; Tang et al. 2013).

Induction of both intra- and extracellular APases is a well-known response to P deficiency by white lupin cluster and non-cluster roots (Gilbert et al. 1999; Miller et al. 2001; Wasaki et al. 2003; Tang et al. 2013). Two *Lupinus albus*-specific

APases have since been identified in both cluster and non-cluster roots. The plasma membrane localised *LaSAP1* (Wasaki et al. 1999, 2003; Miller et al. 2001; Zinn et al. 2009) and root cell secretory APase *LaSAP2* (Ozawa et al. 1995; Miller et al. 2001; Wasaki et al. 2003; Tang et al. 2013). Wasaki et al. (2008) and Tang et al. (2013) showed increased intra- and extracellular APase activity and expression in both cluster rootlets and non-cluster root epidermal cells in response to P deficiency. Transcript accumulation of *LaSAP1* and *LaSAP2* has however been shown to be significantly different between cluster and non-cluster roots (Miller et al. 2001). Non-cluster roots were shown to have a high transcript abundance of *LaSAP1*, but little *LaSAP2*. Cluster roots showed the opposite trend, with a high transcript abundance of *LaSAP2* and reduced *LaSAP1* accumulation (Miller et al. 2001). This effect of P deficiency on increased APase expression and activity can be attributed to the (1) acidification of the vacuole and apoplast and (2) increased mRNA and protein accumulation of *LaSAP1* and *LaSAP2* (Gilbert et al. 1999; Miller et al. 2001; Wasaki et al. 2003; Tang et al. 2013).

The developmental stage of cluster roots was also thought to affect APase expression and activity (Tang et al. 2013). Intracellular APase (*LaSAP1*) was shown to be highly expressed and active in newly emerged cluster roots, rootlet tips and mature cluster roots, with a clear increase and distribution pattern along maturing cluster root axis. During the senescent stage, however, there seemed to be a decrease in LaSAP1 activity, but not gene expression (Tang et al. 2013). Extracellular APase (*LaSAP2*) showed activity and expression increases as cluster roots matured, with a maximum reached during the mature and senescent stage. Through all developmental stages, *LaSAP2* expression was much higher than *LaSAP1* (Tang et al. 2013). These APases thus function to recycle internal P and increase P acquisition during mature and senescent cluster root developmental stages. This increased activity during cluster root maturation also favourably correlates with increased organic acid exudation. This would create an acidified rhizosphere and optimal pH range for APase activity.

3.3.6 Glucanase and Chitinase

Extracellular glucanase and chitinase have also been shown to be exuded from P-deficient cluster roots (Buzynski et al. 2000; Weisskopf et al. 2006a, b). These enzymes have broad antifungal properties and are associated with degradation of fungal cell walls (Weisskopf et al. 2006a). Both glucanase and chitinase were shown to have higher activity during the juvenile stage of cluster root development, immediately preceding the oxidative burst and citrate exudation (Weisskopf et al. 2006a, b). Although not directly acquiring P, these enzymes may keep rhizosphere and pathogenic fungi from scavenging valuable exuded C resources in the form of citrate and malate (Weisskopf et al. 2006a, b).

3.3.7 Isoflavonoid Exudation

Cluster roots have been shown to exude a large variety of different compounds (as previously discussed). Yet not much attention has been given to the exudation of other molecules, particularly phenolics which have been shown to play a role in plant nutrition and plant-microbe interactions (Dinkelaker et al. 1995; Paiva 2000; Weisskopf et al. 2006b). Increased accumulation of plant phenolic compounds, including flavonoids and isoflavonoids, is a well-known response to nutrient stress. Iron and nitrogen stresses have been shown to increase the accumulation of flavonoids and isoflavonoids, with P deficiency shown to induce anthocyanin accumulation (Dixon and Paiva 1995). Flavonoids also affect plant-microbe interactions, attracting symbiotic N-fixing bacteria and mycorrhizal fungi, but also playing a role in defending against soil bacterial and fungal pathogens (Dakora et al. 1993; Paiva 2000; Weisskopf et al. 2006b).

Isoflavonoids are known to be an abundant class of phenolics in legumes (Paiva 2000; Weisskopf et al. 2006a, b). Weisskopf et al. (2006a) identified four diglycosides, six monoglycosides and two aglycones in the exudates of P-deficient cluster roots. These compounds were present in both cluster and non-cluster roots; however, cluster roots showed significantly increased exudation rates (Neumann et al. 2000; Weisskopf et al. 2006b). These flavonoid glycosides exudated from cluster roots have also previously been shown to be present in *Lupinus luteus* and *Lupinus albus* (Shibuya et al. 1991; Franksi et al. 1999). Phosphate deficiency was also shown to increase production and exudation of isoflavonoids in white lupin non-cluster roots (Weisskopf et al. 2006b). This is in line with previous findings in soybean (Murali and Teramura 1985) and bean plants (Juszczuk et al. 2004), where P deficiency induced increased phenolic production.

Phenolic compounds, specifically isoflavonoids, are also involved in plant-microbe interactions and symbiosis signalling during N deficiency. It is thus possible that during P deficiency these signalling pathways could interact (Weisskopf et al. 2006b). Glycosylflavonoid is a phenolic compound known to be involved in the regulation of arbuscular mycorrhizal (AM) association in plants (Weisskopf et al. 2006b). Enhanced exudation of glycosylflavonoid by P-deficient melon plant roots could indicate that flavonoids and isoflavonoids are involved in both P- and N-deficiency signalling (Akiyama et al. 2002).

The distinct exudation pattern of organic acids during cluster root development and function is well known. During cluster root development, organic acid exudation (citrate) is at a maximum during the mature developmental stage, with little (malate) exuded during the pre-emergent and juvenile stages (discussed previously). Isoflavonoid exudation was shown to be increased during the pre-emergent and juvenile stage, decreasing in both the mature and senescent stage of cluster root development (Weisskopf et al. 2006a). This points to a distinct isoflavonoid burst occurring before the oxidative burst, responsible for increased citrate exudation. Isoflavonoids are also well known for their antimicrobial and antifungal properties. This flavonoid burst could thus serve to limit both bacterial

and fungal growth, thereby protecting valuable P-chelating exudates (Dakora and Phillips 1996; Lambers et al. 2006; Weisskopf et al. 2006a, b). Soil fungi are able to use organic acids as an energy C source and could thus scavenge citrate from cluster root exudates, thereby decreasing the acquisition of P (Weisskopf et al. 2006a). Isoflavonoids exuded by white lupin cluster roots were shown to induce fungal sporulation and thus prevent mycelium scavenging of citrate (Weisskopf et al. 2006b). Of the several fungal species tested for growth inhibition by the exuded isoflavonoids, *Fusarium oxysporum* was the most susceptible (Weisskopf et al. 2006a). *Fusarium* fungi are well-known pathogens of lupins and have been shown to be inhibited by flavonoids (Silva et al. 1998; Shield et al. 2000). Soybean roots infected with *Fusarium* fungi have also been shown to increase the accumulation of isoflavonoids in response to infection (Lozovaya et al. 2004).

3.4 Cluster Roots and N Nutrition

Phosphate is a key component in plant growth. Phosphate nutrition is dependent on many factors of both the ability of the plant to acquire the nutrient and the availability in the environment (Høgh-Jensen et al. 2002). The many adaptive responses by plants to P deficiency, including physiological, morphological, biochemical and gene expression changes, show the necessity of this element for plant growth and development (Raghothama 1999). Nodulated legumes use more P, due to energy demand of N_2 fixation when compared to direct NH_3 uptake (Tang et al. 2001). With P being one of the most crucial nutrients for growth, it is not surprising that it plays an important part in nodule structure and function at multiple levels (Le Roux et al. 2009). P deficiency can both directly and indirectly influence nodule growth and functioning.

Direct influence is due to limited P supply and thus limited growth. Indirect influence is due to decreased photosynthate supply from the host plant, due to decreased rates of photosynthesis under P-deficient conditions (Valentine et al. 2011). Nodules are a strong P sink and do not readily release P back to the host plant. During P deficiency root P levels decrease dramatically, while nodule P levels stay constant (Le Roux et al. 2006). Metabolic adaptations to avoid P use must also be considered here. The PEPC pathway is induced under P stress to circumvent the PK P-requiring reaction of the glycolytic pathway (Valentine et al. 2011). For nodules under limited P supply, this pathway is problematic. The PEPC pathway competes for carbon skeletons and causes a shift from organic acid to amino acid metabolism (Le Roux et al. 2008). The problem is further compounded by malate being the carbon substrate of the growing bacteroids. Limited availability of P can also directly influence biological nitrogen fixation (BNF) via an N-feedback mechanism (Valentine et al. 2011). Low levels of P induce increased asparagine synthesis, which in turn inhibits BNF (Høgh-Jensen et al. 2002). Nodule development under limited P supply is reduced and leads to the

forming of smaller nodules. The increase in the surface/volume ratio causes increased O_2 permeability, which leads to decreased BNF (Lea and Miflin 2010).

Nitrogen source may affect cluster root formation. In *Myrica gale* it was observed that urea is more effective at stimulating cluster root formation than NO_3^-. The size of the root cluster was reduced however (Crocker and Schwintzer 1993). In *Gymnostoma papuanum* NO_3^- was more effective than NH_4^+ at promoting cluster root formation (Racette et al. 1990). This can however be due to faster uptake of NH_4^+ leading to increased internal N levels and cluster root suppression. Paungfoo-Lonhienne et al. (2008) found that *Hakea actites* grown with any form of N produced no cluster roots, while plants supplied with no N produced large amounts of cluster roots. When N was however supplied in growth-limiting amounts, N source affected cluster root production.

Nitrogen supply can also effect cluster root growth. Supplying plants with low N and low P stimulates cluster root formation, whereas the supply of low P and high N suppresses cluster root formation (Lamont 2003). This was confirmed for *L. albus* by Dinkelaker et al. (1995). It was shown that cluster root growth was increased during low-N supply and decreased during high-N supply. Sas et al. (2002) showed that *L. albus* plants deficient in P and supplied with NH_4^+ produced the most cluster roots and N_2 fixation the least. Cluster roots grown with NH_4^+ as a N source also produced significantly more cluster root dry biomass when compared to non-cluster roots. Exudates from cluster roots are also dependent on both N and P nutrition. Increased proton extrusion during P deficiency and NH_4^+ supply has been reported for *L. albus* (Sas et al. 2002). The supply of NO_3^- to P-deficient *L. albus* plants caused OH^- extrusion after 21 days of treatment. Phosphate-deficient and adequately supplied plants grown on NH_4^+ furthermore also exuded the same amount of protons, while NO_3^- treatment and N_2 fixation exuded up to 10 times more protons.

4 Nodules Spatio-temporal Nutrient Acquisition

Nitrogen is one of the most abundant elements on Earth, but at the same time, it is the critical limiting element for most plants due to its unavailability. Plants can assimilate N from two main sources (1) the soil, through commercial fertiliser, organic manure and/or the mineralization of organic matter, and (2) the atmosphere through symbiotic N_2 fixation (Vance 2001). Possibly 80% of this biologically fixed N_2 is derived from symbioses involving leguminous plants and species of *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium* (Graham and Vance 2000). In this chapter, they are collectively referred to as rhizobia.

The process of nodulation will be briefly described here; for an in-depth review on the developmental biology of legume nodulation, readers are referred to Gage (2004). Rhizobia are able to sense compounds such as flavonoids and betaines secreted by host plant roots in the rhizosphere and respond in turn by inducing *nod*

genes. Nod genes encode approximately 25 proteins which are essential for bacterial synthesis and export of Nod factor. The Nod factor is a lipooligosaccharide signal and initiates many of the developmental changes seen in the host plant early in the nodulation process such as root hair deformation, membrane depolarization, intracellular calcium oscillations and the initiation of cell division in the root cortex, which establishes a meristem and nodule primordium. In the early stage of the symbiosis, rhizobia must move from the root surface to the inner root tissue where they will populate cells in the emerging nodule. Rhizobia grow and divide inside a tubule called the infection thread which grows down the inside of a root hair and into the body of the epidermal cell. Rhizobia keep the tubule filled with bacteria by growing and dividing inside the infection thread. Bacteria are able to reach the intercellular space between the epidermal cell and the underlying cell layer by fusing of the infection thread with the distal cell wall of the epidermal cell. Branching of the thread as it grows through the root and enters the nodule primordium increases the number of sites from which bacteria can enter nodule cells, ensuring that a sufficient number of nodule cells are colonised. Once the bacteria have entered the nodule cells, they continue to differentiate and synthesise proteins required for nitrogen fixation and for the maintenance of the symbiosis (Gage 2004). The intracellular compartments enclosing the rhizobia are called symbiosomes, and the conditions inside these symbiosomes are conducive to maintaining rhizobial respiration and permit the oxygen-sensitive nitrogenase enzyme complex to convert unreactive atmospheric N to ammonia (Oldroyd et al. 2001). This ammonia is then converted to asparagine, glutamine or similar field N products and is exported to the legume host. In return, photosynthetically derived sucrose is converted to a suitable carbon source such as malate and provided to the rhizobia (Prell and Poole 2006).

4.1 Spatial Distribution of Nodules

The location of nodules within single roots and across the root system is not a random process (Remmler et al. 2014). Bhuvaneswari et al. (1980) identified a zone where lateral roots are most susceptible to rhizobial infection and where most of the nodules are later formed. This zone has been described as a small region just above the root tip where root hairs have begun differentiating but not yet matured. The root hairs in this location have cell walls that are plastic enough to allow deformation and penetration of the rhizobia via the infection thread (Remmler et al. 2014).

In an entire root system, it has been noted that not all roots bear nodules (Remmler et al. 2014). Sagan and Gresshoff (1996) have found that nodules in *Pisum sativum* plants are formed high in the root system in a collection located close to the crown of the plant (all the above-ground parts, including stems, leaves and reproductive structures). Two possible explanations exist for this occurrence. The first one is that inoculants are usually applied to seeds or the growing medium, and because rhizobia have a low ability to migrate in the soil (Wadisirisuk

et al. 1989), they will infect only the roots near the crown of the seedlings (Cardoso et al. 2009; Remmler et al. 2014). The second explanation for this clustering of nodules near the crown is the autoregulation of nodules (Ferguson et al. 2010; Remmler et al. 2014). In this systemic regulatory mechanism, the first nodules to be formed on the root system would send an ascending signal to the shoot. The decryption of this signal by a CLAVATA-like leucine-rich kinase would result in the production of an inhibitory signal descending to the root, which would prevent new nodules from developing on the younger roots now developing further down in the growth medium or substrate (Ferguson et al. 2010; Remmler et al. 2014).

The spatial distribution of nodules also controlled at the genotype level as was found in *Glycine max* (soybean), where different cultivars present different nodulation profiles and the nodules' position in the root system is only slightly influenced by different growth conditions (Burias et al. 1990; Remmler et al. 2014). Schulze et al. (2006) showed that nodules of *Lupinus albus* plants were located in the vicinity of cluster roots where P uptake is presumably highest.

4.2 The Nutritional Control of Root Development Over Time

The variable supply of water and nutrients across space and time frequently limits growth of belowground structures (Robinson 1994; Forde and Lorenzo 2001). Primary root growth appears to be much less sensitive to nutritional effects than secondary or higher-order roots – a general rule of thumb for plants (Forde and Lorenzo 2001).

The development of N-fixing nodules on legume roots is a highly regulated process whereby the number of nodules on a root system is controlled by a mechanism called autoregulation, in which previously formed or forming nodules suppress the development of further nodules (Schulze and Kondorosi 1998). Split-root experiments have established that autoregulation acts systemically and that the signal originates from the shoot (Kosslak and Bohlool 1984). Combined N also has a strong suppressive effect on nodule formation whereby legumes preferentially utilise an N source instead of forming the N-fixing symbiosis (Carroll and Matthews 1990).

NO_3^- inhibits nodule formation without interfering with plant growth. It has been shown that sensitivity of nodulation to NO_3^- is strongly dependent on plant species and genotype (Carroll and Matthews 1990). Nitrate suppresses the N-fixing symbiosis at various stages of the nodulation process such as rhizobial infection and also inhibits nitrogenase activity in established nodules and triggers early nodule senescence (Carroll and Matthews 1990). Malik et al. (1987) have shown that with a delay of 18 h, the NO_3^- treatment after rhizobial inoculation showed a vastly diminished inhibitory effect in soybean. Wilson found that soybean nodule formation was only inhibited in those parts of the root directly exposed to NO_3^- supply. The inhibition of nodulation is primarily a localised response that has been confirmed by a number of studies (Carroll and Matthews 1990).

The systemic or autoregulatory effect of NO_3^- cannot be ruled out: when the concentration of NO_3^- supplied to the NO_3^- fed half of the split-root system is sufficiently high, nodule development in NO_3^- free half can be inhibited (Carroll and Gresshoff 1983; Hinson 1975). Other evidence for systemic effects of NO_3^- came from experiments in which NO_3^- was supplied either above or below the site of inoculation and was still effective in inhibiting inoculation (Malik et al. 1987). There is genetic evidence for a relationship between autoregulation of nodulation (systemic phenomenon) and NO_3^- inhibition of nodulation (Day et al. 1989). It may be that NO_3^- interacts with the autoregulation process at more than one level, accounting for both localised and systemic effects.

External supply of NO_3^- triggers a rapid induction of a number of genes, including those for NR, nitrite reductase and NO_3^- transporters. It has been shown that NR activity is not required for the induction to occur, clearly implicating the NO_3^- ion as the signal molecule (Deng et al. 1989; Pouteau et al. 1989). Studies using NR-deficient mutants have shown that localised NO_3^- inhibition of nodulation in legumes is independent of NO_3^- assimilation (Carroll and Gresshoff 1986; Jacobsen 1984). Localised developmental responses to NO_3^- and in all probability other nutrient ions as well are mediated via specific sensors or receptors on the plasma membrane or elsewhere and their associated signal transduction pathways. This points to the existence of nutritionally regulated transcription factors or other regulatory proteins that could modulate developmental processes in response to changes in external nutrient supply.

Pea and soybean mutants have been isolated which are NO_3^- tolerant for nodulation; all NO_3^--tolerant mutants isolated so far are defective in the autoregulatory mechanism and also display a super-nodulation phenotype. This suggests that NO_3^- inhibition of nodulation acts through an interaction with the autoregulatory mechanism, which is thought to depend on the production of a phloem-mobile inhibitory signal in the shoot (Carroll and Matthews 1990). Systemic regulation of nodulation is controlled by a particular class of CLE peptides that are predominantly induced by microbial infection (Djordjevic et al. 2015).

NO_3^--tolerant modulating mutants also have an altered root phenotype (increased number of lateral roots and an increased shoot-root ratio) even when not inoculated with rhizobium (Day et al. 1986; Postma et al. 1988). The NO_3^--tolerant mutants have a constitutive 'high-N' phenotype, and it is possible that the regulatory mechanisms responsible for autoregulation and NO_3^- regulation of nodulation have evolved from pre-existing mechanisms for the regulation of root development and shoot-root partitioning that may be common to nonlegumes. The mechanisms that allow legumes to switch on this competency for nodulation in low-N environments are not known (Djordjevic et al. 2015). Crosstalk or convergence between different signal transduction pathways is becoming a well-established mechanism in plants by which signals from independent stimuli are integrated at the biochemical and genetic levels (Genoud and Metraux 1999). It is also possible for the systemic and localised signals to act on different stages of the developmental process, so that integration may only be achieved at the whole plant level.

Sensing of the plant's nutrient status takes place in the shoot in cases where root's response is systemic and the shoot is the source of long-distance signals that regulate both physiological and developmental processes. Plants can monitor nutrient supply in at least two ways – directly through localised changes in nutrient concentration in the external soil solution or indirectly through changes in the internal nutrient status of the plant itself. Direct sensing allows the plant to respond to short-term changes in nutrient availability and provides roots with spatial information regarding distribution of nutrients within the soil profile which in turn allows plants to concentrate developmental responses to soil regions where nutrient acquisition will be maximised. Indirect pathway allows a plant to integrate its nutritional signals with those coming from other physiological processes, such as photosynthesis (Forde and Lorenzo 2001).

4.3 Long-Term Temporal Development of Nodules

In a comprehensive study on seasonal development of both below- and above-ground organs of *Trifolium pratense*, Chmelikova et al. (2015) found that nodule activity was influenced by season to a much greater extent than soil conditions. Both Voisin et al. (2002) and Hatch et al. (2007) found that N_2-fixing activity decreased with legume age. N_2-fixing activity depended on growth rate and phenology during the growth period and generally decreased with flowering and maturation (Voisin et al. 2002; Cupina et al. 2010), and it was found that nodule numbers decreased when numbers of inflorescences increased.

These authors also found that nodule activity (measured as proportion of pink nodules) decreased during the season. Several authors have reported on molybdenum (Mo) increasing the number and weight of nodules thereby improving the symbiotic process of N_2 fixation of legumes. Shaw et al. already reported in 1966 that the chief effect of Mo on nodulation appeared to be a longer period of effective N_2 fixation, which meant that over time more nodules remained active (pink) and did not become senescent or moribund (green or brown).

4.4 Nutritional Influences of Phosphorus (P), Potassium (K) and Sulphur (S) on Biological Nitrogen Fixation

Legumes that acquire N by BNF generally have a higher requirement of P, K and S than those which only rely on soil N (Israel 1987; Sulieman et al. 2013). These nutrients can affect BNF directly by modulating nodule growth, nodule formation and functioning (Duke et al. 1980; Almeida et al. 2000; Varin et al. 2010).

Due to the high ATP requirements for nitrogenase function, P availability is critical for nodule activity (Ribet and Drevon 1995; Al-Niemi et al. 1997), and it

also plays a role in signal transduction, membrane biosynthesis and nodule development and function (Al-Niemi et al. 1997).

Duke et al. (1980) demonstrated a direct effect of K status on BNF mediated by its influence on nodule growth and function, activity of enzymes involved in ammonia assimilation, amino acid interconversions, carbon supply and energy transduction. Varin et al. (2010) showed a close relationship between S supply and nitrogenase and leghaemoglobin content in nodules. Schere et al. (2006) determined that S deficiency reduced BNF in pea (*Pisum sativum*) and lucerne (*Medicago sativa*) as a consequence of decreased ferredoxin and leghaemoglobin concentrations as well as reduced ATP supply.

Besides the direct impact of P, K and S in these aspects of carbon and N metabolism, it is generally accepted that their main effect on BNF is mediated by responses of host plant growth (Almeida et al. 2000; Hogh-Jensen 2003; Varin et al. 2010). In this sense, when nutrient deficit reduces plant growth, an N feedback is triggered that downregulates nodule development and activity. This mechanism also seems responsible for the regulation of BNF when other stresses, such as drought, salt, toxic metals and pathogen attack, are involved (Lea et al. 2007).

A meta-analysis of studies investigating the effects of P, K and S availability on plant legume growth and BNF has found that there exists a larger sensitivity of nodule mass compared to shoot mass in response to P, K and S deficiency (Divito and Sadras 2014). Reports on the effect of these nutrients on nodule number are controversial, especially for P. There have been reports that P deficiency may increase (Schulze et al. 2006), decrease (Pereira and Bliss 1989) or have no effect (Drevon and Hartwig 1997) on nodule number per unit shoot mass. It is widely accepted that P-deficient plants tend to have a larger number of smaller nodules, and Ribet and Drevon (1995) proposed that this strategy increases the nodule surface/volume ratio, thereby facilitating oxygen diffusion into the nodule, which is critical for effective BNF (Layzell et al. 1990). Using *L. albus*, Schulze et al. (2006) showed that not only the number of nodules increased with increases with P deficiency but also that nodules were located in the vicinity of cluster roots where P uptake is presumably highest. In contrast to these responses to P, nodule number and mass are equally affected by deficit of K and S.

When investigating trait responses, special consideration should be given to sampling dates. This meta-analysis has shown that differences in nodule mass reduction in response to P deficiency decrease with plant age. Qiao et al. (2007) determined that 2 weeks after transplanting, early nodule formation was not affected by external P supply in soybean. They proposed that P from seed reserves could support rhizobial infection and nodule initiation. In the period from 3 to 4 weeks after sowing, P deficit markedly decreased nodule formation, but did not affect the growth of host plants. After 5 weeks, both nodule formation and plant growth were depressed, although the effect was greater on nodule formation. To date, no consistent explanations have been proposed to account for this pattern.

The interaction between plant age and nodule type might further bias the conclusions regarding nutrient effect on BNF and related traits. Nodules can be determinate or indeterminate depending on the host plant species (Hirsch 1992).

Indeterminate nodules continuously produce new cells from a persistent meristem to replace older senescent cells. They are found in species such as clover, lucerne and pea. By contrast, in determinate nodules found in most monocarpic legumes, the end of the nodules life cycle coincides with pod filling (Puppo et al. 2005). Therefore, the relations between plant growth and nodule growth and activity could be influenced by the interaction between plant age and nodule type. In the analyses of Divito and Sadras (2014), no attempt was made to account for plant age or nodule type, but these factors warrant further investigation.

5 Mycorrhizal Spatio-temporal Nutrient Acquisition

The majority of plant families (92%) are associated with a specialised group of soil fungi which reside and develop within and around plant roots and are collectively termed mycorrhizas (Wang and Qiu 2006). Fungi assist this mutualistic partnership through exploitation of the soil environment gaining access to vital nutrients and returning these to the host plant roots in exchange for photosynthetically derived carbon compounds. Almost 40 years ago, it was proposed that plants were able to establish themselves outside an aquatic environment with the aid of symbiotic fungi; this was supported by fossils over 400 million years old, first discovered in the Rhynie Chert in Scotland (Pirozynski and Malloch 1975). Aseptate hyphae, vesicles, spores and branched hyphal arbuscular-like structures were identified in fossilised rhizome material (Remy et al. 1994; Taylor et al. 1995) and resembled the fungal structures which characterise arbuscular mycorrhizal (AM) associations today. Subsequent DNA nucleotide substitution analysis dates the appearance of AM associations to approximately 510 million years ago, representing an ancestral association common across a wide range of plant families from gametophytes of mosses and lycopods, gymnosperms, pteridophytes and angiosperms and in diverse environments, bearing witness to their ability to establish under harsh and changing conditions (Cairney 2000). Given the diversity of host plants, the *Glomeromycota* fungi are surprisingly represented by approximately 300 species (Öpik et al. 2010) possibly due their obligatory nature and inability to independently complete their life cycle (Cairney 2000; Smith and Read 2008) as well as our inability to readily distinguish between species morphologically (Smith and Read 2008). AM associations are characterised by a well-developed intraradical hyphal proliferation forming finely branched arbuscules within cortical cells, vesicles, spores and a network of intercellular hyphae. This network extends out of the root into the surrounding soil environment (Maherali and Klironomos 2012).

Woody trees, shrubs and some herbaceous plant taxa associate with ectomycorrhizal (ECM) fungi which include members from the *Basidiomycota*, *Ascomycota* and some *Zygomycota* phyla, encompassing approximately 6,000 fungal species (Smith and Read 2008). Their point of origin in geological time has been debated; the only fossil record dates to ca. 50 million years ago (LePage et al. 1997), while other researchers believe separate lineages diverged around

500 million years ago (Cairney 2000). ECM associations are characterised by the development of a fungal mantle or sheath around host plant roots with radiating rhizomorphs and mycelial threads. Internal hyphae develop into a netlike structure which surrounds root cells forming a 'Hartig' net (Smith and Read 2008). Some of these diverse fungi have retained saprotrophic abilities which enable them to degrade organic material (Read and Perez-Moreno 2003).

Ericoid mycorrhizal (ERM) fungi associate with a narrow group of plants belonging to the *Ericales*. The majority of these fungi belong to restricted groups of the *Ascomycota*, with a few *Basidiomycota*. ERM associations are dominant in nutrient poor, acidic environments rich in complex organic compounds (Perotto et al. 2002). They are recognised by tightly packed intracellular hyphal coils with limited extraradical growth (Smith and Read 2008). The fungi express many saprotrophic traits which allow them to access nutrients from organic matter (Perotto et al. 2002). The *Ericales* and their associated fungi are thought to have arisen in the southern hemisphere during the Gondwanan era, approximately 140 million years ago (Cairney 2000).

Specificity of fungal partners ranges from highly specific as in some of the ERM systems to more generalist as observed in AM systems. Despite their divergent origins, different association types and diverse hosts, mycorrhizal systems exist today in all ecosystems (Smith and Read 2008) each contributing to the success of land plants and the ever-changing plant community structure. The prevalence of any one mycorrhizal type in an ecosystem is largely dependent on the host plant and fungal identity, with some influence of environmental conditions (Brundrett 2002).

5.1 Mycorrhizal Benefits

The mycorrhizal association is generally considered to be beneficial to host plants, and many review articles have addressed the symbiotic potential of improving the plants' ability to survive and tolerate adverse growing conditions such as drought, salinity, heavy metals and pathogens (Smith and Read 2008). Enhanced nutrient uptake either directly or indirectly is one mechanism by which host plants access these benefits. Many of the ECM and ERM fungi have distinct enzymatic activities which have been studied in vitro indicating their ability to access nutrients from complex organic molecules such as cellulose, starch and proteins (Read and Perez-Moreno 2003), amongst others. These enzymes access nutrients not normally accessible to the plants, and as a result smaller molecules are absorbed by the hyphae and transported through a compartmented regulatory system involving both the fungal cytoplasm and vacuoles, with mixing being restricted through polarisation of the solutes, therefore providing for both uni- and bidirectional movement (Ashford and Allaway 2002; Smith et al. 2001, 2003). Poorly mobile soil nutrients such as P, Zn, Cu, Fe and Mo rely on diffusion for root uptake; this results in the formation of depletion zones (Liu et al. 2000). Extraradical hyphae

have been shown to absorb nutrients up to 25 cm away from roots, well beyond the depletion zone (Cardoso and Kuyper 2006).

Rhizomorphic structures of ECM fungi are well designed for long-distance nutrient and water transport with vessel-type hyphae which as a result of partially or completely dissolved septa reduced the flow resistance. These F-type rhizomorphs such as found in *Suillus bovinus* (Agerer 2001) are well adapted to explore the soil environment. Rhizomorphs are not formed by AM fungi but the role of the extraradical hyphae is of equal importance. Hyphal diameter ranges from 2 to 27 μM with varying wall thickness. The main thick walled anchor hyphae give rise to finely branched absorptive hyphae; anastomosis occurs linking plants of different species together belowground (Giovannetti et al. 2004).

5.2 Temporal Changes in Seasons and Habitats

AM fungi have limited dispersal capacity due to the production of large spore types which may show poor germination. The living hyphal network is regarded as being the most important for rapid colonisation and nutrient acquisition for seedlings (Smith and Read 2008). However, the establishment of the hyphal network and its consequent benefits may also impose carbon costs on host resources, to the extent where AM spatial and temporal development can inhibit the development of other symbionts. This was found in the tripartite symbiosis with roots, N_2-fixing nodules and AM fungi in *Phaseolus vulgaris* (Mortimer et al. 2008). In this regard, the growth of nodules was suppressed by the early development of AM colonisation, especially under low-P supply. This early AM establishment during P limitation imposed a greater photosynthetic and respiratory cost on AM plants, which may have reduced the carbon availability to the nodule development. However, once the AM colonisation rate reached a plateau phase, the efficiency of P nutrition also increased in this established AM symbiosis. These P nutritional benefits of the established AM symbiosis then led to improved nodular growth and enhanced N_2 fixation. These findings indicate that the spatial and temporal development of the AM fungi was the dominant symbiotic sinks for host C in the tripartite symbiosis (Mortimer et al. 2008). For other mycorrhizas the large carbon costs related to temporal and spatial changes may be associated with fruiting structures, rather than mostly hyphal development and function.

ECM fungi often produce large sexual structures producing spores in quantity aiding dispersal over long distances (Bahram et al. 2015). Mostly mycorrhizal fungi remain hidden belowground; ECM fungi are observed above-ground as sporocarps making it difficult to accurately predict temporal changes in populations. More recent molecular approaches combined with sporocarp surveys conducted by Van der Linde et al. (2012) indicated that despite lack of sporocarps, species were identified molecularly in the soil 4 years later. A meta-analysis by Bahram et al. (2015) of the limited studies on ECM and AM fungi suggests in the upper soil horizons, spatial and temporal variation was higher due to the heterogeneous

nature of the environmental conditions. AM fungal spore densities can fluctuate from as little as one to five spores to over 100 spores per gramme of soil (Smith and Read 2008). Spore densities can vary seasonally depending on individual species (Bever et al. 2001); how rapid host plant roots become colonised will determine the effectiveness of the association.

5.3 Spatial Changes in Mycelial Networks

In undisturbed soil the common mycorrhizal network (CMN) is an important and often ignored extraradical component of the mycorrhizal system allowing for greater exploitation of the soil environment for nutrient sources and establishment of colonisation in adjacent plants forming common linkages belowground. Seedlings of *Quercus serrata* were able to establish ECM associations in predominantly AM forests up to 18 m away from a host tree (Matsuda et al. 2013), although direct evidence could not be provided for hyphal interaction due to the presence of spore banks and sclerotia. Young temperate forests are often colonised by a few ECM fungi some of which produce fruiting bodies, and these are often found fruiting around the trees. As the forest matures, diversity increases (Paey et al. 2011), and interactions between mycorrhizal species may result in the exclusion of other species. Tyler (1994) reported that *Russula* and *Lactarius* species in a hornbeam (*Carpinus betulus*) forest occurred by excluding each other, while *Laccaria* and *Cortinarius* species were unaffected. This may be as a result of resource availability and ability of the respective fungi to utilise various nutrient sources. This assumes that late-stage ECM fungi such as *Russula* are able to degrade more complex organic molecules such as cellulose and lignin, while early-stage ECM fungi are confined to more mineralised sources of nutrients (Paey et al. 2011). This has been supported by studies that have shown that some early-stage (ruderal or pioneer) ECM fungi such as *Laccaria proxima* and *L. bicolor* were unable to utilise protein as an N source, while proteolytic ability of *Thelephora terrestris* was variable (Finlay et al. 1992). More recently ECM spatial distribution has been attributed to root density rather than degradative ability (Paey et al. 2011) being related to number of host trees in a cluster. Changes in root density occur throughout forest stands at the margins, within the soil profile and during early establishment. In tree stands where roots are widely spaced, ECM fungi with limited mycelial exploratory ability are unable to colonise the roots. These ECM morphotypes are characterised by smooth mantles with few emanating hyphae requiring these short-distance exploratory ECM types to be in close contact with the resource substrate and include species of *Russula*, *Lactarius*, *Tomentella* and *Tuber*, successful colonisation resulting mainly from spore banks (Agerer 2001: Paey et al. 2011). As root density increases, ECM morphotypes are dominated by medium- and long-distance exploratory types such as *Laccaria*, *Cortinarius*, *Thelephora* and *Scleroderma*; this results in increased competition for nutrients and a need to exploit hidden or inaccessible resources (Agerer 2001: Paey et al. 2011).

Ericoid mycorrhizal roots are commonly associated with limited extraradical mycelial networks, substrate contact being a requirement for nutrient sourcing through degradative enzymes. ERM fungi are well known to produce enzymes that degrade complex organic molecules such as cellulose and proteins (Nasholm and Persson 2001; Perotto et al. 2002).

5.4 Spatial Changes Within the Soil Profile

Mycorrhizal investigations have mainly been limited to the top 5–20 cm of the soil profile (Bahram et al. 2015). In a Norway spruce (*Picea abies*) plantation, Baier et al. (2006) investigated the distribution of ECM morphotypes within the soil profile. *Cenococcum geophilum*, *Lactarius* and *Tomentella* species predominantly preferred the organic horizons, while the humic A horizon was exploited by *Russula* and *Lactarius* species. Similarly an investigation in *P. patula* plantations recorded a predominance of *Scleroderma citrinum* in accumulated organic layers, with 97% of roots colonised indicating a short-circuited nutrient cycle due to poor nutrient resources and very low pH in the mineral horizon (Dames et al. 2002). This circumvention of the accepted cycling process in ECM forests has been reviewed by Wu (2011) given their ability to utilise both inorganic and organic sources of nutrients (Lindahl et al. 2002; Lindahl and Taylor 2004; Nygren et al. 2007; Read and Perez-Moreno 2003).

Nutrient cycling interactions between decomposer and mycorrhizal fungi should not be disregarded. In forest soil the outcome may be antagonistic, and removal of ECM fungi from the system, as elegantly shown by Gadgil and Gadgil in 1975, results in an increase in the rate of decomposition. The contribution of mycorrhizal fungi to decomposition is often overlooked. Some ECM fungi are regarded as facultative saprotrophs and have retained the ability to degrade organic material using oxidative mechanisms in co-metabolic processes in the presence of other C sources such as glucose (Lindahl et al. 2007; Lindahl and Tunlid 2015). The mycelia of exploratory ECM fungi are finely branched and rapidly contribute to biomass turnover. Rhizomorphic ECM fungi are able to recycle their own biomass, while other melanised ECM fungal mycelia appear more resistant to decomposition and contribute to the organic matter (Clemmensen et al. 2013).

Investigations into the distribution of AM fungal spores within the soil profile generally show a decrease with increasing soil depth, higher spore densities occurring within the top 20 cm (Smith and Read 2008). Investigators have tended to concentrate their sampling in this region, as this correlates to the presence of host plant feeder roots. The decrease in AM spore density is less pronounced in intensively managed agricultural soils subjected to soil disturbance and tillage, possibly a result of mixing and other agricultural practices. AM fungal diversity may increase with soil depth, indicating the maintenance of a subsoil reservoir (Oehl et al. 2005). Vertical variation in ECM communities has been reported (Bahram et al. 2015) suggesting that moisture and temperature stability can play

a role in maintaining mycorrhizal populations in lower soil horizons. Variability in the upper soil layers can also be attributed to the heterogeneous nature of soil nutrient resources and environmental conditions (Bahram et al. 2015). The presence of AM fungal spores however does not necessarily correlate with the ability of the fungus to access nutrients as they function as infective propagules (Smith and Read 2008).

5.5 Spatial Changes in Ecosystem Diversity

Host relations with mycorrhizal fungi are complex and are not limited to single partners. Parties can interact with multiple partners; one host plant can form associations with several different mycorrhizal fungi, and conversely a single fungal species can interact with many host plants. The combination of partners that are more favourable is likely to be prevalent. Host plants discriminate between fungal partners forming partnership with more effective fungal species which are rewarded with more carbohydrates; the selected fungal partners ensure the transfer of nutrients and enforce the cooperation (Kiers et al. 2011). It is widely accepted that belowground the common mycorrhizal networks are pivotal in transferring nutrients to multiple connected host plants and are formed by diverse fungal species. The networks extend the foraging capabilities of the fungi, and the transfer of nutrients such as P is driven towards the largest C sources (Hammer et al. 2011). Exploitation of the CMN by host plants that do not invest in the symbiosis is therefore controlled. This belowground selection influences spatial distribution of plant communities and may drive plant species diversity and coexistence or exclude some host plants that are not linked to the CMN (Ettema and Wardle 2002).

6 Genetic Regulation of Spatio-temporal Nutrient Acquisition

Roots have the capability to forage for optimal soil environments, including deepening to explore lower soil layers or growing towards patches with more moisture and nutrients. In this way, roots have evolved genetic mechanisms to recognise and further coordinate physiology and development in order not only to cope with but also to thrive under stress conditions. Root cells have sensing and signalling pathways that recognise optimal and toxic environments that trigger mechanisms to coordinate root growth and development through membrane receptors and sensors, hormones and cell signalling cascades. Mechanisms also exist to elicit reactions to these signals (such as regulatory gene networks centred around transcription factors) as well as to explore optimal conditions and to neutralise harmful situations (e.g. importers, metabolite efflux, mucilage exudation).

Recent advances in molecular genetics and analytical chemistry now allow us to comprehensively explore the physiological mechanisms through comparative genomics, transcriptomics, proteomics and metabolomics not only in model species but also directly in crops. These studies are providing us with a larger picture, albeit quite incomplete still, of the genetic and physiological mechanisms involved in root growth and development in space and time.

6.1 Regulation of Root Architecture and Spatio-temporal Nutrient Acquisition by Roots

Root architecture and growth are, to a great extent, the phenotypical output of constant genetic responses to nutrient availability in the medium. Below, we summarise the recent developments of our understanding of genetic regulation of root architecture, nutrient acquisition and utilisation and root symbioses. This section explores some of the recent developments in our understanding of how roots respond to soil cues and elicit phenotypical changes. We further evaluate experimental designs and data analyses that might prompt a more comprehensive understanding of root biology.

6.1.1 Root Architecture and Growth

There is a consensus that root architecture remodelling plays a great role in the efficiency of nutrient acquisition. Phenotyping and germplasm characterisation via gene association techniques are a key step in identifying genes involved in these traits. However, assessing relevant root architecture traits is particularly challenging because of the difficulties in tridimensionally phenotyping roots growing in soil and especially at a high throughput (Kuijken et al. 2015; Topp et al. 2013; Lynch and Brown 2012). Recent methods have been proposed to overcome this difficulty (Galkovskyi et al. 2012; Zhu et al. 2011; Clark et al. 2011, 2013), but the challenge remains a major hindrance for genotype selection in crop breeding programmes, especially under field conditions.

Ultimately, genes are the culprits of how organisms respond to the environment and shape their organs. This principle also applies to how roots scavenge the soil and grow towards high-resource patches. A review on genes involved in root development and growth in response to nutrient deficiencies and symbiotic associations has been recently published (Li et al. 2015a; Giehl and von Wirén 2014). Genes involved in root architecture phenotypical plasticity in response to nutrient deficiencies include transcription factors, such as the AP2/ERF transcription factor WRI4/PRD in *Arabidopsis* (Camacho-Cristóbal et al. 2008), the phosphate starvation response MYB-family PHR regulators of root hair development in *Arabidopsis* (Khan et al. 2014) and the rice OsMYB2P-1 regulator of root architecture under low

P (Guo et al. 2015), the bHLH regulator that induces tolerance to low P in monocot crops (Li et al. 2011; Yi et al. 2005). Membrane transporters also play a role in this process, such as the *Arabidopsis* and *Medicago* NRT1.1 and NRT2.1 nitrate transporters (Mounier et al. 2014; Yendrek et al. 2010; Walch-Liu and Forde 2008) and a proton pump (the *Arabidopsis* P-ATPase AHA2). Signalling cascade proteins known to act on root architecture include the *Arabidopsis* CLV1 receptor kinase and CLV3/ESR-related (CLE) signal peptides (Araya et al. 2014a, b) and the P signalling protein SPX1 in legumes (Yao et al. 2014a, b). Protein ubiquitination and degradation contribute to define root architecture phenotype through processes involving SUMO E3 ligase SIZ1 in *Arabidopsis* (Miura et al. 2005, 2011) and EL5 in rice (Koiwai et al. 2007; Mochizuki et al. 2014; Nishizawa et al. 2015). A cell wall expansin (GmEXPB2: Li et al. 2015b; Zhou et al. 2014); the tryptophan aminotransferase TAR2, which is an auxin biosynthesis enzyme in *Arabidopsis* (Ma et al. 2014); and the OsARF12, an auxin response factor in rice (Wang et al. 2014a, b; Qi et al. 2012), also contribute to defining root architecture. Often, these genes do not only affect root anatomy but also the physiology of specific nutrient homeostasis and stress responses.

Moreover, miRNAs have been found to play a role in root architecture. MicroRNAs are (antisense) small RNAs that downregulate gene expression of their targets post-transcriptionally by recognising complementary (sense) transcripts and calling RNA degradation machinery. The role of miRNAs on root development and nutrient uptake is starting to be unveiled. miR393 expression is induced by nitrate and controls root architecture by targeting for degradation transcripts that code for bHLH transcription factors and auxin receptors TIR1 and AFB1-3, thus decreasing auxin response in roots (Vidal et al. 2010; Turner et al. 2013). Expression of transcripts resistant to miR393 degradation therefore might have positive effects on osmotic-related stress in the root (Chen et al. 2012a, b, 2015; Iglesias et al. 2014). miR160 also controls root architecture. Overexpression of miR160 leads to auxin hypersensitivity in soybean (Turner et al. 2013) by suppressing the expression of certain repressor ARF transcription factors (Nizampatnam et al. 2015). Interestingly, the ectopic expression of both of these miRNAs in soybean roots resulted in the inhibition of nitrogen fixation nodule development (Turner et al. 2013; Nizampatnam et al. 2015). The miR169/NF-Y module has also been recognised as influencing root architecture and nutrient uptake, possibly via auxin regulation (Sorin et al. 2014; Qu et al. 2015). Indeed, N and P starvation in wheat repressed miR169 and induced expression of NFYA transcription factors, while constitutive overexpression of *TaNFYA-B1* led to increased N and P uptake and higher yields under low-nutrient conditions (Qu et al. 2015). By the same token, miR167 targets auxin-related transcripts and has been linked to root architecture, osmotic stress responses and nodulation in soybean (Wang et al. 2015a, b; Kinochita et al., 2012).

6.1.2 Nutrient Acquisition and Use Efficiency

The genetic regulation of nutrient homeostasis relies on sensing specific nutrients and the activation of an extensive regulatory gene network and epigenetic responses that largely reshape the genomic landscape of the cell, thereby providing opportunities for cell response (including coping and tolerance) to nutritional cues. Adding to the complexity of mechanisms involved in defining root architecture, we should not forget that shoot-root communication pathways also exert great influence on root growth and development. Indeed, the root is often the first organ that detects nutrient stresses; however, the shoot also plays an important part in nutrient homeostasis and use efficiency. Ideally, the plant under nutritional stress is able to activate a highly efficient physiological response to better use the nutrient without any impact on the yield. Below, we describe some of the recent, most important findings in genetic regulation of nutrient acquisition and use efficiency in roots.

6.1.3 Nitrogen

Nitrogen is the most limiting macronutrient for crop production. Remarkably, N can be taken up as ammonium and nitrate as well as in organic forms, such as amino acids and urea (see recent reviews on N uptake and assimilation: Krapp 2015; Xu et al. 2012). It is not surprising that the plant's nutritional status exerts large influence on gene expression (Fig. 7). Indeed, around 7% of all genes in maize respond to nitrogen levels (Yang et al. 2011). A comparative transcriptome analysis of four low-N tolerant and three sensitive genotype groups of sorghum revealed a complex response to low-N conditions with the tolerant genotypes increasing root biomass and consistently differentially expressed 115 genes, including the induction of nitrate (NRTs) and amino acid (LHT1) transporters in the low-N tolerant group (Gelli et al. 2014). Furthermore, finding gene network hubs that coordinate tolerance to low-N conditions may uncover regulatory genes mastering this trait (Wei et al. 2013). Indeed, in poplar this approach revealed 11 networks revolving around two transcription factors (*PtaRAP2.11* and *PtaNAC1*) and an F-box protein (*PtaHWS*), which is possibly part of a SCF complex mediating protein degradation. Individual constitutive expression of these three genes in poplar had a positive impact on the biomass of roots and shoots, thus further showing that root architecture and nitrogen use efficiency go hand in hand. Remarkably, the expression of each of the genes identified induced the other two in transgenic plants, which indicates they are involved in a common (albeit unknown) mechanism of low-N tolerance (Dash et al. 2015).

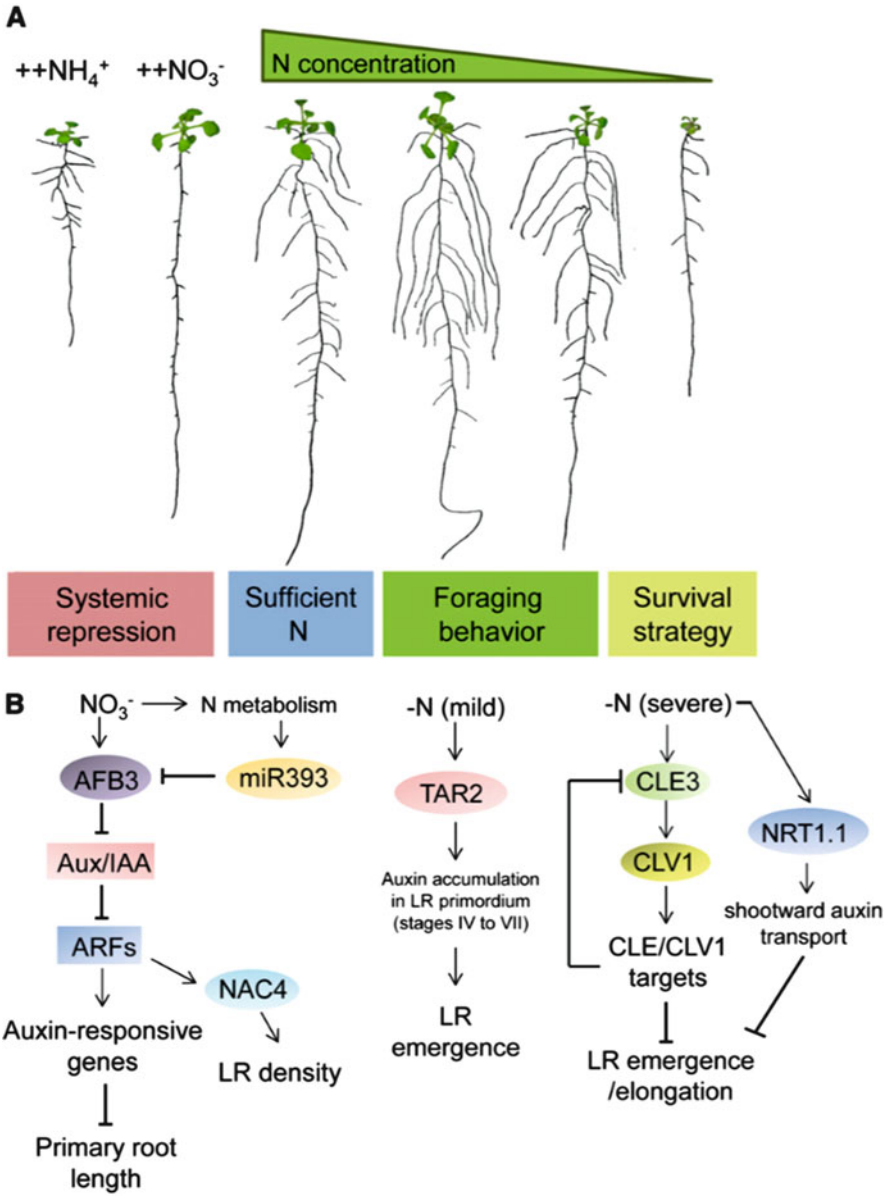

Fig. 7 Root system architecture (RSA) responses to nitrogen availability. (**a**) Excess supply of ammonium ($++NH_4^+$) or nitrate ($++NO_3^-$) leads to a systemic repression of root growth, where high ammonium inhibits mostly PR elongation and high nitrate represses mainly LR elongation. Compared with sufficient nitrogen supply, mild nitrogen deficiency (2N) increases the lengths of PR and LRs, whereas severe nitrogen deficiency inhibits PR elongation as well as LR emergence and elongation. These distinct RSA responses likely reflect different strategies of the plants to cope with limited nitrogen availability. (**b**) Examples of signalling pathways involved in modulating RSA responses to the supply of nitrate to otherwise nitrogen-deficient plants and mild or severe nitrogen deficiency. *Source*: From Giehl and von Wirén (2014)

6.1.4 Phosphate

Phosphate is a common limiting macronutrient in many soil types, imposing the requirement of P fertilisation in large amounts for optimal yields. On the other hand, P fertilisation often causes environmental pollution (e.g. eutrophication of the Gulf of Mexico due to fertiliser run-off along the Mississippi River), and P reserves are limited with its production expected to start declining in 2030 (Fess et al. 2011). A major breakthrough on P acquisition was the identification of the *PSTOL1* protein kinase in traditional rice germplasm, which confers tolerance to low-P conditions (Gamuyao et al. 2012). This gene might be key to producing genotypes that are more P use efficient. It will be interesting to assess potential functional homologs and their impact in the yield of other crops (Azevedo et al. 2015).

6.1.5 Potassium

Potassium is a macronutrient that plays a major role in regulating the osmotic potential in plant cells. Increasing K uptake and low-K tolerance will help decrease K needs in agricultural systems. While high- and low-affinity K transporters (KT/KUP/HAK transporters) have been known for some time, the regulatory genes that activate their expression are more elusive. Recently, the AP2/ERF transcription factor RAP2.11 was found to coordinate HAK5 expression in *Arabidopsis* (Kim et al. 2012). A thorough discussion on breeding strategies for increasing K use efficiency has been recently published (Shin 2014).

6.1.6 Micronutrients

Globally, micronutrient deficiency is a major hindrance to crop yields and food production. We are now starting to understand how micronutrient deficiencies affect gene expression in roots of crop species. For example, iron deficiency modulated the expression of more than 90 genes in roots of tomato cv. 'Marmande superprecoce', including membrane transporters, regulatory proteins and flavonoid biosynthetic genes (Zamboni et al. 2012). However, transcriptomic profiles of single genotypes are limited in furthering our understanding of molecular mechanisms related to micronutrient deficiency responses. Follow-up studies using contrasting (ideally isogenic) genotypes will be invaluable to further understand the regulation mechanisms of each micronutrient. Similar studies can be found for other essential micronutrients. However, beyond a list of differentially expressed genes, it is important to consider how these genes behave in various levels of deficiency and to compare genotypes with discrepant tolerance levels.

6.1.7 Combinations of Nutrient Starvation

While single-nutrient starvation analysis is a more straightforward approach (Gruber et al. 2013), combinatorial starvation experimental data tend to be convoluted to analyse. In nature as well as in agricultural systems, however, multiple nutrient limitations happen at the same time. Thus, it is important that scientists recognise the effects of multiple mild nutritional deficiencies in production environments and that research reflects this reality in order to more accurately simulate field conditions in experimental settings. There are several efforts in this direction. A recent study combining Fe and S deficiencies showed synergistic interaction at the transcriptional and metabolic levels in tomato roots and shoots growing on nutrient solution (Zuchi et al. 2015). Interestingly, Fe deficiency reduced S content in both roots and shoots and vice versa. Furthermore, the expression of the Fe transporter *IRT1* gene was induced in dual Fe plus S deficiency in comparison to single Fe deficiency. The mechanism behind this synergy is unknown. This work was performed on seven-day seedlings of a single genotype (cv. Gimar) exposed to seven-day mineral depletion in a hydroponic system (Zuchi et al. 2015). Thus, it is important to understand the limitations of this study regarding genotype-specific responses and the experimental conditions used. It will be interesting to further assess whether these differences hold true in different genotypes. Furthermore, instead of a single-level deficiency, this study could be expanded to include a full transcriptome analysis (e.g. RNA-seq) of a time course and nutrient level combinations in order to generate a full picture of gene networks that connect both nutrients. For this type of analysis, characterisation of nutrient-efficient and nutrient-inefficient genotypes would be very useful in these studies.

6.1.8 Aluminium Toxicity

Aluminium toxicity is one of the most limiting components of crop yield in acidic soils, which constitute around half of the arable land worldwide. Short-term exposure (4 h) to 30 μM Al^{3+} of soybean roots led to differential expression of more than 600 genes in a resistant genotype (cv. 'Jiyu 70'). As expected, this work revealed induced expression of MATE transporters homologous to organic acid exporters known to precipitate Al^{3+} in the soil as well as the C_2H_2 transcription factor STOP1, a key regulator of Al^{3+} tolerance (You et al. 2011). A largely unexplored facet is the effect of mycorrhization on increasing Al^{3+} tolerance, although several efforts are emerging (Zhang et al. 2015; Seguel et al. 2013).

6.1.9 Heavy Metals and Other Toxic Compounds

Root systems can be directly exposed to environmental hazards and contaminants in the soil, such as heavy metals and organic toxins. On the other hand, low heavy-

metal levels generally induce root growth. For example, in an experiment of Pb-spiked soil, tomato roots grew better (32% more dry weight at 57 mg/kg) than the control with no Pb spiking (Hou et al. 2015a). However, as the stress level increases, root cells undergo a massive transcriptional response. A study assessing the tomato root transcriptome using an Agilent 4x44K microarray in a dosage series found that approximately 4K genes (around 10% of the genes analysed) were differentially expressed underexposure to four heavy metals (Cd, Cr, Hg and Pb), and of these, 864 genes were specific to Pb stress (Hou et al. 2015a, b). In another study, three-day exposure of rice seedlings to 100 μM Cu and Cd rendered 882 and 604 specific response genes in roots, respectively, while 568 were differentially expressed in both treatments in comparison to the control (Lin et al. 2013). A time-course study would be invaluable to create regulatory gene networks to identify central hubs that coordinate toxicity tolerance. Another aspect of stress response is at the epigenetic level, and studies are starting to contemplate this scenario in crops. A recent study assessed the genome-wide dynamics of DNA methylation in maize roots under 3 mM Pb stress for 12, 24 and 48 h (Ding et al. 2014). The study found that 140 genes differentially methylated in all three sampling times. Matching this result with differential expression data (or alternative splicing dynamics) could provide clues regarding the genomic mechanisms involved in regulating genetic hubs that control stress responses to heavy-metal toxicity.

6.1.10 Root Symbioses

Mutualistic symbiosis requires the coordination of a large gene set (Gutjahr and Parniske 2013). Although this symbiosis emerged early in the evolutionary history of land plants (ca. 400 million years ago), it has recently been discovered that many symbiotic proteins were already present in the last common ancestor between land plants and green algae (Delaux et al. 2015). Arbuscular mycorrhizal associations are notable for cooperating with P acquisition to plants, but the plant normally suppresses the association when under P-sufficient conditions. On the other hand, although mycorrhizal N acquisition is well documented, a recent finding using mutants of the model legume *Medicago truncatula* demonstrated that low-N conditions sustain mycorrhization even under sufficient P (Breuillin-Sessoms et al. 2015; Javot et al. 2011). Furthermore, some plant groups, like legumes, are also able to interact with bacteria to sustain symbiotic nitrogen fixation when under low N. This process also requires a coordinated set of genes to recognise and interact with the bacteria in the soil, allowing the bacteria to become established intracellularly and further develop nodules that hold nitrogen fixation. Some genes and mechanisms involved in this process are known; however, the full picture of how the genes are coordinated in order to fully and successfully develop and maintain these symbioses is far from complete. Therefore, improving symbiotic N and P nutrition in crops through more efficient nitrogen fixation in legumes as well as optimal mycorrhization in crops has been largely unattained through breeding programmes, but the realisation of these goals will potentially reduce

production costs while allowing for more sustainable production systems and increasing yields.

6.2 *Perspectives in Molecular Genetic Studies of Root Systems*

It is evident that our knowledge about the genetic networks that control root architecture and nutrient sensing is still very limited and that a plethora of genes and systems are involved. The identification of biomarkers that denote the nutritional status of a plant as well as resolution of the regulatory hubs of gene networks involved in enhanced nutrient use efficiency is largely missing. Technologically, this goal can now be achieved with full transcriptomic tools (e.g. RNA-seq or complete chips). However, the focus now should be on proper experimental designs. Our understanding of these processes will only be achieved when experiments are properly designed to capture global gene expression not of single genotypes but rather by comparing contrastive genotypes, i.e. either isogenic lines or genotype groups that fall into classes of a trait. Reports of extensive lists of differentially expressed genes have been rarely useful to anyone, since it rarely leads to the functional characterisation of the most striking differentially expressed genes.

Also, although transcriptional regulation is the main mechanism controlling gene expression, it is important to keep in mind that the cell has additional means to fine-tune its genetic response to environmental cues (Stauffer and Maizel 2014). Alternative splicing is a possible mechanism, but it has not been fully explored in root stresses (Li et al. 2013). Another important process is post-translational protein modifications. For example, the *Arabidopsis* NRT1.1 is a dual-affinity nitrate transporter that relies on protein phosphorylation to define its high affinity under low-nitrate conditions (Sun et al. 2014). Finally, the ultimate goal of systems biology is the integration of various -omics data into sensible mechanisms that explain the phenotypical plasticity of living organisms. This goal is still far from being achieved given that studies trying to find correlations between metabolites, enzymes and transcriptional modulations often lead to inconclusive results (e.g. Krapp et al. 2011). Therefore, more advanced analysis models are necessary to integrate the complex data sets generated by each of these techniques while taking into account each data type.

6.3 *Significance of Understanding Root Genetic Regulation to Crop Breeding*

The progress made in our understanding of how plant architecture and nutrient economy are genetically regulated will be key to create effective breeding strategies to increase tolerance to soil stresses and improve nutrient use efficiency in crops (Meister et al. 2014). The discovery of microRNA-target modules that act upon root architecture and nutrient efficiency is expected to enable the breeding of more resilient crops for high yields under low-input agricultural systems. Although results have been demonstrated in transgenic plants, if non-transgenic crops are desired, plant breeding programmes can use this knowledge to seek natural genetic variations in specific regions of the gene (e.g. the target region of the miRNA in order to create miRNA-resistant transcripts) or even to induce target-specific genome editing using either the CRISPR-Cas9 system, which can yield transgene-free progeny after the segregation of the editing cassette (Andersen et al. 2015), or even without introducing the editing cassette by using a preassembled CRISPR-Cas9 ribonucleoprotein complex (Woo et al. 2015).

Whatever the technology chosen, it is important to keep in mind that most of the molecular genetic mechanisms currently known in plants have been marshalled in model organisms and that crop species often present distinct mechanisms. This is a lesson we are painfully learning in translational genetics and crop breeding. A comparative functional approach is required to understand common mechanisms across plant species as well as species-specific regulations (Sanchez et al. 2011). While in the past few decades, there was a need to explore the genetics and genomics of a single plant model like *Arabidopsis* to start building our understanding of plant molecular genetics, we have since advanced to alternative models within plant families and are now moving on to genomic exploration of non-model species of economic importance (Benedito 2007; Valentine et al. 2011). Furthermore, as recurrently stated above, the use of single genotypes to describe molecular mechanisms in a species is becoming obsolete and uninformative as it becomes necessary to explore the intraspecific genetic diversity related to biological processes through comparative genomes of cultivated genotypes and their closely related wild relatives (e.g. Branca et al. 2011; Koenig and Weigel 2015; Stanton-Geddes et al. 2013; Aflitos et al. 2014).

7 Conclusions

It is clear that the root acquisition of nutrients is not a simple physiological event. Owing to the spatial distribution and temporal availability of nutrients, plant root systems have evolved a spectacular array of functional and morphological adaptations, both from within the root system itself and in association with symbiotic soil microbes. Although genetic regulation of these adaptations has greatly advanced

our understanding of the forms and functions of these root alterations, this may only represent the start of many unexplored root alterations. In particular, the regulation of spatial and temporal root modifications requires much more investigation, both for fundamental understanding and for crop improvement of nutrient acquisition.

References

Aflitos S, Schijlen E, de Jong H, de Ridder D, Smit S, Finkers R, Wang J, Zhang G, Li N, Mao L, Bakker F, Dirks R, Breit T, Gravendeel B, Huits H, Struss D, Swanson-Wagner R, van Leeuwen H, van Ham RC, Fito L, Guignier L, Sevilla M, Ellul P, Ganko E, Kapur A, Reclus E, de Geus B, van de Geest H, Te Lintel Hekkert B, van Haarst J, Smits L, Koops A, Sanchez-Perez G, van Heusden AW, Visser R, Quan Z, Min J, Liao L, Wang X, Wang G, Yue Z, Yang X, Xu N, Schranz E, Smets E, Vos R, Rauwerda J, Ursem R, Schuit C, Kerns M, van den Berg J, Vriezen W, Janssen A, Datema E, Jahrman T, Moquet F, Bonnet J, Peters S (2014) Exploring genetic variation in the tomato (*Solanum* section *Lycopersicon*) clade by whole-genome sequencing. Plant J 80(1):136–148. doi:10.1111/tpj.12616

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Akiyama K, Matsuoka H, Hayashi H (2002) Isolation and identification of a phosphate deficiency-induced C-glycosylflavonoid that stimulates arbuscular mycorrhizal formation in melon roots. Mol Plant Microbe Interact 15:334–340

Almeida JPF, Hartwig UA, Frehner M, Nösberger J, Luscher A (2000) Evidence that P deficiency induces N feedback regulation of symbiotic N2 fixation in white clover (Trifolium repens L.). J Exp Bot 51:1289–1297

Al-Niemi TS, Kahn ML, McDermott TR (1997) P metabolism in the bean-Rhizobium tropici symbiosis. Plant Physiol 113:1233–1242

Aloni R, Alon E, Langhans M, Ullrich C (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Andersen MM, Landes X, Xiang W, Anyshchenko A, Falhof J, Østerberg JT, Olsen LI, Edenbrandt AK, Vedel SE, Thorsen BJ, Sandøe P, Gamborg C, Kappel K, Palmgren MG (2015) Feasibility of new breeding techniques for organic farming. Trends Plant Sci 20 (7):426–434. doi:10.1016/j.tplants.2015.04.011

Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, Tsuchiya YN, Sawa S, Fukuda H, von Wirén N, Takahashi H (2014a) CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. Proc Natl Acad Sci U S A 111(5):2029–2034. doi:10.1073/pnas.1319953111

Araya T, von Wirén N, Takahashi H (2014b) CLE peptides regulate lateral root development in response to nitrogen nutritional status of plants. Plant Signal Behav 9(7), e29302. doi:10.4161/psb.29302

Ashford AE, Allaway WG (2002) The role of the motile tubular vacuole system in mycorrhizal fungi. Plant Soil 244:177–187

Azevedo GC, Cheavegatti-Gianotto A, Negri BF, Hufnagel B, da Costa e Silva L, Magalhaes JV, Garcia AA, Lana UG, de Sousa SM, Guimaraes CT (2015) Multiple interval QTL mapping and searching for *PSTOL1* homologs associated with root morphology, biomass accumulation and phosphorus content in maize seedlings under low-P. BMC Plant Biol 15:172. doi:10.1186/s12870-015-0561-y

Bahram M, Peay KG, Tedersoo L (2015) Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. New Phytol 205:1454–1463

Baier R, Ingenhaag J, Blaschke H, Göttlein A, Agerer R (2006) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Krast.) stand of the Bavarian Limestone Alps. Mycorrhiza 16:197–206

Bartelheimer M, Steinlein T, Beyschlag W (2006) Aggregative root placement: a feature during interspecific competition in inland sand-dune habitats. Plant Soil 280:101–114

Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Can J For Res 29:260–273

Beier C (2004) Climate change and ecosystem function – full scale manipulations of CO_2 and temperature. New Phytol 162:243–245

Benedito VA (2007) Time to crop: jumping from biological models to crop biotechnology. Crop Breed Appl Biotechnol 7:1–10, http://www.sbmp.org.br/cbab/siscbab/uploads/bd6b9d90-4937-41b0.pdf

Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. Bioscience 51:923–931

Bhuvaneswari TV, Turgeon BG, Bauer WD (1980) Early events in the infection of soybean (Glycine max L. Merr.) by Rhizbium japonicum. I. Localization of infectible root cells. Plant Physiol 64:1027–1031

Bilbrough CJ, Caldwell MM (1997) Exploitation of springtime ephemeral N pulses by six great basin plant species. Ecology 78:231–243

Blouin M, Barot S, Roumet C (2007) A quick method to determine root biomass distribution in diameter classes. Plant Soil 290:371–381

Bolland MDA, Sweetingham MW, Jarvir RJ (2000) Effects of applied phosphorus on the growth of *Lupinus luteus*, *L. angustifolius* and *L. albus* in acidic soils in the south-west of Western Australia. Aust J Exp Agric 40:79–92

Branca A, Paape TD, Zhou P, Briskine R, Farmer AD, Mudge J, Bharti AK, Woodward JE, May GD, Gentzbittel L, Ben C, Denny R, Sadowsky MJ, Ronfort J, Bataillon T, Young ND, Tiffin P (2011) Whole-genome nucleotide diversity, recombination, and linkage disequilibrium in the model legume *Medicago truncatula*. Proc Natl Acad Sci U S A 108(42):e864–e870. doi:10.1073/pnas.1104032108

Breuillin-Sessoms F, Floss DS, Gomez SK, Pumplin N, Ding Y, Levesque-Tremblay V, Noar RD, Daniels DA, Bravo A, Eaglesham JB, Benedito VA, Udvardi MK, Harrison MJ (2015) Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the Ammonium Transporter 2 family protein AMT2;3. Plant Cell 27(4):1352–1366. doi:10.1105/tpc.114.131144

Brown G, Mitchell DT (1986) Influence of fire on the soil phosphorus status in sandplain lowland fynbos, south western Cape. S Afr J Bot 52:67–72

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Buczko U, Kuchenbuch RO (2013) Spatial distribution assessment of maize roots by 3D monolith sampling. Commun Soil Sci Plant Anal 44:2127–2151

Burias N, Planchon C, Paul MH (1990) Phenotypic and genotypic distribution of nodules on soybean root system inoculated with *Bradyrhizobium japonicum* strain G49. Agronomie 10:57–62

Buzynski A, Pislewska M, Wojtaszek P (2000) Active chitinases in the apoplastic fluids of healthy white lupin (*Lupinus albus* L.) plants. Acta Physiol Plant 22:31–38

Cairney JWG (2000) Evolution of mycorrhiza systems. Naturwissenschaften 87:467–475

Camacho-Cristóbal JJ, Rexach J, Conéjéro G, Al-Ghazi Y, Nacry P, Doumas P (2008) *PRD*, an Arabidopsis *AINTEGUMENTA*-like gene, is involved in root architectural changes in response to phosphate starvation. Planta 228(3):511–522. doi:10.1007/s00425-008-0754-9

Cardoso IM, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agric Ecosys Environ 116:72–84

Cardoso JD, Gomes DF, Goes KCGP, Fonseca NS Jr, Dorigo OF, Hungria M, Andrade DS (2009) Relationship between total nodulation and nodulation at the root crown of peanut, soybean and common bean plants. Soil Biol Biochem 41:1760–1763

Carroll BJ, Gresshoff PM (1983) Nitrate inhibition of nodulation and nitrogen-fixation in white clover. Z Pflanzenphysiol 110:77–88

Carroll BJ, Gresshoff PM (1986) Isolation and initial characterization of constitutive nitrate reductase-deficient mutants nr328 and nr345 of soybean (*Glycine max*). Plant Physiol 81:572–576

Carroll BJ, Matthews A (1990) Nitrate inhibition of nodulation in legumes. In: Gresshoff PM (ed) Molecular biology of symbiotic nitrogen fixation. CRC Press, Boca Raton, FL, pp 159–180

Celenza JL, Grisafi PL, Fink GR (1995) A pathway for lateral root formation in *Arabidopsis thaliana*. Genes Dev 9:2131–2142

Chen H, Li Z, Xiong L (2012a) A plant microRNA regulates the adaptation of roots to drought stress. FEBS Lett 586(12):1742–1747. doi:10.1016/j.febslet.2012.05.013

Chen YL, Dunbabin VM, Diggle AJ, Siddique KHM, Rengel Z (2012b) Assessing variability in root traits of wild *Lupinus angustifolius* germplasm: basis for modelling root system structure. Plant Soil 354:141–155

Chen Z, Hu L, Han N, Hu J, Yang Y, Xiang T, Zhang X, Wang L (2015) Overexpression of a miR393-resistant form of transport inhibitor response protein 1 (mTIR1) enhances salt tolerance by increased osmoregulation and Na^+ exclusion in *Arabidopsis thaliana*. Plant Cell Physiol 56(1):73–83. doi:10.1093/pcp/pcu149

Chmelikova L, Wolfrum S, Schmid H, Hejcman M, Hulsbergen K-J (2015) Seasonal development of above- and below-ground organs of Trifolium pratense in grass-legume mixture on different soils. J Plant Nutr Soil Sci 178:13–24

Clark RT, MacCurdy RB, Jung JK, Shaff JE, McCouch SR, Aneshansley DJ, Kochian LV (2011) Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiol 156(2):455–465. doi:10.1104/pp.110.169102

Clark RT, Famoso AN, Zhao K, Shaff JE, Craft EJ, Bustamante CD, McCouch SR, Aneshansley DJ, Kochian LV (2013) High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant Cell Environ 36(2):454–466. doi:10.1111/j.1365-3040.2012.02587.x

Clarkson DT (1996) Root structure and sites of ion uptake. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker, pp 483–510

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Findlay RD, Wardle DA, Lindahl BD (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forests. Science 339:1615–1618

Crocker LJ, Schwintzer CR (1993) Factors affecting formation of cluster roots in *Myrica gale* seedlings in water culture. Plant Soil 152:287–289

Cupina B, Krstic D, Mikic A, Eric P, Vuckovic S, Pejic B (2010) The effect of field pea (*Pisum sativum* L.) companion crop management on red clover (*Trifolium pratense* L.) establishment and productivity. Turk J Agric For 34:275–283

Curt T, Prevosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. Plant Soil 255:265–279

Dakora FD, Phillips DA (1996) Diverse functions of isoflavonoids in legumes transcend antimicrobial definitions of phytoalexins. Physiol Mol Plant Path 49:1–20

Dakora FD, Joseph CM, Phillips DA (1993) Alfalfa (*Medicago sativa* L.) root exudates contain isoflavanoids in the presence of *Rhizobium meliloti*. Plant Phys 101:819–824

Dames JF, Scholes MC, Straker CJ (2002) Nutrient cycling in a *Pinus patula* plantation in the Mpumalanga Province, South Africa. Appl Soil Ecol 20:211–226

Dash M, Yordanov YS, Georgieva T, Kumari S, Wei H, Busov V (2015) A systems biology approach identifies new regulators of poplar root development under low nitrogen. Plant J 84 (2):335–346. doi:10.1111/tpj.13002

Davidson EA, Matson PA, Vitousek PM, Riley R, Dunkin K, Garcia-Mendez G, Maass JM (1993) Processes regulating soil emissions of NO and N2O in a seasonally dry tropical conditions. Plant Soil 344:347–360

Day DA, Lambers H, Bateman J, Carroll BJ, Gresshoff PM (1986) Growth comparisons of a supernodulating soybean (*Glycine max*) mutant and its wild-type parent. Physiol Plant 68:375–382

Day DA, Carroll BJ, Delves AC, Gresshoff PM (1989) Relationship between autoregulation and nitrate inhibition of nodulation in soybeans. Physiol Plant 75:37–42

de Dorlodot S, Forster B, Pagè L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12:360–1385

Delaux PM, Radhakrishnan GV, Jayaraman D, Cheema J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, Rothfels CJ, Sederoff HW, Stevenson DW, Surek B, Zhang Y, Sussman MR, Dunand C, Morris RJ, Roux C, Wong GK, Oldroyd GE, Ané JM (2015) Algal ancestor of land plants was preadapted for symbiosis. Proc Natl Acad Sci U S A 112(43):13390–13395. doi:10.1073/pnas.1515426112

Deng MD, Moreaux T, Cabosche M (1989) Tungstate, a molybdenum analog inactivating nitrate reductase, deregulates the expression of the nitrate reductase structural gene. Plant Physiol 91:304–309

Ding H, Gao J, Qin C, Ma H, Huang H, Song P, Luo X, Lin H, Shen Y, Pan G, Zhang Z (2014) The dynamics of DNA methylation in maize roots under Pb stress. Int J Mol Sci 15 (12):23537–23554. doi:10.3390/ijms151223537

Dinkelaker B, Hengeler C, Marschner H (1995) Distribution and function of proteoid roots and other root clusters. Bot Acta 108:183–200

Divito GA, Sadras VO (2014) How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. Field Crop Res 156:161–171

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097

Djordjevic MA, Mohd-Radzman NA, Imin N (2015) Small-peptide signals that control root nodule number, development, and symbiosis. J Exp Bot 66:5171–5181

Dolan L (2001) The role of ethylene in root hair growth in Arabidopsis. J Plant Nutr Soil Sci 164:141–145

Drevon JJ, Hartwig UA (1997) Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa. Planta 201:463–469

Drew MC (1975) Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. New Phytol 75:479–490

Duke SH, Collins M, Soberalske RM (1980) Effects of potassium fertilization on nitrogen fixation and nodules enzymes of nitrogen metabolism in alfalfa. Crop Sci 20:213–219

Eissenstadt DM, Yanai RD (1997) The ecology of root lifespan. In: Fitter AM (ed) Advances in ecological research, vol 27. Academic, San Diego, pp 1–60

Eissenstat DM, Caldwell JP (1988) Seasonal timing of root growth in favourable microsites. Ecology 69:870–873

Ettema CH, Wardle DA (2002) Spatial soil ecology. Trends Ecol Evol 17:177–183

Ferguson BJ, Indrasummer A, Hayashi S, Lin M, Lin Y, Reid DE, Gresshoff PM (2010) Molecular analysis of legume nodule development and autoregulation. J Integr Plant Biol 52:61–76

Fess TL, Kotcon JB, Benedito VA (2011) Crop breeding for low input agriculture: a sustainable response to feed a growing world population. Sustainability 3(10):1742–1772. doi:10.3390/su3101742

Finlay RD, Frostegård Å, Sonnerfeldt A-M (1992) Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in pure culture and in symbiosis with *Pinus contorta* Dougl. Ex Loud. New Phytol 120:105–115

Forde B, Lorenzo H (2001) The nutritional control of root development. Plant Soil 232:51–68

Franksi R, Bednarek P, Wojtaszek P, Stobiecki M (1999) Identification of flavonoid diglycosides in yellow lupin (*Lupinus luteus* L.). J Mass Spectrom 34:486–495

Fransen B, Blijjenberg J, de Kroon H (1999) Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches. Plant Soil 211:179–189

Fukaki H, Tasaka M (2009) Hormone interactions during lateral root formation. Plant Mol Biol 69:437–449

Gadgil RL, Gadgil PD (1975) Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. N Z J For Sci 5:33–44

Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 68:280–300

Gahoonia TS, Nielsen NE (2004) Barley genotypes with long root hairs sustain high grain yields in low-P field. Plant Soil 262:55–62

Gahoonia TS, Alib O, Sarkerc A, Nielsena NE, Rahmand MM (2006) Genetic variation in root traits and nutrient acquisition of lentil genotypes. J Plant Nutr 29:643–655

Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, Harer J, Benfey PN, Weitz JS (2012) GiA Roots: software for the high throughput analysis of plant root system architecture. BMC Plant Biol 12:116. doi:10.1186/1471-2229-12-116

Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase PSTOL1 from traditional rice confers tolerance of phosphorus deficiency. Nature 488(7412):535–539. doi:10.1038/nature11346

Gelli M, Duo Y, Konda AR, Zhang C, Holding D, Dweikat I (2014) Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genomics 15:179. doi:10.1186/1471-2164-15-179

Genoud T, Metraux JP (1999) Crosstalk in plant cell signalling: structure and function of the genetic network. Trends Plant Sci 4:503–507

George TS, Gregory PJ, Robinson JS, Buresh RJ (2002) Changes in phosphorus concentrations and pH in the rhizosphere of some agroforestry and crop species. Plant Soil 246:65–73

George TS, Fransson A, Hammond JP, White PJ (2011) Phosphorus nutrition: rhizosphere processes, plant response and adaptations. In: Bünemann EK (ed) Phosphorus in action, vol 26: Soil biology. Springer, Berlin, pp 245–271

Gibson SI (2005) Control of plant development and gene expression by sugar signalling. Curr Opin Plant Biol 8:93–102

Giehl RFH, Nicolaus von Wirén N (2014) Root nutrient foraging. Plant Physiol 166:509–517

Gilbert GA, Knight JD, Vance CP, Allan DL (1999) Acid phosphatase activity in phosphorus deficient white lupin roots. Ann Bot 85:921–928

Gilbert GA, Knight JD, Vance CP, Allan DL (2000) Proteoid root development of phosphorus deficient white lupin is mimicked by auxin and phosphonate. Ann Bot 85:921–928

Giovannetti M, Sbrana C, Avio L, Strani P (2004) Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. New Phytol 164:175–181

Graham PH, Vance CP (2000) Nitrogen fixation in perspective: an overview of research an extension needs. Field Crop Res 65:93–106

Grift T, Novais J, Bohn M (2011) High-throughput phenotyping technology for maize roots. Biosyst Eng 110:40–48

Gross KL, Pregitzer KS, Burton AJ (1995) Spatial variation in nitrogen availability in three successional plant communities. J Ecol 83:357–367

Gruber BD, Giehl RF, Friedel S, von Wirén N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. Plant Physiol 163(1):161–179. doi:10.1104/pp.113.218453

Guo M, Ruan W, Li C, Huang F, Zeng M, Liu Y, Yu Y, Ding X, Wu Y, Wu Z, Mao C, Yi K, Wu P, Mo X (2015) Integrative comparison of the role of the *PHOSPHATE RESPONSE1* subfamily

in phosphate signaling and homeostasis in rice. Plant Physiol 168(4):1762–1776. doi:10.1104/pp.15.00736
Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617. doi:10.1146/annurev-cellbio-101512-122413
Hammer DC, Pallon J, Wallander H, Olsson PA (2011) Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. FEMA Microbiol Ecol 76:236–244
Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorous starvation. J Exp Bot 59:93–109
Hatch DJ, Goddlass G, Joynes A, Shepherd MA (2007) The effect of cutting, mulching and applications of farmyard manure on nitrogen fixation in a red clover/grass sward. Bioresour Technol 98:3243–3248
Hauck AL, Novais J, Grift TE, Bohn MO (2015) Characterization of mature maize (*Zea mays* L.) root system architecture and complexity in a diverse set of Ex-PVP inbreds and hybrids. SpringerPlus 4:424. doi:10.1186/s40064-015-1187-0
Hernandez G, Ramirez M, Valdes-Lopez O, Tesfaye M, Graham MA, Czechowski T, Schlereth A, Wandrey M et al (2007) Phosphorus stress in common bean: root transcript and metabolic responses. Plant Physiol 144:752–767
Hinsinger P, Bengough A, Vetterlein D, Young I (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152
Hinson K (1975) Nodulation responses from nitrogen applied to soybean half-root systems. Agron J 67:799–804
Hirsch AM (1992) Tansley Review 40. Developmental biology of legume nodulation. New Phytol 122:211–237
Hocking P, Jeffrey S (2004) Cluster root production and organic anion exudation in a group of old world lupin and a new world lupin. Plant Soil 258:135–150
Hodge A (2003) Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. New Phytol 157:303–314
Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol 162:9–24
Hodge A (2006) Plastic plants and patchy soils. J Exp Bot 57:401–411
Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (1998) Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. New Phytol 139:479–494
Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (1999) Plant, soil fauna and microbial responses to N-rich organic patches of contrasting temporal availability. Soil Biol Biochem 31:1517–1530
Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187
Hogh-Jensen H (2003) The effect of potassium deficiency on growth and N2-fixation in Trifolium repens. Physiol Plant 119:440–449
Høgh-Jensen H, Schjoerring JK, Soussana J (2002) The influence of phosphorus deficiency on growth and nitrogen fixation of white clover plants. Ann Bot 90:745–753
Hou J, Bai L, Xie Y, Liu X, Cui B (2015a) Biomarker discovery and gene expression responses in *Lycopersicon esculentum* root exposed to lead. J Hazard Mater 299:495–503. doi:10.1016/j.jhazmat.2015.07.054
Hou J, Liu X, Wang J, Zhao S, Cui B (2015b) Microarray-based analysis of gene expression in *Lycopersicon esculentum* seedling roots in response to cadmium, chromium, mercury, and lead. Environ Sci Technol 49(3):1834–1841. doi:10.1021/es504154y
Hutchings MJ, John EA (2004) The effects of environmental heterogeneity on root system architecture models to meet future, agricultural challenges. Adv Agron 85:181–219
Hutchings MJ, John EA, Wijesinghe DK (2003) Toward understanding the consequences of soil heterogeneity for plant populations and communities. Ecology 84:2322–2334

Hynynen J, Niemistö P, Viherä-Aarnio A, Brunner A, Hein S, Velling P (2010) Silviculture of birch (Betula pendula Roth and Betula pubescens Ehrh.) in northern Europe. Forestry 83 (1):103–119

Iglesias MJ, Terrile MC, Windels D, Lombardo MC, Bartoli CG, Vazquez F, Estelle M, Casalongué CA (2014) MiR393 regulation of auxin signaling and redox-related components during acclimation to salinity in Arabidopsis. PLoS One 9(9), e107678. doi:10.1371/journal.pone.0107678

Israel DW (1987) Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Plant Physiol 84:835–840

Jacobsen E (1984) Modification of symbiotic interaction of pea (*Pisum sativum* L.) and *Rhizobium leguminosarum* by induced mutations. Plant Soil 82:427–438

Jaillard B, Schneider A, Mollier A, Pellerin S (2000) Modelling the mineral uptake by plants based on the bio-physical-chemical functioning of the rhizosphere. In: Maillard P, Bonhomme R (eds) Fonctionnement des peuplements végétaux sous contraintes environnementales. INRA, Paris, pp 253–287

Jain A, Poling MD, Karthikeyan AS, Blakeslee JJ, Peer WA, Titapiwatanakun B, Murphy AS, Raghothama KG (2007) Differential effects of sucrose and auxin on localized phosphate deficiency-induced modulation of different traits of root system architecture in *Arabidopsis*. Plant Physiol 144:232–247

Javot H, Penmetsa RV, Breuillin F, Bhattarai KK, Noar RD, Gomez SK, Zhang Q, Cook DR, Harrison MJ (2011) *Medicago truncatula mtpt4* mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. Plant J 68 (6):954–965. doi:10.1111/j.1365-313X.2011.04746.x

Jobbágy EG, Jackson RB (2001) The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeochemistry 53:51–77

Johnson JF, Allan DL, Vance CP (1994) Phosphorus stress-induced proteoid roots show altered metabolism in *Lupinus albus*. Plant Physiol 104:657–665

Johnson JF, Allan DL, Vance CP (1996) Phosphorus deficiency in *Lupinus albus*: altered root development and enhanced expression of phosphoenolpyruvate carboxylase. Plant Physiol 12:31–41

Jones DL (1998) Sorption of organic acids in acid soils and its implications in the rhizosphere. Eur J Soil Sci 49:447–455

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 32:5–33

Jordan-Meille L, Pellerin S (2008) Shoot and root growth of hydroponic maize (*Zea mays* L.) as influenced by K deficiency. Plant Soil 304:157–168

Jumpponen A, Högberg P, Huss-Danell K, Mulder CPH (2002) Interspecific and spatial differences in nitrogen uptake in monocultures and two-species mixtures in north European grasslands. Funct Ecol 16:454–461

Jung JKH, McCouch S (2013) Getting to the roots of it: genetic and hormonal control of root architecture. Front Plant Sci 186:1–32

Juszczuk IM, Wiktorowska A, Malusa E, Rychter AM (2004) Changes in the concentrations of phenolic compounds and exudation induced by phosphate deficiency in bean plants (*Phaseolus vulgaris* L.). Plant Soil 267:41–49

Kai M, Takazumi K, Adachi H, Wasaki J, Shinano T, Osaki M (2002) Cloning and characterization of four phosphate transporter cDNAs in tobacco. Plant Sci 163:837–846

Kania A, Neumann G, Cesco S, Pinton R, Römheld V (2001) Use of plasma membrane vesicles for examination of phosphorus deficiency-induced root excretion of citrate in cluster roots of white lupin (*Lupinus albus* L.). In: Horst WJ, Schenk MK, Bürket (eds) Plant nutrition: food security and sustainability of agro-ecosystems through basic and applies research, vol 14. Kluwer Academic, Dordrecht, pp 546–547

Kania A, Langlade N, Martinoia E, Neumann G (2003) Phosphorus deficiency-induced modifications in citrate catabolism and in cytosolic pH as related to citrate exudation in cluster roots of white lupin. Plant Soil 248:117–127

Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG (2007) Phosphate starvation responses are mediated by sugar signalling in *Arabidopsis*. Planta 225:907–918

Keertisinghe G, Hocking PJ, Ryan PR, Delhaize E (1998) Effect of phosphorous supply on the formation and function of proteoid roots of white lupin (*Lupinus albus* L.). Plant Cell Environ 21:467–478

Khan GA, Bouraine S, Wege S, Li Y, de Carbonnel M, Berthomieu P, Poirier Y, Rouached H (2014) Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1;H3 in Arabidopsis. J Exp Bot 65(3):871–884. doi:10.1093/jxb/ert444

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A et al (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882

Kihara T, Wada T, Suzuki Y, Hara T, Koyama H (2003) Alteration of citrate metabolism in cluster roots of white lupin. Plant Cell Physiol 44:901–908

Kim MJ, Ruzicka D, Shin R, Schachtman DP (2012) The Arabidopsis AP2/ERF transcription factor RAP2.11 modulates plant response to low-potassium conditions. Mol Plant 5 (5):1042–1057. doi:10.1093/mp/sss003

Kinoshita N, Wang H, Kasahara H, Liu J, Macpherson C, Machida Y, Kamiya Y, Hannah MA, Chua NH (2012) IAA-Ala Resistant3, an evolutionarily conserved target of miR167, mediates Arabidopsis root architecture changes during high osmotic stress. Plant Cell 24(9):3590–3602. doi:10.1105/tpc.112.097006

Kochian LV, Hoekenga OA, Pineros MA (2004) How to crop plants tolerate acid soils? Mechanisms of aluminium tolerance and phosphorus deficiency. Annu Rev Plant Biol 55:459–493

Koenig D, Weigel D (2015) Beyond the thale: comparative genomics and genetics of Arabidopsis relatives. Nat Rev Genet 16(5):285–298. doi:10.1038/nrg3883

Koiwai H, Tagiri A, Katoh S, Katoh E, Ichikawa H, Minami E, Nishizawa Y (2007) RING-H2 type ubiquitin ligase EL5 is involved in root development through the maintenance of cell viability in rice. Plant J 51(1):92–104. doi:10.1111/j.1365-313X.2007.03120.x

Kong X, Zhang M, De Smet I, Ding Z (2014) Designer crops: optimal root system architecture for nutrient acquisition. Trends Biotechnol 32:597–598

Köpke U (1981) A comparison of methods for measuring root growth of field crops. Z Acker-Pflanzenbau 150:39–49

Kosslak RM, Bohlool BB (1984) Suppression of nodule development of one side of a split-root system of soybeans caused by prior inoculation of the other side. Plant Physiol 75:125–130

Krapp A (2015) Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. Curr Opin Plant Biol 25:115–122. doi:10.1016/j.pbi.2015.05.010

Krapp A, Berthomé R, Orsel M, Mercey-Boutet S, Yu A, Castaings L, Elftieh S, Major H, Renou JP, Daniel-Vedele F (2011) Arabidopsis roots and shoots show distinct temporal adaptation patterns toward nitrogen starvation. Plant Physiol 157(3):1255–1282. doi:10.1104/pp.111.179838

Kristensen HL, Thorup-Kristensen K (2004) Root growth and nitrogen uptake of three catch crops in deep soil layers. Soil Sci Soc Am J 68:529–537

Kuchenbuch RO, Ingram KT (2004) Effects of soil bulk density on seminal and lateral roots of young maize plants (*Zea mays* L.). J Plant Nutr Soil Sci 167:229–235

Kuchenbuch RO, Ingram KT, Buczko U (2006) Effects of decreasing soil water content on seminal and lateral roots of young maize plants. J Plant Nutr Soil Sci 169:841–848

Kuchenbuch R, Gerke H, Buczko U (2009) Spatial distribution of maize roots by complete 3D soil monolith sampling. Plant Soil 315:297–314

Kuijken RC, van Eeuwijk FA, Marcelis LF, Bouwmeester HJ (2015) Root phenotyping: from component trait in the lab to breeding. J Exp Bot 66(18):5389–5401. doi:10.1093/jxb/erv239

Lambers H, Juniper D, Cawthray GR, Veneklaas EJ, Martinez-Ferri E (2002) The pattern of carboxylate exudation in *Banksia grandis* (Proteaceae) is affected by the form of phosphate added to the soil. Plant Soil 238:111–122

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann Bot 98:693–713

Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. Trends Ecol Evol 23:95–103

Lamont B (1982) Mechanisms for enhancing nutrient uptake in plants, with particular reference to the Mediterranean, South Africa and Australia. Bot Rev 48:597–689

Lamont BB (2003) Structure, ecology and physiology of cluster roots – a review. Plant Soil 48:1–19

Langlade NB, Messerli G, Weisskopf L, Plaza S, Tomasi N, Smutny J, Neumann G, Martinoia E, Massonneau A (2002) ATP citrate lyase: cloning, heterologous expression and possible implication in root organic acid metabolism and excretion. Plant Cell Environ 25:1561–1569

Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D et al (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. Plant Cell 19:3889–3900

Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennett M, Laplaze L (2013) Lateral root development in Arabidopsis: fifty shades of auxin. Trends Plant Sci 18:450–458

Layzell DB, Hunt S, Palmer GR (1990) Mechanisms of nitrogenase inhibition in soybean nodules. Pulse-modulated spectroscopy indicates that nitrogenase activity is limited by O_2. Plant Physiol 92:1101–1107

Le Roux MR, Ward CL, Botha FC, Valentine AJ (2006) The route of pyruvate synthesis under Pi starvation in legume root systems. New Phytol 169:399–408

Le Roux MR, Kahn S, Valentine AJ (2008) Organic acid accumulation inhibits N2-fixation in P-stressed lupin nodules. New Phytol 177:956–964

Le Roux MR, Kahn S, Valentine AJ (2009) Nitrogen and carbon costs of soybean and lupin root systems during phosphate starvation. Symbiosis 48:102–109

Lea PJ, Miflin BJ (2010) Nitrogen assimilation and its relevance to crop improvement. Ann Plant Rev 42:1–40

Lea PJ, Sodek L, Parry MAJ, Shewry PR, Halford NG (2007) Asparagine in plants. Ann Appl Biol 150:1–26

Lei M, Liu Y, Zhang B, Zhao Y, Wang X, Zhou Y (2011) Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis. Plant Physiol 156:1116–1130

LePage BA, Currah RS, Stockey RA, Rothwell GW (1997) Fossil ectomycorrhizae from the middle Eocene. Am J Bot 84:410–412

Li L, Tang C, Rengel Z, Zhang FS (2003) Chickpea facilitates phosphorus uptake by intercropping wheat from an organic phosphorus source. Plant Soil 248:305–312

Li Z, Gao Q, Liu Y, He C, Zhang X, Zhang J (2011) Overexpression of transcription factor ZmPTF1 improves low phosphate tolerance of maize by regulating carbon metabolism and root growth. Planta 233(6):1129–1143. doi:10.1007/s00425-011-1368-1

Li W, Lin WD, Ray P, Lan P, Schmidt W (2013) Genome-wide detection of condition-sensitive alternative splicing in Arabidopsis roots. Plant Physiol 162(3):1750–1763. doi:10.1104/pp.113.217778

Li X, Zeng R, Liao H (2015a) Improving crop nutrient efficiency through root architecture modifications. J Integr Plant Biol 58:193–202. doi:10.1111/jipb.12434

Li X, Zhao J, Tan Z, Zeng R, Liao H (2015b) *GmEXPB2*, a cell wall β-expansin gene, affects soybean nodulation through modifying root architecture and promoting nodule formation and development. Plant Physiol 169:2640–2653. doi:10.1104/pp.15.01029

Ligaba A, Yamaguchi M, Shen H, Sasaki T, Yamamoto Y, Matsumoto H (2004) Phosphorus deficiency enhances plasma membrane H^+-ATPase activity and citrate exudation in greater purple lupin (*Lupinus pilosus*). Funct Plant Biol 31:1075–1083

Lin CY, Trinh NN, Fu SF, Hsiung YC, Chia LC, Lin CW, Huang HJ (2013) Comparison of early transcriptome responses to copper and cadmium in rice roots. Plant Mol Biol 81(4-5):507–522. doi:10.1007/s11103-013-0020-9

Lindahl BD, Taylor AFS (2004) Occurrence of N-acetylhexosaminidase-genes in ectomycorrhizal basidiomycetes. New Phytol 164:193–199

Lindahl BD, Tunlid A (2015) Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. New Phytol 205:1443–1447

Lindahl BD, Taylor AFS, Findlay RD (2002) Defining nutritional constraints on carbon cycling in boreal forests-towards a less 'phytocentric' perspective. Plant Soil 242:123–135

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in boreal forest. New Phytol 173:611–620

Liu A, Hamel C, Hamilton RI, Ma BL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrients levels. Mycorrhiza 9:331–336

Liu J, Samac DA, Bucciarelli B, Allan DL, Vance CP (2001) Signalling of phosphate deficiency-induced gene expression in white lupin requires sugar and phloem transport. Plant J 41:257–268

Liu J, Samac DA, Bucciarelli B, Allan DL, Vance CP (2005) Signalling of phosphate deficiency-induced gene expression in white lupin requires sugar and phloem transport. Plant J 41:257–268

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287

Lopez-Hernandez D, Brossard M, Frossard E (1998) P-isotopic exchange values in relation to P mineralization in soils with very low P-sorbing capacities. Soil Biol Biochem 30:1663–1670

Lozovaya VV, Lygin AV, Zernova OV, Li SX, Hartman GL, Windholm JM (2004) Isoflavonoid accumulation in soybean hairy roots upon treatment with *Fusarium solani*. Plant Physiol Biochem 42:671–679

Luo Y, Su B, Currie WS, Dukes JS, Finzi AC, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Lynch JP (1995) Root architecture and plant productivity. Plant Physiol 109:7–13

Lynch JP, Brown KM (2001) Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. Plant Soil 237:225–237

Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. Philos Trans R Soc Lond B Biol Sci 367(1595):1598–1604. doi:10.1098/rstb.2011.0243

Ma Y, Jakowitsch J, Maier TL, Bayer MG, Muller NE, Schenk HEA, Loffelhardt W (2001) ATP citrate lyase in the glaucocystophyte alga *Cyanophora paradoxa* is a cytosolic enzyme: characterisation of the gene for the large subunit at the cDNA and genomic levels. Mol Genet Genomics 266:231–238

Ma W, Li J, Qu B, He X, Zhao X, Li B, Fu X, Tong Y (2014) Auxin biosynthetic gene TAR2 is involved in low nitrogen-mediated reprogramming of root architecture in Arabidopsis. Plant J 78(1):70–79. doi:10.1111/tpj.12448

Maherali H, Klironomos JN (2012) Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. PLoS One 7:e36695

Malik NSA, Calvert HE, Bauer WD (1987) Nitrate-induced regulation of nodule formation in soybean. Plant Physiol 84:266–271

Marschner H (1998) Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. Field Crops Res 56:203–207

Massonneau A, Langlade N, Leon S, Smutny J, Vogt E, Neumann G, Martinoia E (2001) Metabolic changes associated with cluster root development in white lupin (*Lupinus albus* L.). Planta 213:523–542

Matsuda Y, Takano Y, Shimada H, Yamanaka T, Ito S (2013) Distribution of ectomycorrhizal fungi in a *Chamaecyparis obtusa* stand at different distances from a mature *Quercus serrata* tree. Mycosciecne 54:260–264

Meisinger JJ, Hargrove WL, Mikkelsen RL, Williams JR, Benson VW (1991) Effects of cover crops on ground water quality. In Cover crops for clean water: The proceedings of an international conferences. West Tennessee Exp Stn, 9–11 April, 1991. Jackson, Tennessee

Meister R, Rajani MS, Ruzicka D, Schachtman DP (2014) Challenges of modifying root traits in crops for agriculture. Trends Plant Sci 19(12):779–788. doi:10.1016/j.tplants.2014.08.005

Meng ZB, You XD, Suo D, Chen YL, Tang C, Yang JL (2013) Root-derived auxin contributes to the phosphorous-deficiency-induced cluster-root formation in white lupin (*Lupinus albus*). Physiol Plant 148:481–489

Mia MW, Yamauchi A, Kono Y (1996) Root system structure of six food legume species. Jpn J Crop Sci 65:131–140

Miller SS, Liu J, Allan DL, Menzhuber CJ, Feorova M, Vance CP (2001) Molecular control of acid phosphatase secreted into the rhizosphere of proteoid roots from phosphorous stressed white lupin. Plant Physiol 127:594–606

Miura K, Rus A, Sharkhuu A, Yokoi S, Karthikeyan AS, Raghothama KG, Baek D, Koo YD, Jin JB, Bressan RA, Yun DJ, Hasegawa PM (2005) The Arabidopsis SUMO E3 ligase SIZ1 controls phosphate deficiency responses. Proc Natl Acad Sci U S A 102(21):7760–7765

Miura K, Lee J, Gong Q, Ma S, Jin JB, Yoo CY, Miura T, Sato A, Bohnert HJ, Hasegawa PM (2011) SIZ1 regulation of phosphate starvation-induced root architecture remodeling involves the control of auxin accumulation. Plant Physiol 155(2):1000–1012. doi:10.1104/pp.110.165191

Mochizuki S, Jikumaru Y, Nakamura H, Koiwai H, Sasaki K, Kamiya Y, Ichikawa H, Minami E, Nishizawa Y (2014) Ubiquitin ligase EL5 maintains the viability of root meristems by influencing cytokinin-mediated nitrogen effects in rice. J Exp Bot 65(9):2307–2318. doi:10.1093/jxb/eru110

Mommer L, Visser E, van Ruijven J, de Caluwe H, Pierik R, de Kroon H (2011) Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous forest. Ecology 74:130–139

Morcuende R, Bari R, Gibon Y, Zheng W, Pant BD, Bläsing O, Usadel B, Czechowski T, Udvardi MK, Stitt M, Scheible WR (2007) Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. Plant Cell Environ 30:85–112

Mortimer PE, Perez-Fernandez MA, Valentine AJ (2008) Photosynthetic and respiratory C costs of N and P nutrition in the dual symbiosis of a mycorrhizal legume. Soil Biol Biochem 40:1091–1027

Mounier E, Pervent M, Ljung K, Gojon A, Nacry P (2014) Auxin-mediated nitrate signalling by NRT1.1 participates in the adaptive response of Arabidopsis root architecture to the spatial heterogeneity of nitrate availability. Plant Cell Environ 37(1):162–174. doi:10.1111/pce.12143

Müller M, Schmidt W (2004) Environmentally induced plasticity of root hair development in *Arabidopsis*. Plant Physiol 134:409–419

Müller R, Nilsson L, Nielsen LK, Nielsen TH (2005) Interaction between phosphate starvation signalling and hexokinase-independent sugar sensing in *Arabidopsis* leaves. Physiol Plant 124:81–90

Müller R, Morant M, Jarmer H, Nilsson L, Nielsen TH (2007) Genome-wide analysis of the *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism. Plant Phys 143:156–171

Murali NS, Teramura AH (1985) Effects of ultraviolet-B irradiance on soybean. IV. Influence of phosphorus nutrition on growth and flavonoid concentrations. Physiol Plant 63:413–416
Nasholm T, Persson J (2001) Plant acquisition of organic nitrogen in boreal forests. Physiol Plant 111:419–426
Neukirchen D, Himken M, Lammel J, Czypionka-Krause U, Olfs H-W (1999) Spatial and temporal distribution of the root system and root nutrient content of an established *Miscanthus* crop. Eur J Agron 11:301–309
Neumann G, Martinoia E (2002) Cluster root-an underground for survival in extreme environments. Trends Plant Sci 7:162–167
Neumann G, Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant Soil 211:121–130
Neumann G, Massonneau A, Martonoia E, Römheld V (1999) Physiological adaptations to phosphorous deficiency during proteoid root development in white lupin. Planta 208:373–382
Neumann G, Massonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E (2000) Physiological aspects of cluster root function and development in phosphorous-deficient white lupin (*Lupinus albus* L.). Ann Bot 85:909–919
Nielsen KL, Miller CR, Beck D, Lynch JP (1999) Fractal geometry of root systems: field observations of contrasting genotypes of common bean (*Phaseolus vulgaris* L.) grown under different phosphorus regimes. Plant Soil 206:181–190
Nishizawa Y, Mochizuki S, Koiwai H, Kondo K, Kishimoto K, Katoh E, Minami E (2015) Rice ubiquitin ligase EL5 prevents root meristematic cell death under high nitrogen conditions and interacts with a cytosolic GAPDH. Plant Signal Behav 10(3), e990801. doi:10.4161/15592324.2014.990801
Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS (2013) Responses of root architecture development to low phosphorus availability: a review. Ann Bot 112:391–408
Nizampatnam NR, Schreier SJ, Damodaran S, Adhikari S, Subramanian S (2015) microRNA160 dictates stage-specific auxin and cytokinin sensitivities and directs soybean nodule development. Plant J 84(1):140–153. doi:10.1111/tpj.12965
Nordström A, Tarkowski P, Tarskowska D, Norbaek R, Astot C, Dolezal K (2004) Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin regulated development. Proc Natl Acad Sci U S A 101:8039–8044
Nygren CMR, Edqvist J, Elfstrand M, Heller G, Taylor AFS (2007) Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. Mycorrhiza 17:241–248
O'Leary B, Park J, Plaxton WC (2011) The remarkable diversity of plant PEPC (phosphoenol carboxylase): recent insights into the physiological functions and post-translational controls of non-photosynthetic PEPCs. Biochem J 436:15–34
Oehl F, Oberson A, Sinaj S, Frossard E (2001) Organic phosphorus mineralization studies using isotopic dilution techniques. Soil Sci Soc Am J 65:780–787
Oehl F, Sieverding E, Ineichen K, Ris E, Boller T, Wiemken A (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed ecosystems. New Phytol 165:273–283
Oldroyd GE, Engstrom EM, Long SR (2001) Ethylene inhibits the Nod factor signal transduction pathway of *Medicago truncatula*. Plant Cell 13:1835–1849
Öpik M, Vanatoa A, Vanatoa E, Moora M, Davidson J, Kalwij JM, Reier U, Zobel M (2010) The online database MaarjAM reveal global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241
Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root system architecture. Annu Rev Plant Biol 58:93–113
Ozawa K, Osaki M, Matsui H, Homma M, Tadano T (1995) Purification and properties of acid phosphatase secreted from lupin roots under phosphorus deficiency conditions. Soil Sci Plant Nutr 41:461–469

Paey KG, Kennedy PG, Bruns TD (2011) Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecol 4:233–240
Paiva NL (2000) An introduction to the biosynthesis of chemicals used in plant-microbe interactions. J Plant Growth Regul 19:131–143
Pate J, Watt M (2001) Roots of *Banksia* spp. (Proteaceae) with special reference to functioning of their specialized root clusters. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Marcel Dekker, New York, pp 989–1006
Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, Webb RI, Gamage HK, Carroll BJ, Schenk PM, Schmidt S (2008) Plants can use protein as nitrogen source without assistance from other organisms. Proc Natl Acad Sci 11:4524–4529
Penaloza E, Munoz G, Salvo-Garrido H, Silva H, Corcuera LJ (2005) Phosphate deficiency regulates phosphoenolpyruvate carboxylase expression in proteoid root clusters of white lupin. J Exp Bot 56:145–153
Pereira PAA, Bliss FA (1989) Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorus under field and environmentally-controlled conditions. Plant Soil 115:75–82
Perotto S, Girlanda M, Martino E (2002) Ericoid mycorrhizal fungi: some new perspectives and old acquaintances. Plant Soil 244:41–53
Pierret A, Doussan C, Capowiez Y, Bastardie F, Pagès L (2007) Root functional architecture: a framework for modelling the interplay between roots and soil. Vadoze Zone J 6:269–281
Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotrophism. Biosystems 6:153–164
Plaxton WC, Carswell MC (1999) Metabolic aspects of the phosphate starvation response in plants. In: Lerner HR (ed) Plant responses to environmental stresses: from phytohormones to genome reorganisation. Dekker, New York, pp 349–372
Postma JG, Jacobsen E, Feenstra WJ (1988) Three pea mutants with an altered nodulation studied by genetic analysis and grafting. J Plant Phys 132:424–430
Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiol 166:590–602
Pouteau S, Cherel I, Vaucheret H, Caboche M (1989) Nitrate reductase mRNA regulation in *Nicotiana plumbaginifolia* nitrate deficient mutants. Plant Cell 1:1111–1120
Pregitzer KS, King JS (2005) Effects of soil temperature on nutrient uptake. In: Bassirirad H (ed) Nutrient acquisition by plants – an ecological perspective. Springer, Berlin, pp 277–310
Prell J, Poole P (2006) Metabolic changes of rhizobia in legume nodules. Trends Microbiol 14:161–168
Priha O, Hallantie T, Smolander A (1999) Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of Pinus sylvestris, Picea abies and Betula pendula seedlings by microscale methods. Biol Fertil Soils 30:14–19
Puppo A, Groten K, Bastian F, Carzaniga R, Soussi M, Lucas MM, de Felipe MR, Harrison J, Vanacker H, Foyer HC (2005) Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural ageing process. New Phytol 165:683–701
Purnell HM (1960) Studies of the family Proteaceae. I. Anatomy and morphology of the roots of some Victorian species. Aust J Bot 8:38–50
Qi Y, Wang S, Shen C, Zhang S, Chen Y, Xu Y, Liu Y, Wu Y, Jiang D (2012) *OsARF12*, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (*Oryza sativa*). New Phytol 193(1):109–120. doi:10.1111/j.1469-8137.2011.03910.x
Qiao Y, Tang C, Han X, Miao S (2007) Phosphorus deficiency delays the onset of nodule function in soybean. J Plant Nutr 30:1341–1353
Qu B, He X, Wang J, Zhao Y, Teng W, Shao A, Zhao X, Ma W, Wang J, Li B, Li Z, Tong Y (2015) A wheat CCAAT box-binding transcription factor increases the grain yield of wheat with less fertilizer input. Plant Physiol 167(2):411–423. doi:10.1104/pp.114.246959
Raghothama KG (1999) Phosphate acquisition. Plant Boil 50:665–693

Read D, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance. New Phytol 157:475–492
Reader RJ, Jalili A, Grime JP, Matthews N (1992) A comparative study in seedling rooting depth in dryings oil. J Ecol 81:543–550
Racette S, Louis I, Torrey JG (1990) Cluster root formation by Gymnostoma papuanum (Casuarinaceae) in relation to aeration and mineral nutrient availability in water culture. Can J Bot 68:2564–2570
Remmler L, Clairmont L, Rolland-Lagan A-G, Guinel FC (2014) Standardized mapping of nodulation patterns in legume roots. New Phytol 202:1083–1094
Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci U S A 91:11841–11843
Ribet J, Drevon JJ (1995) Increase in permeability to oxygen and in oxygen uptake of soybean nodules under limiting phosphorus nutrition. Physiol Plant 94:298–304
Richardson AE, George TS, Jakobsen I, Simpson RJ (2007) Plant utilization of inositol phosphates. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates: linking agriculture and the environment. CABI, Wallingford, pp 242–260
Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009a) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339
Richardson AE, Hocking PJ, Simpson RJ, George TS (2009b) Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci 60:124–143
Robinson D (1994) The responses of plants to non-uniform supplies of nutrients. New Phytol 127:635–674
Robinson D (1996) Variation, co-ordination and compensation in root systems in relation to soil variability. Plant Soil 187:57–66
Robinson D (2001) Root proliferation, nitrate inflow and their carbon costs during nitrogen capture by competing plants in patchy soil. Plant Soil 232:41–50
Rodriguez-Medina C, Atkins CA, Mann AJ, Jordan ME, Smith PMC (2011) Macromolecular composition of phloem exudate for white lupin (Lupinus albus L.). BMC Plant Biol 11:1–19
Roumet C, Urcelay C, Díaz S (2006) Suites of root traits differ between annual and perennial species growing in the field. New Phytol 170:357–368
Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC, Gurevitch J (2001) A metaanalysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562
Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–560
Ryel RJ, Caldwell MM, Manwarring JH (1996) Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. Plant Soil 184:299–309
Sagan M, Gresshoff PM (1996) Developmental mapping of nodulation events in pea (Pisum sativum L.) using supernodulating plant genotypes and bacterial variability reveals both plant and Rhizobium control of nodulation regulation. Plant Sci 117:167–179
Sanchez DH, Pieckenstain FL, Szymanski J, Erban A, Bromke M, Hannah MA, Kraemer U, Kopka J, Udvardi MK (2011) Comparative functional genomics of salt stress in related model and cultivated plants identifies and overcomes limitations to translational genomics. PLoS One 6(2), e17094. doi:10.1371/journal.pone.0017094
Sas L, Rengel Z, Tang C (2001) Excess cation uptake, and extrusion of protons and organic acid anions by *Lupinus albus* under phosphorus deficiency. Plant Sci 160:1191–1198
Sas L, Rengel Z, Tang C (2002) The effect of nitrogen nutrition on cluster root formation and proton extrusion by *Lupinus albus*. Ann Bot 89:435–442
Schere HW, Pacyna S, Manthey N, Schulz M (2006) Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N_2 fixation. Plant Soil Environ 52:72–77

Schmidt IK, Jonasson S, Shaver GR, Michelsen A, Nordin A (2002) Mineralization and distribution of nutrients in plants and microbes in four arctic ecosystems: responses to warming. Plant Soil 242:93–106

Schulze M, Kondorosi A (1998) Regulation of symbiotic root nodule development. Ann Rev Genet 32:33–57

Schulze J, Temple G, Temple SJ, Beschow H, Vance CP (2006) Nitrogen fixation by white lupin under phosphorus deficiency. Ann Bot 98:731–740

Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F (2013) The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza 23 (3):167–183. doi:10.1007/s00572-013-0479-x

Selivanov IA, Utemova LD (1969) Root anatomy of sedges in relation to their mycotrophy. Trans Perm State Ped Inst 68:45–55

Shane MW, De Vos M, De Roock S, Lambers H (2003) Shoot P regulates cluster-root growth and citrate exudation in *Lupinus albus* grown with a divided root system. Plant Cell Environ 26:265–273

Shane MW, Cramer MD, Funayama-Noguchi S, Millar AH, Day DA, Lambers H (2004) Developmental physiology of cluster root carboxylate synthesis and exudation in harsh hakea. Expression of phosphoenolpyruvate carboxylase and alternative oxidase. Plant Physiol 135:549–560

Shane MW, Dixon KW, Lambers H (2005) Cluster roots: a curiosity in context. Plant Soil 274:101–125

Shane MW, Cawthray GR, Cramer MD, Kuo J, Lambers H (2006) Specialised 'dauciform' roots of Cyperaceae are structurally distinct, but functionally analogous to 'cluster roots'. Plant Cell Environ 10:1989–1999

Shane MW, Fedosejevs ET, Plaxton WC (2013) Reciprocal control of anaplerotic phosphoenolpyruvate carboxylase by in vivo monoubiquitination and phosphorylation in developing proteoid roots of phosphate-deficient harsh hakea. Plant Physiol 161:1–11

Sharratt BS, McWilliams DA (2005) Microclimatic and rooting characteristics of narrow-row versus conventional-row corn. Agron J 97:1129–1135

Shaw NH, Gates CT, Wilson JR (1966) Growth and chemical composition of Townsville Lucerne (Stylosanthes humilis). 1. Dry matter yield and nitrogen content in response to superphosphate. Aust J Exp Agric 6:150–156

Shemesh H, Arbiv A, Gersani M, Ovadia O, Novoplansky A (2010) The effects of nutrient dynamics on root patch choice. PLoS One 5, e10824

Shen J, Li H, Neumann G, Zhang F (2005) Nutrient uptake, cluster-root formation and exudation of proton and citrate in Lupinus albus as affected by localized supply of phosphorus in a split-root system. Plant Sci 168:837–845

Shibuya Y, Tahara S, Kimura Y, Mizutani J (1991) New isoflavone glucosides from white lupin (Lupinus albus L.). Z Naturforsch 46:513–518

Shield IF, Scott T, Stevenson HJ, Leach JE, Todd AD (2000) The causes of over-winter plant losses of autumn-sown white lupin (*Lupinus albus*) in different regions of the UK over three seasons. J Agric Sci 135:173–183

Shin R (2014) Strategies for improving potassium use efficiency in plants. Mol Cells 37 (8):575–584. doi:10.14348/molcells.2014.0141

Silberbush M, Barber SA (1983) Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic mathematical model. Plant Soil 74:93–100

Silva AMS, Weidenborner M, Cavaleiro JAS (1998) Growth control of different Fusarium species by selected flavones and flavonoid mixtures. Fungal Biol 102:638–640

Skene KR (1998) Cluster-roots: some ecological considerations. J Ecol 86:10601064

Skene KR (2000) Pattern formation in cluster-roots: some developmental and evolutionary considerations. Ann Bot 85:901–908

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Smith FA, Jakobsen I, Smith SE (2000) Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. New Phytol 147:357–366

Smith SE, Dickson S, Smith FA (2001) Nutrient transfer in arbuscular mycorrhizas: how are fungal and plant processes integrated? Aus J Plant Physiol 28:683–694

Smith FW, Mudge SR, Rae AL, Glassop D (2003) Phosphate transport in plants. Plant Soil 248:71–83

Sorin C, Declerck M, Christ A, Blein T, Ma L, Lelandais-Brière C, Njo MF, Beeckman T, Crespi M, Hartmann C (2014) A miR169 isoform regulates specific NF-YA targets and root architecture in Arabidopsis. New Phytol 202(4):1197–1211. doi:10.1111/nph.12735

Stanton-Geddes J, Paape T, Epstein B, Briskine R, Yoder J, Mudge J, Bharti AK, Farmer AD, Zhou P, Denny R, May GD, Erlandson S, Yakub M, Sugawara M, Sadowsky MJ, Young ND, Tiffin P (2013) Candidate genes and genetic architecture of symbiotic and agronomic traits revealed by whole-genome, sequence-based association genetics in *Medicago truncatula*. PLoS One 8(5), e65688. doi:10.1371/journal.pone.0065688

Stauffer E, Maizel A (2014) Post-transcriptional regulation in root development. Wiley Interdiscip Rev RNA 5(5):679–696. doi:10.1002/wrna.1239

Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in Arabidopsis roots. Plant Cell 19:2169–2185

Stock WD, Lewis OAM (1986) Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. J Ecol 74:317–328

Subbaiah CC, Palaniappan A, Duncan K, Rhoads DM, Huber SC, Sachs MM (2006) Mitochondrial localization and putative signalling function of maize sucrose synthase. J Biol Chem 281:15625–15635

Sulieman S, Ha CV, Schulze J, Tran LSP (2013) Growth and nodulation of symbiotic *Medicago truncatula* at different levels of phosphorus availability. J Exp Bot 64:2701–2712

Sun J, Bankston JR, Payandeh J, Hinds TR, Zagotta WN, Zheng N (2014) Crystal structure of the plant dual-affinity nitrate transporter NRT1.1. Nature 507(7490):73–77. doi:10.1038/nature13074

Tang C, Hinsinger P, Drevon JJ, Jaillard B (2001) Phosphorous deficiency impairs early nodule functioning and enhances proton release in roots of *Medicago truncatula* L. Ann Bot 88:131–138

Tang H, Li X, Zu C, Zhang F, Shen J (2013) Spatial distribution and expression of intracellular and extracellular acid phosphatases of cluster roots at different developmental stages in white lupin. Plant J 170:1243–1250

Taylor TN, Remy W, Hass H, Kerp H (1995) Fossil arbuscular mycorrhizae from the Early Devonian. Mycologia 87:560–573

Tesfaye M, Liu JQ, Allan DL, Vance CP (2007) Genomic and genetic control of phosphate stress in legumes. Plant Physiol 144:594–603

Thorup-Kristensen K, Magid J, Jensen LS (2003) Catch crops and green manures as biological tools in nitrogen management in temperate zones. Adv Agron 79:227–302

Tomasi N, Kretzschmar T, Expen L, Weisskopf L, Fuglsang AT, Palmgren MG, Neumann G, Varanini Z, Pinton R, Martinoia E, Cesco S (2009) Plasma membrane H^+-ATPase-dependent citrate exudation from cluster roots of phosphate-deficient white lupin. Plant Cell Environ 32:465–475

Topp CN, Iyer-Pascuzzi AS, Anderson JT, Lee CR, Zurek PR, Symonova O, Zheng Y, Bucksch A, Mileyko Y, Galkovskyi T, Moore BT, Harer J, Edelsbrunner H, Mitchell-Olds T, Weitz JS, Benfey PN (2013) 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. Proc Natl Acad Sci U S A 110(18):E1695–E1704. doi:10.1073/pnas.1304354110

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant Soil 341:75–87

Turner M, Nizampatnam NR, Baron M, Coppin S, Damodaran S, Adhikari S, Arunachalam SP, Yu O, Subramanian S (2013) Ectopic expression of miR160 results in auxin hypersensitivity, cytokinin hyposensitivity, and inhibition of symbiotic nodule development in soybean. Plant Physiol 162(4):2042–2055. doi:10.1104/pp.113.220699

Tyler G (1994) Spatial sporophore pattern of ectomycorrhizal fungi in a hornbeam (*Carpinus-betulus l*) forest. For Ecol Manage 65(2–3):165–170

Uhde-Stone C, Gilbert G, Johnson JMF, Litjens R, Zinn KE, Temple SJ, Vance CP, Allan DL (2003) Acclimation of white lupin to phosphorus deficiency involves enhanced expression of genes related to organic acid metabolism. Plant Soil 248:99–116

Valentine AJ, Benedito VA, Kang Y (2011) Nitrogen metabolism in plants in the post genomic era. Ann Plant Rev 42:208–236

Van der Linde S, Holden E, Parkin P, Alexander IJ, Anderson IC (2012) Now you see it, now you don't: the challenge of detecting, monitoring and conserving ectomycorrhizal fungi. Fungal Ecol 5:633–640

Van Wilgen BW, Le Maitre DC (1981) Preliminary estimates of nutrient levels in fynbos vegetation and the role of fire in nutrient cycling. S Afr J For 119:24–28

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397

Vance CP (2008) Plants without arbuscular mycorrhizae. Plants without arbuscular mycorrhizae. In: White PJ, Hammond JP (eds) The ecophysiology of plant-phosphorous interactions, vol 7. Springer, Berlin, pp 117–142

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorous acquisition and use: critical adaptation by plants for securing non-renewable resource. New Phytol 157:423–447

Varin S, Cliquet JB, Personeni E, Avice JC, Lemauviel-Lavenant S (2010) How does sulphur availability modify N acquisition of white clover (Trifolium repens L). J Exp Bot 61:225–234

Vidal EA, Araus V, Lu C, Parry G, Green PJ, Coruzzi GM, Gutiérrez RA (2010) Nitrate-responsive miR393/AFB3 regulatory module controls root system architecture in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 107(9):4477–4482. doi:10.1073/pnas.0909571107

Voisin AS, Salon C, Munier-Jolain NG, Ney B (2002) Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (Pisum sativum L.). Plant Soil 242:251–262

Wadisirisuk P, Danso SKA, Hardarson G, Bowen GD (1989) Influence of Bradyrhizobium japonicum location and movement on nodulation and nitrogen fixation in soybeans. Appl Environ Microb 55:1711–1716

Wahlström EM, Hansen EM, Mandel A, Garbout A, Kristensen HL, Munkholm LJ (2015) Root development of fodder radish and winter wheat before winter in relation to uptake of nitrogen. Eur J Agron 71:1–9

Walch-Liu P, Forde BG (2008) Nitrate signalling mediated by the NRT1.1 nitrate transporter antagonises L-glutamate-induced changes in root architecture. Plant J 54(5):820–828. doi:10.1111/j.1365-313X.2008.03443.x

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang EL, Smith CJ (2004) Modelling the growth and water uptake function of plant root systems: a review. Aust J Agric Res 55:501–523

Wang S, Zhang S, Sun C, Xu Y, Chen Y, Yu C, Qian Q, Jiang DA, Qi Y (2014a) Auxin response factor (OsARF12), a novel regulator for phosphate homeostasis in rice (*Oryza sativa*). New Phytol 201(1):91–103. doi:10.1111/nph.12499

Wang Z, Straub D, Yang H, Kania A, Shen J, Ludewig U (2014b) The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus* L.) identified by transcriptome sequencing. Physiol Plant 151:323–338

Wang Y, Li K, Chen L, Zou Y, Liu H, Tian Y, Li D, Wang R, Zhao F, Ferguson BJ, Gresshoff PM, Li X (2015a) MicroRNA167-directed regulation of the auxin response factors GmARF8a and

GmARF8b is required for soybean nodulation and lateral root development. Plant Physiol 168 (3):984–999. doi:10.1104/pp.15.00265

Wang Z, Rahman ABMM, Wang G, Ludewig U, Shen J, Neumann G (2015b) Hormonal interactions during cluster-root development in phosphate-deficient white lupin (*Lupinus albus* L.). Plant J 177:74–82

Wasaki J, Omura M, Osaki M, Ito H, Matsui H, Shinano T et al (1999) Structure of a cDNA for an acid phosphatase from phosphate-deficient lupin (*Lupinus albus* L.) roots. Soil Sci Plant Nutr 45:439–449

Wasaki J, Yamamura T, Shinano T, Osaki M (2003) Secreted acid phosphatase is expressed in cluster roots of lupin in response to phosphorus deficiency. Plant Soil 248:129–136

Wasaki J, Kojima S, Maruyama H, Haase S, Osaki M, Kandeler E (2008) Localization of acid phosphatase activities in the roots of white lupin plants grown under phosphorus-deficient conditions. Soil Sci Plant Nutr 54:95–102

Watt M, Evans JR (1999) Proteoid roots. Physiology and development. Plant Physiol 121:317–323

Wei H, Yordanov Y, Kumari S, Georgieva T, Busov V (2013) Genetic networks involved in poplar root response to low nitrogen. Plant Signal Behav 8(11), e27211. doi:10.4161/psb.27211

Weisskopf L, Tomasi N, Santelia D, Martinoia E, Langlade NB, Tabacchi R, Abou-Mansour E (2006a) Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage. New Phytol 171:657–668

Weisskopf L, Abou-Mansour E, Fromin N, Tomasi N, Santelia D, Edelkott I, Neumann G, Aragno M, Tabacchi R, Martinoia E (2006b) White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. Plant Cell Environ 29:919–927

Williamson LC, Ribrioux SP, Fitter AH, Leyser HM (2001) Phosphate availability regulates root system architecture in *Arabidopsis*. Plant Physiol 126:875

Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS (2015) DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol 33(11):1162–1164. doi:10.1038/nbt.3389

Wu QS (2011) Mycorrhizal efficacy of trifoliate orange seedlings on alleviating temperature stress. Plant Soil Environ 10:459–464

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153–182. doi:10.1146/annurev-arplant-042811-105532

Yamagashi M, Zhou K, Osaki M, Miller SS, Vance CP (2011) Real-time RT-PCR profiling of transcription factors including 34 MYBs and signalling components in white lupin reveals their P status dependent and organ specific expression. Plant Soil 342:481–493

Yan F, Zhu Y, Muller C, Zorb C, Schubert S (2002) Adaptation of H^+-pumping and plasma membrane H^{+-}ATPase activity in proteoid roots of white lupin under phosphate deficiency. Plant Physiol 129:50–63

Yang XS, Wu J, Ziegler TE, Yang X, Zayed A, Rajani MS, Zhou D, Basra AS, Schachtman DP, Peng M, Armstrong CL, Caldo RA, Morrell JA, Lacy M, Staub JM (2011) Gene expression biomarkers provide sensitive indicators of *in planta* nitrogen status in maize. Plant Physiol 157 (4):1841–1852. doi:10.1104/pp.111.187898

Yao ZF, Liang CY, Zhang Q, Chen ZJ, Xiao BX, Tian J, Liao H (2014a) SPX1 is an important component in the phosphorus signalling network of common bean regulating root growth and phosphorus homeostasis. J Exp Bot 65(12):3299–3310. doi:10.1093/jxb/eru183

Yao Z, Tian J, Liao H (2014b) Comparative characterization of GmSPX members reveals that GmSPX3 is involved in phosphate homeostasis in soybean. Ann Bot 114(3):477–488. doi:10.1093/aob/mcu147

Yendrek CR, Lee YC, Morris V, Liang Y, Pislariu CI, Burkart G, Meckfessel MH, Salehin M, Kessler H, Wessler H, Lloyd M, Lutton H, Teillet A, Sherrier DJ, Journet EP, Harris JM, Dickstein R (2010) A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in *Medicago truncatula*. Plant J 62 (1):100–112. doi:10.1111/j.1365-313X.2010.04134.x

Yi K, Wu Z, Zhou J, Du L, Guo L, Wu Y, Wu P (2005) OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. Plant Physiol 138(4):2087–2096

You J, Zhang H, Liu N, Gao L, Kong L, Yang Z (2011) Transcriptomic responses to aluminum stress in soybean roots. Genome 54(11):923–933. doi:10.1139/g11-060

Yu P, White PJ, Hochholdinger F, Li C (2014) Phenotypic plasticity of the maize root system in response to heretogeneous nitrogen availability. Planta 240:667–678

Zamboni A, Zanin L, Tomasi N, Pezzotti M, Pinton R, Varanini Z, Cesco S (2012) Genome-wide microarray analysis of tomato roots showed defined responses to iron deficiency. BMC Genomics 13:101. doi:10.1186/1471-2164-13-101

Zhang Y-L, Lynch JP, Brown KM (2003) Ethylene and phosphorus availability have interacting yet distinct effects on root hair development. J Exp Bot 54:2351–2361

Zhang WH, Ryan PR, Tyerman SD (2004) Citrate-permeable channels in the plasma membrane of cluster roots from white lupin. Plant Physiol 136:3771–3783

Zhang S, Zhou J, Wang G, Wang X, Liao H (2015) The role of mycorrhizal symbiosis in aluminum and phosphorus interactions in relation to aluminum tolerance in soybean. Appl Microbiol Biotechnol 99(23):10225–10235. doi:10.1007/s00253-015-6913-6

Zhou L, Yamagishi M, Osaki M, Masuda K (2008) Sugar signalling mediates cluster root formation and phosphorous starvation-induced gene expression in white lupin. J Exp Bot 59:2749–2756

Zhou J, Xie J, Liao H, Wang X (2014) Overexpression of β-expansin gene *GmEXPB2* improves phosphorus efficiency in soybean. Physiol Plant 150(2):194–204. doi:10.1111/ppl.12077

Zhu JM, Kaeppler SM, Lynch JP (2005a) Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant Soil 270:299–310

Zhu Y, Yan F, Zör C, Schubert S (2005b) A link between citrate and proton release by proteoid roots of white lupin (Lu*pinus albus* L.). Plant Cell Physiol 46:892–901

Zhu J, Ingram PA, Benfey PN, Elich T (2011) From lab to field, new approaches to phenotyping root system architecture. Curr Opin Plant Biol 14(3):310–317. doi:10.1016/j.pbi.2011.03.020

Zinn KE, Liu J, Allan DL (2009) White lupin (*Lupinus albus*) response to phosphorus stress: evidence for complex regulation *LaSAP1*. Plant Soil 322(1):1–15

Zuchi S, Watanabe M, Hubberten HM, Bromke M, Osorio S, Fernie AR, Celletti S, Paolacci AR, Catarcione G, Ciaffi M, Hoefgen R, Astolfi S (2015) The interplay between sulfur and iron nutrition in tomato. Plant Physiol 169:2624–2639. doi:10.1104/pp.15.00995

The Biodiversity of the Yucatan Peninsula: A Natural Laboratory

Rafael Durán-García, Martha Méndez-González, and Alfonso Larqué-Saavedra

Contents

Abstract Advances of the biodiversity of the Yucatán Peninsula, Mexico, are presented. The area is known for settlement and development of the Mayan culture that had a profound understanding of their environment and has maintained a long-standing tradition in the use of natural resources spanning many centuries. The vegetation of this region is tropical and the territorial extension currently constitutes the second largest forest land in Latin America and it is formed by a very diverse terrestrial, freshwater and marine plant communities with more than 10 types or associations of vegetation such as forests, mangroves, coastal dune brushwood, hammocks, savannahs and diverse associations of fresh-water and marine hydrophytes. The coastal and marine environments of the peninsula include areas of coral reefs, coastal lagoons and sea grass beds. It is estimated that the peninsula has 2,300 species of vascular plants, grouped into 956 genera and 161 botanical families. Of the total number 203 are endemic, representing 8.82% of the Yucatan flora. There are more than 130 vegetative species used for construction; 145 plant species have been registered as food sources, 88 of which are native. So far, 680 plant species have been registered for medicinal use, 97 native plant species are ornamental; 36 species as bee plants, 27 species for handcrafted objects and 24 native species used

R. Durán-García • M. Méndez-González • A. Larqué-Saavedra (✉)
Centro de Investigación Científica de Yucatán A.C. (CICY), Unidad de Recursos Naturales, Calle 43 No. 130, Colonia Chuburná de Hidalgo, CP 97200 Mérida, Yucatán, Mexico
e-mail: rduran@cicy.mx; makandas@hotmail.com; larque@cicy.mx

Progress in Botany (2017) 78: 237–258, DOI 10.1007/124_2016_8,
Published online: 8 June 2016

as firewood; 12 native species have been reported as forage plants, 10 species as acaricides and 5 species are used as dye plants. The faunal wealth is over 7,300 species of wildlife with 5,765 species of invertebrates and 1,551 species of vertebrates. In the case of the invertebrates, the record shows 2,500 insect species, 350 arachnids, 715 crustaceans and 769 mollusks. The vertebrates reported are 700 fish species, 22 amphibians, 140 reptiles, 543 birds and 118 mammals. In the region, at least 81 species of 48 families and 21 taxonomic orders of terrestrial vertebrates are used. Fifteen species of reptiles (11 families), 38 species of birds belonging to 18 families are hunted, mainly as food, 28 species of mammals of 18 families are used and exploited. At least 40 species of wild vertebrates are used for medicinal purposes, of which 8 are reptiles, 15 birds and 17 mammals. With respect to marine resources are commercially exploited, 62 teleost species, 3 elasmobranchiae, 4 mollusks and 4 crustaceans. The Yucatan Peninsula has 39 protected natural areas, covering a surface area of 2,977,752 ha of terrestrial ecosystems, which represent 21.6% of the total surface area of the peninsula contributing significantly to the conservation of large extensions of forest and other tropical ecosystems together with the biodiversity inhabiting. The construction of the Seed Bank in 2013 by the Scientific Research Center of Yucatan for the conservation and management of the biodiversity found in the Mayan area is giving special attention to native plant species which are of medicinal agricultural, ecological, and forestry importance and is given special attention to endemic species and those which are known to be in danger of extinction.

1 Introduction

The Yucatan Peninsula, located in the extreme eastern portion of Mexico comprising the states of Campeche, Quintana Roo, and Yucatan, as well as northern parts of Belize and Guatemala, is recognized as a unique province from a biotic, geographic, and geomorphological point of view (Barrera 1962; Rzedowski 1978; Ferrusquía-Villafranca 1993; Morrone 2002). Historically known as the area of Mayan settlement and culture, Yucatan harbors great biological, ecological, and cultural diversity (Dupuy et al. 2015), displaying significant floral and faunal affinity with neighboring regions such as Central America and the Caribbean Basin (Estrada-Loera 1991; Durán et al. 1998; Ibarra-Manríquez et al. 2002).

Yucatan's vegetation is tropical with a few predominant forest types, such as high evergreen rainforest, medium-sized semi-evergreen and semi-deciduous forest, as well as low-stature deciduous forest (Miranda 1958; Flores and Espejel 1994; Olmsted et al. 1999). The Peninsula's forest currently constitutes the second largest forested area in Latin America (Dupuy et al. 2015). Additional, less extensive plant communities, such as palm groves, coastal sand dune brushwood and mangroves, seasonally flooded forests, grasslands, and wetlands, contribute within the forest

matrix to the region's vegetation mosaic (Espejel 1982; Trejo-Torres et al. 1993; Olmsted et al. 1999).

The region has a long history of natural resources use by the Maya people, who have exploited the native flora and fauna as part of their livelihood (Galletti 1994; Hernández-X 1959; Méndez and Durán 1997; Terán et al. 1998; Santos et al. 2005). However, over the past four decades, the natural ecosystems have been severely affected by human activities, in particular, agricultural, urban, and tourism development along with highway construction, requiring large regional transformation both of forestland and wetlands (Hernández-Barrios 2002; Andrade 2010). In addition, due to its geographic location, the Yucatan Peninsula is frequently hit by devastating hurricanes with subsequent flooding and fire events (Jáuregui et al. 1980).

In view of such natural and anthropogenic pressures on the ecosystems and their biodiversity, diverse conservation initiatives have been developed, among which ecosystem conservation by protecting natural areas is highly important. In addition, species conservation is pursued by ex situ establishment of botanical gardens. Beyond that, sustainable use of the biological wealth is being developed to allow human populations to persist within their living ecosystems.

Such features allow us to consider this region as a natural laboratory for exploring ecological and evolutionary processes, including interaction with strong historical human influences, which have molded the ecosystems and landscapes into their current form.

2 Physical Context

According to the Köppen classification as modified by García (2004), the climate of the Yucatan Peninsula is tropical with warm, humid, sub-humid, and semi-arid sub-variants characterized by summer rains. The highest temperatures occur from May through August (Orellana et al. 1999). Climatic determinants of the region are (a) ocean currents, (b) circulation of trade winds, (c) tropical waves, (d) tropical low-pressure systems from the Atlantic Ocean and the Caribbean Sea, and (e) cold fronts or northern winds (Orellana et al. 2010). The average annual temperature in the peninsula ranges from 24°C to 28°C, with two thermal zones (west and east) separated by an isothermal boundary of 26°C, which runs north to south from about Progreso City to the southern central portion of Calakmul reserve (bordering Guatemalan Peten). Rainfall presents a gradient from a dry zone with $<$600 mm in the northwest to a humid zone with $>$1,500 mm in the south to southeast around Laguna de Términos (bordering the coastal plain of the Gulf). The largest portion of the area receives between 1,000 and 1,200 mm of rainfall (Orellana et al. 1999).

The Yucatan Peninsula consists of a tectonic block without folds, originating in the Paleozoic and limited by the Motagua fault. This continental Pangean founda-

tion was separated from the Louisiana-Texas Block, when the Gulf of Mexico began to open. The current Yucatan position dates from the end of the Triassic (~200 million years). A thick layer of marine sediments from the Late Paleozoic has accumulated on top of that foundation, followed by continental sedimentation in the Jurassic, which in turn lies under an extensive deposit of evaporates and a final layer of limestone rocks (Schmitter-Soto et al. 2002). Limestone of the Tertiary period dominates most of the Peninsula. The lack of clays and the presence of sedimentary rocks or marlstone from the Upper Tertiary on top of the limestone rocks allow infiltration of rainwater, which gradually generates karst reliefs (García and Graniel 2010). The current surface geology is mainly formed by Cenozoic sediments, predominantly those of limestone origin (Rebolledo 2010).

In the coastal areas, calcareous sediments from the Quaternary form barrier beaches and flooded lagoons, as well as shallow bays with saline intrusions. The subsoil of the coastal area consists of soluble carbonate rocks of marine origin with abundant ducts of dissolution and fractures (Duch-Gary 1991), as immature soils can result from the accumulation of calcareous nutrient-poor material without consolidation (García and Graniel 2010).

As a result of these geological characteristics, there are virtually no surface water currents in the peninsula. Instead, rainwater filters into the ground, dissolving the limestone carbonates and giving rise to underground water deposits in caves and grottos, i.e., the so-called cenotes. The underground water moves away from the areas of high rainfall, i.e., from the south towards the northwest and north, where it is discharged by subterranean rivers filling coastal estuaries and lagoons (Graniel 2010). The water table ranges at depths from −6 to −90 m (Rebolledo 2010). The Peninsula's only surface rivers are located in the southern-most portion of the region.

The Yucatan Peninsula is characterized by its extremely shallow soils and large outcrops of bare rock and high stone content, particularly in the state of Yucatan (Bautista 2010). Here, soils are not homogenously distributed. Rather, they form differently sized "patches," extending from square-meter to hectare scales, being part of a Leptosol matrix (in the FAO-UNESCO classification system), dominating across almost 80% of the Yucatan landscape. Other, less dominant, soil groups include Histosols, Vertisols, Gleysols, Nitiosols, Phaeozems, Luvisols, Arenosols, Cambisols, and Regosols (Bautista 2010). These soils, having developed during centennial to millennial time scales, are fundamental to the regional vegetation and agriculture, decomposition of organic residues and nutrient cycling, and water and air quality, while providing habitats for numerous organisms. Representing non-renewable resources, the soils have to be regarded as their own ecosystems (Bautista 2010). Yucatan environments are determined by geoforms, climate, topography, and soil and vegetation types along with socio-economic influences. On such grounds, landscape units have been determined as (a) dry and semi-dry warm plains; (b) sub-humid and humid warm plains; (c) plateaus; and (d) karst valleys (García et al. 2010).

3 Biodiversity

3.1 Ecosystems and Communities

3.1.1 Terrestrial Ecosystems

The ecosystems of the Yucatan Peninsula have received much attention from numerous scientists since the middle of the last century due to its differences from the rest of Mexico. In particular: (a) the almost total absence of geological elevations on the peninsula; (b) the almost total absence of surface currents of water, such as rivers or streams, except for those located in the south; (c) the karst type calcareous substrate with its micro-spatial heterogeneity, and (d) the scarcity of soil with poor accumulation of organic material, barely few centimeters of width (García and Graniel 2010).

Within the framework of the climatic, edaphic, and geomorphological heterogeneity of the region, the terrestrial ecosystems of the Yucatan Peninsula are formed by a diverse mosaic of terrestrial, freshwater, and marine plant communities. More than ten types or associations of vegetation can be found in the region: forests, mangroves, coastal dune brushwood, hammocks, savannahs, and diverse associations of freshwater and marine wetlands (Flores and Espejel 1994; Olmsted et al. 1999).

According to the rainfall gradient, from the southern portion of the peninsula, along the border between Mexico and Guatemala, some high evergreen forest communities can be found. These plant communities are the most complex from a structural point of view and present a greater floral and faunal diversity. Some common species are *Swietenia macrophylla, Manilkara zapota, Brosimum alicastrum, Bursera simaruba*, and *Ceiba pentandra*.

The medium-stature semi-evergreen and semi-deciduous forests are the most widespread plant communities in the region, covering most of Campeche and Quintana Roo, and the southern part of Yucatan. Although diverse in their composition and structure, the medium-stature forests do not have the vegetative diversity of the high forests, particularly regarding epiphytic species. The representative species of this vegetation are *Manilkara zapota, Metopium brownei, Lysiloma latisiliquum, Piscidia piscipula, Vitex gaumeri*, and *Ceiba pentandra*.

Deciduous and semi-deciduous low forests also occupy a large area, spanning the northwest portion of the peninsula within the state of Yucatan, and somewhat in Campeche and along coastal Quintana Roo. Such forests possess high tree species diversity, due to high stone content of the shallow soils. On the other hand, the understory communities are structurally and floristically poor. Some common species of trees are *Alvaradoa amorphoides, Jatropha gaumeri, Gymnopodium floribundum, Caesalpinia gaumeri*, and *Bursera simaruba*.

The Yucatan Peninsula has a very characteristic type of vegetation consisting of flooded forests which develop on shallow ground where deficient drainage allows

the accumulation of rainwater for prolonged periods. The period of flooding alternates with a long period of drought, which causes substantial stress to the vegetation. Hence, these communities are formed by the very few species of trees and bushes capable of resisting the changing hydrological conditions (Olmsted l and Durán 1986). The epiphytic component is moderately represented, while the understory community is almost completely absent.

Alternating communities of coastal sand dunes and mangroves exist all along the coastal area of the entire region, adjoining inland with an extensive wetland zone. The latter is associated with the coastal lagoons surrounding the peninsula, where a diversity of emerging and floating hydrophilic salt, brackish, or fresh water plant prevail (Espejel 1982; Trejo-Torres et al. 1993; Zaldivar et al. 2010). In the sand dune communities two areas are distinguishable, i.e., the pioneer zone, occupied by species which grow directly on the beach and are exposed to the action of wind and tides, and the brushwood zone, where the substrate is more stable and the vegetation develops into a low-stature forest (Espejel 1982). Mangrove communities occupy a large territorial extension in the Peninsula and present a diversity of forms, which include the strip mangrove directly exposed to the sea waves, the basin mangrove farther away from the sea, around lagoons, and water bodies in the coastal zone, high mangrove on soils rich in organic matter under regular tidal change, and dwarf mangrove on loamy soils, poor in organic matter and almost anoxic. Mangrove communities comprise 1–3 mangrove species (*Rhizophora mangle, Avicennia germinans*, and *Laguncularia racemosa*) and may also contain some epiphytic (e.g., *Tillandsia* spp.) and climbing species (e.g., *Rhabdadenia biflora*) (Trejo-Torres et al. 1993; Zaldivar et al. 2010).

As a result of the topographic and edaphic heterogeneity within the forest matrix, additional vegetation types can occupy small landscape patches that enhance floral diversity. Patches include flooded forest, grasslands, and wetlands as well as hammocks and palm groves (Olmsted et al. 1999).

3.1.2 Aquatic Ecosystems

The coastal and marine territory of the Yucatan Peninsula presents an environmental variability deriving from temporal rainfall, wind, and temperature patterns. The seasonality of physico-chemical sea water characteristics generates significant spatio-temporal fluctuations in salinity, which together with the geomorphological structure creates high habitat diversity (Pech et al. 2010). The coastal and marine environments include coral reefs, coastal lagoons, and sea grass beds. The coastal lagoons are heterogeneous in hydrological terms, warranting the important connectivity with terrestrial ecosystems, while offering breeding, feeding, and refuge sites of high sea productivity for fish, crustaceans, and mollusks (Pech et al. 2010).

In the zone of sea grasses, turtle grass (*Thalassia testudinum*), manatee grass (*Syringodium filiforme*), and shoal weed (*Halodule wrightii*) are particularly

abundant. Depending on water depth, sediment type, currents, and turbidity, sea grasses develop in patches of variable dimensions, where fish, mollusks, and crustaceans reproduce and feed. The meadow-like patches increase water transparency and mitigate currents, as extensive systems of roots and rhizomes stabilize and retain sand, thereby preventing erosion. Moreover, the leaves function as a vital substrate for a large number of epibionts (Ayala 2010). The distribution and extension of these ecosystems is still uncertain despite the number of studies (e.g., Vargas et al. 1981; Yáñez-Arancibia and Lara-Domínguez 1983; Solís-Weiss and Carreño 1986; Gallegos et al. 1992; Gutiérrez-Aguirre et al. 2000).

A particularly important reef area is located along the east coast of the Yucatan Peninsula, from Contoy Island to Xcalak, and includes the Chinchorro Bank atoll (Jordán-Dahlgren 1993; Jordán-Dahlgren and Rodríguez-Martínez 2003). The reef is part of a larger Mesoamerican Barrier Reef System (MBRS) that extends about 1,000 km, from northern Yucatan to Belize, Guatemala and the Bahia Islands off the coast of Honduras (García-Salgado et al. 2008). A range of 45–56 hermatypic coral species have been reported for the Caribbean area of Mexico (Carricart-Ganivet and Horta-Puga 1993; Spalding et al. 2001), as well as 335 species associated with the reefs all along the Yucatan coast. According to Roberts et al. (2002), the Western Caribbean is considered to be one of the 18 most important centers of endemic reef species in the world.

One of the characteristics of the Yucatan Peninsula is the presence of *cenotes* ("caves with a body of water," i.e., sinkholes). Although the actual number of cenotes in the peninsula is not known, between 7,000 and 8,000 have been estimated for the state of Yucatan alone (Beddows et al. 2007). The aquatic cenote fauna has been studied since the beginning of the last century, emphasizing conspicuous groups such as fishes and macrocrustaceans. However, little is known about the composition of microfaunal groups, such as the copepods, of which 43 species have been reported, ostracods (18), decapods (8), amphipods (5), isopods (2), remipedia (1), and thermosbaenacea (1), with several taxa of aquatic invertebrates yet to be studied (Suárez-Morales and Rivera-Arriaga 1998). The fish component exceeds 50 species (Navarro-Mendoza 1988; Gamboa-Pérez 1992). Twenty-three native fresh water fish species inhabit the cenotes of Yucatan, spread across 14 genera and 6 families, 7 of which are endemic. The Cichlidae (bream) and Poeciliidae (Yucatan molly) families are the most representative, with 11 and 6 species, respectively (Chumba and Barrientos 2010). Suárez-Morales and Rivera-Arriaga (1998) report that several endemic species are threatened with extinction and need to be considered for protection and conservation; these include: *Cyprinodon beltrani*, *C. labiosus*, *C. maya*, *C. simus*, *C. verecundus*, *Olgilbia pearsei*, and *Ophisternon infernale*. Given the intense recreational use of many cenotes, it is important to value them as part of a limnic system that must be protected and preserved in its entirety rather than in isolated or fragmented ways (Suárez-Morales and Rivera-Arriaga 1998).

3.2 *Plant and Animal Diversity*

3.2.1 Floristic Wealth

The Yucatan Peninsula is well known for its floristic wealth. The botanical exploration of this region dates back to the late nineteenth and early twentieth century with work by the North Americans A.C. Schott, C.F. Millspaugh, G.F. Gaumer, P. Standley, and C. Lundell (Dupuy et al. 2015). More recently, a second period of exploration began in the 1980s carried out by researchers from Mexican institutions, continuing up until today. Botanists have come from the National Institution of Research in Biotic Resources (INIREB acronym in Spanish), Institute of Biology of the National Autonomous University of Mexico (IBUNAM – acronym in Spanish), Research Center of Quintana Roo (CIQRO – acronym in Spanish), Yucatan Center for Scientific Research of (CICY – acronym in Spanish), the State Universities of Yucatan, Campeche, and Quintana Roo, and the College of the Southern Frontier (ECOSUR – acronym in Spanish). As a result, numerous botanical publications give testimony of the floral wealth in this region (Millspaugh 1895, 1896; Standley 1930; Lundell 1934; Sousa and Cabrera 1983; Sosa et al. 1985; Durán et al. 2000; Arellano-Rodríguez et al. 2003; Gutiérrez-Baéz 2003; Carnevali et al. 2010), and of their phytogeographical relationships to other parts of the Americas (Estrada-Loera 1991; Durán et al. 1998; Ibarra-Manríquez et al. 2002; Espadas-Manrique et al. 2003).

It is estimated that the Mexican part of the Yucatan Peninsula harbors 2,300 species of vascular plants, grouped into 956 genera and 161 botanical families (Carnevali et al. 2012). While there is only one genus strictly endemic *Plagiolophus* Greenm. (Asteraceae), two others *Goldmanella* Greenm. (Asteraceae) and *Asemnantha* Hook. f. (Rubiaceae) extend to Belize and Guatemala as part of the Biotic Province of the Yucatan Peninsula. At the species level, 203 vascular plants are endemic to the Peninsula, representing 8.82% of the Yucatan flora (Table 1). The richest families are Fabaceae, Poaceae, Asteraceae, Orchidaceae, and Euphorbiaceae (Table 2). The most diverse genera include *Ipomoea* L. (Convolvulaceae), *Croton* L., Euphorbia L., (Euphorbiaceae), *Cyperus* L., and Rhynchospora Vahl (Cyperaceae) (Carnevali et al. 2012).

Besides its diversity, the vascular flora of the Yucatan Peninsula shares a greater number of species with The Antilles, in comparison with any other region of Mexico.

3.2.2 Faunal Wealth

The efforts of travelers, naturalists, and scientists, tracking and collecting examples of the wildlife, have contributed to increasing the body of data regarding the biological wealth present in different ecosystems and taxonomic groups. In the same way, have contributed to the development of collections deposited in

Table 1 Wealth of vascular plant species in the Yucatan Peninsula (Carnevali et al. 2012)

Plant groups	Families	Génera	Species	Endémic
Ferns and related plants	15	32	65	0
Gymnosperms	2	2	2	1
Angiosperms	144	922	2,233	202
Total	*161*	*956*	*2,300*	*203*

Table 2 Families of vascular plants with greater wealth of species in the Yucatan Peninsula

Family	Number of genera	Number of species
Fabaceae	78	225
Poaceae	70	214
Asteraceae	79	142
Orchidaceae	65	131
Euphorbiaceae	20	112
Cyperaceae	15	109
Convolvulaceae	12	76
Malvaceae	37	72
Rubiaceae	35	68
Apocynaceae	30	60

museums, research centers and higher education institutions in Mexico and other parts of the world. With the information currently available, it is possible to calculate the number of species known for Yucatan and to define their habitats and distribution (García-Contreras 2008). As an outcome, about 7,300 wildlife species are estimated to inhabit the Yucatan Peninsula, with 5,765 invertebrate and 1,551 vertebrate species. Invertebrates comprise 2,500 insect, 350 arachnid, 715 crustacean, and 769 mollusk species (Table 3), as compared to vertebrates with 700 fish, 22 amphibian, 140 reptile, 543 bird, and 118 mammal species (Table 3).

Nevertheless, collections carried out have undoubtedly been insufficient in some cases. Significant gaps exist in two fundamental areas: (1) minor taxonomic groups, such as lack of records of seven orders of insects (Delfín et al. 2010), and (2) underexplored parts of the Peninsula like the Calakmul area.

3.2.3 Species at Risk

Some species merit special attention, if endemic and "rare" in terms of spatial distribution, or if threatened by human activities, e.g., ecosystem degradation or loss. Unfortunately, due to limited information and too small collections, definition of current conservation status and prioritization for species registration in the Mexican Threatened Lists (NOM-059-SEMARNAT-2010) or The International Trade in Endangered Species of Wild Fauna and Flora List (CITES) are impeded. Nonetheless, as was mentioned previously, Carnevali et al. (2012) report on the existence of 203 endemic plant species, with 40 listed in the NOM-059

Table 3 Diversity of invertebrates and vertebrates registered in the three states of the Yucatan Peninsula

	Campeche	Quintana Roo	Yucatan	Peninsula
Invertebrates				
Marine sponges	23	30	21	50
Hydromedusas	nd	88	66	90
Echinoderms	74	174	102	180
Ophiuroids	22	nd	nd	nd
Siphonophorae	nd	nd	33	35
Corals	nd	54	50	80
Mollusks	660	675	365	769
Helminths	nd	150	50	150
Nematodes	nd	152	nd	160
Opisthobranchs (gastropod mollusks)	nd	nd	64	70
Chaetognatha	nd	16	5	16
Polychaete worms	322	230	22	600
Crustaceans	240	226	714	715
Arachnids	342	343	347	350
Insects	nd	1,977	2,247	2,500
Total	*1,683*	*4,115*	*4,086*	*5,765*
Vertebrates				
Fresh water fish	54	89	23	100
Salt water fish	356	580	457	600
Amphibians	21	22	18	22
Reptiles	99	106	87	140
Bird fauna	489	481	453	543
Mammals	105	114	89	118
Marine mammals	15	15	28	28
Total	*1,139*	*1,407*	*1,155*	*1,551*

Source: State Studies on the Biodiversity of Campeche, Quintana Roo, and Yucatan

(SEMARNAT 2010) to be prioritized for conservation (e.g., *Guaiacum sanctum, Cordia dodecandra, Thrinax radiata, Pseudophoenix sargentii, Beaucarnea pliabilis,* and *Pterocereus gaumeri*). Furthermore, Tetetla Rangel et al. (2012) recognize the existence of 195 rare woody-plant species in the region, 11 of which are endemic and 4 categorized as at risk. The authors modelled the distribution of 130 of these species, locating zones with high diversity of rare species and whether they overlapped with protected areas and with deteriorated zones, in view of determine priority conservation areas. Durán and Trejo (2010) plausibly estimate 280–350 rare species in the region. Although the Yucatan Peninsula does not contain a particularly high diversity of species, in comparison with other parts of Mexico, the ecosystems, species, and genetic variation present in local populations agree with characteristics of this biogeographic province, in view of prioritized number of ecosystems and rare and endemic species.

Factors exist in the region which threaten the integrity of the natural ecosystems and give arguments for preserving a large number of floral and faunal species, as habitats have been reduced, fragmented, or deteriorated during short time periods, with the consequence of population declines. Upon population fragmentations (as for spider monkey), developmental and evolutionary processed may seriously be affected, so that risk of extinction may mean such of losing natural capital (Ramos-Fernández and Ayala-Orozco 2002). Similar cases have been documented with some species of cacti, in both the coastal dune vegetation and low deciduous forest, where urgent protective measures are required, accompanied by permanent recording and monitoring programs (Durán and Méndez 2010b). With respect to local extinctions, historical documents (Leopold 1972; Villa 1959) mention the presence of species such as the Caribbean monk seal (*Monachus tropicalis*), the manatee (*Trichechus manatus*), and the pilot whale (*Globicephala macrorhynchus*), which were apparently common in the waters off the Yucatan Peninsula, but are now considered to be virtually extinct. Other historical widespread species, such as the tapir and the great curassow, whose area of distribution has been severely reduced, can now only be found in certain areas in the south of the region.

3.2.4 Flora and Fauna Used by Humans

Based on their cosmovision and a profound understanding of their environment, the Mayan populations of the peninsula have maintained a long-standing tradition in the use of natural resources spanning many centuries. Ethnobiological studies have shown that Mayan communities have employed a high diversity of plant and animal species in order to satisfy their basic needs of food, health, and housing (Durán and Méndez 2010a; Villalobos-Zapata and Mendoza 2010; Pozo et al. 2011). Within the Useful Species Database developed by researchers at the Yucatan Center for Scientific Research (Durán et al. 2012), there are more than 130 plant species registered as being use for the construction of homes, furniture, and tools. Similarly, 145 plant species have been registered as food sources, 88 of which are native. To date, 680 plant species have been registered for medicinal use (e.g., *Hamelia patens, Ocimum campechianum, Cissampelos pareira*, and *Pluchea carolinensis*), 97 for ornamentals (mainly trees as *Tabebuia rosea, Tecoma stans*, *Plumeria rubra* and palm trees *Pseudophoenix sargentii, Roystonea regia,* and *Thrinax radiata*), 36 for honey production (e.g., *Gymnopodium floribundum, Viguiera dentata,* and *Piscidia piscipula*), 27 for handcraft (e.g., *Bursera simaruba, Guaiacum sanctum*, and *Cordia dodecandra*), and 24 for firewood (*Acacia gaumeri*, *Mimosa bahamensis*, and *Havardia albicans*). Twelve species are used as forage plants (e.g., *Leucaena leucocephala, Brosimum alicastrum,* and *Guazuma ulmifolia*), 10 species as acaricides (e.g., *Petiveria alliacea*), and 5 species as dye plants (e.g., *Indigofera suffruticosa*) (Fig. 1).

In some rural communities of the Yucatan Peninsula the inhabitants still depend, to a certain extent, on wild animals for their subsistence, given that they constitute an important element in their diet, are used as medicine and serve for trading

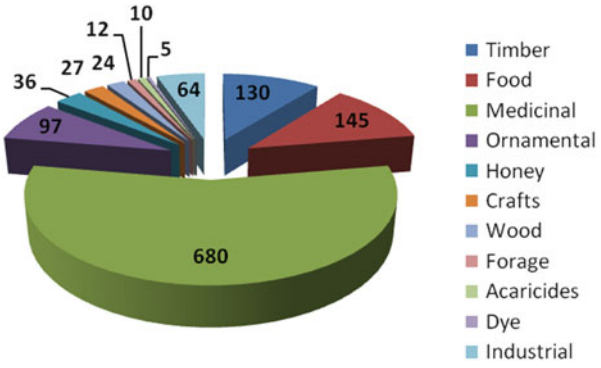

Fig. 1 Species diversity of useful plants in Yucatan. *Source*: Useful species database of the CICY (Durán et al. 2012)

(Segovia et al. 2010). Subsistence-based harvesting requires understanding animal reproductive cycles, age, and feeding patterns (Segovia et al. 2010), knowledge common to many Mayans. At least 81 species of terrestrial vertebrates are used, including reptiles, birds, and mammals of 48 families and 21 taxonomic orders (Chablé and Delfín 2010). Fifteen species of reptiles (11 families) are exploited, of which the most common are the Emydidae, Phrynosomatidae, Boidae, and Viperidae. In the case of birds, 38 species belonging to 18 families are hunted, mainly for food; they come from the Cracidae, Phasianidae, Tinamidae, and Columbidae and include the ocellated turkey (*Meleagris ocellata*), plain chachalaca (*Ortalis vetula*), Yucatan bobwhite (*Colinus nigrogularis*), and the doves (*Columbina talpacoti*, *Zenaida asiatica*, *Patagioenas flavirostris*, and *Leptotila verreauxi*). As for mammals, 28 species of 18 families are used; the most popular families are: Dasyproctidae, Aotidae, Geomyidae, Tayassuidae, Cervidae, Dasypodidae, Felidae, and Procyonidae. The animals used for food with greatest frequency are: white-tailed deer (*Odocoileus virginianus*), collared peccary (*Pecari tajacu*), rabbit (*Sylvilagus floridanus*), white-nosed coati (*Nasua narica*), and spotted paca (*Agouti paca*) (Jorgenson 1992; Chablé-Santos and Delfín-González 2004; Segovia et al. 2010; Santos-Fita et al. 2012). At least 40 species of wild vertebrates used for medicinal purposes have been reported, of which 8 are reptiles, 15 are birds, and 17 are mammals (Chablé and Delfín 2010). Table 4 presents the most commonly hunted species in the Yucatan Peninsula.

Unfortunately, some species commonly used as food sources are of culinary value, such as the spotted paca (*Agouti paca*) and the ocellated turkey (*Meleagris ocellata*) are being under great pressure from overexploitation, livestock development, urbanization, forest fires, and hurricanes (Calmé 2011).

With respect to marine resources, 62 teleost species, 3 elasmobranchiae, 4 mollusks, and 4 crustaceans are known to be commercially harvested (Flores et al. 2010). The most valuable resources include octopus (*Octopus maya*), shrimp (*Farfantepenaeus duorarum*), and lobster (*Panulirus argus*); however, the most productive species are shellfish (*Strombus gigas*, *S. costatus*, *Pleuroploca gigantea*, *Turbinella angulata*, *Busycon perversum*, and *Fasciolaria tulipa*), crab (*Callinectes rathbunae* and *C. sapidus*), the sharks (*Rhizoprionodon terraenovae*, *Carcharhinus*

Table 4 Faunal species most commonly hunted in the Yucatan Peninsula

Scientific name	Common name	Mayan name
Mammals		
Agouti paca	Spotted paca	Haaleb
Dasyprocta punctata	Central American agouti	Tsab
Dasypus novemcinctus	Armadillo	Weech
Mazama americana	Red brocket	Yuc
Mazama pandora	Yucatan Brown brocket	Yuc
Nasua narica	White-nosed coati	Chi'ik
Odocoileus virginianus	White-tailed deer	Ceh
Panthera onca	Jaguar	Chac-mol
Pecari tajacu	Collared peccary	Kitam
Pecari tayassu	White-lipped peccary	Kitam
Procyon lotor	Raccoon	Kulú
Sylvilagus sp.	Rabbit	Tu'ul
Birds		
Crax rubra	Great curassow	K'anbul
Meleagris ocellata	Ocellated turkey	Kuuts
Ortalis vetula	Plain chachalaca	Baach
Penelope purpurascens	Crested guan	Kaax

Sources: Calmé (2011) and Segovia et al. (2010)

spp., and *Sphyrna* spp.), stingray (*Dasyatis americana*, *Aetobatus narinari*, and *Rhinoptera bonasus*), and scaled fish (grouper, bar jack, bass, and sawfish) (Flores et al. 2010; Sosa-Cordero and Ramírez-González 2011).

In terms of commercial value, octopus is the main artisanal fishing activity in Campeche and Yucatan. The red octopus (*Octopus maya*) is endemic to the bay of Campeche and the north coast of Yucatan. In Campeche this is the only species of octopus exploited and represents 10% of the total volume of capture (Flores et al. 2010). Populations of these species have been severely affected by illegal fishing and excessive capture of species such as the so-called queen conch and octopus. Growth in tourism, besides increasing the demand for marine products, in some cases also deteriorates habitats which are fundamental for the persistence of coral reef species. Moreover, the excessive development of tourist-urban complexes is carried out at the expense of wetlands and mangroves which provide areas of reproduction for many fish and invertebrate species of commercial interest.

3.2.5 Environmental Services of Biodiversity

Without a doubt, the Yucatan Peninsula provides a great diversity of environmental services to society, among which is the provision of goods as raw materials, food, or medicines. More subtle services relate to climate regulation, air, soil, and water

quality, sustaining soil fertility and productivity, preventing soil erosion, and conserving biodiversity as a value per se (Dupuy et al. 2015).

Given that the area is vulnerable to the recurring threat of northerly winds and hurricanes, it is important to note that the terrestrial and aquaculture communities established in the coastal areas, such as reefs, mangroves, coastal dunes, and wetlands help to mitigate a great extent the impact of these meteorological phenomena.

In addition, it is important to consider the cultural services which depend on the perceptions and idiosyncrasies of the people and the socio-cultural context; in this region these acquire great relevance given the presence and preponderance of the Mayan culture, and include esthetic, educative, recreational, and spiritual or religious benefits (De Groot et al. 2005; Balvanera et al. 2009).

4 Threats to Plant Diversity

The Yucatan Peninsula, like the whole southeast region of Mexico, has lost a large part of its forests due to anthropogenic impact, such as (a) land use change to crop and livestock farming, urbanization, and tourism development; (b) overexploitation and extraction of economically important species; (c) exploitation of raw materials for domestic purposes; (d) construction of road infrastructure and services; and (e) frequent incidence of forest fire (cf. Dupuy et al. 2015). Unfortunately, such impacts resulted from insufficiently planned and coordinated human activities, and the absence of environmental policies and land use regulations (Ramírez-Carrillo 2010), neglecting definition of floral and faunal conservation areas. Major constraints arise from the continuing human population growth on the peninsula, which has about quadrupled since 1970 to above four million inhabitants in 2010 (Dupuy et al. 2015). Estimated 80% of the native vegetation has been perturbed, deforested, or degraded in such context (García-Contreras 2014). Real estate development has concentrated population in the main cities of the area, and excessive tourism such as encountered in Cancun and the Riviera Maya was developed at the expense of forests, mangroves, and coastal dunes. Such activities greatly threaten biodiversity, even within protected natural areas. Ecological reserves, such as Sian Ka'an, Holbox, and Punta Laguna, are examples of natural protected areas that have conflicts with adjacent tourist developments.

5 Conservation

To date, the Yucatan Peninsula has 39 natural protected areas, covering 2,977,752 ha of terrestrial ecosystems, which represent 21.6% of the total surface area of the peninsula dedicated to wildlife conservation, contributing significantly to the conservation of forest and other tropical ecosystems, also the biodiversity and

the environmental services they provide (Dupuy et al. 2015). It is important to note that, besides their function of conserving biodiversity, the Natural Protected Areas (PNA's) in Mexico have served as a focal point for the development of activities aimed at sustainable biodiversity use. Although all the vegetation types of the region are represented in such areas, efforts are insufficient to sustain ecological processes or viable species populations (CONABIO-CONANP-TNC-PRONATURA-FCF, UAN L. 2007; CONABIO-PNUD 2009; Durán and Ramos-Pacheco 2010; Koleff and Urquiza-Haas 2011). In order to contribute to the maintenance of viable populations of wild flora and fauna it's necessary to increase the number of PNA's, and other instruments of conservation, such as management units for the conservation of wildlife (UMA's), programs of sustainable forest management and forest certification, as well as the program of payments for environmental services (PES).

Two initiatives have been launched in the Yucatan Peninsula in such context: The Small Grants Program (SGP) for forest management, agriculture, apiculture, aquaculture, and sustainable tourism (Durán et al. 2012), and the Mesoamerican-Mexico Biological Corridor seeking to maintain ecological connectivity (Eccardi 2008).

In the Yucatan Peninsula, additional conservation strategies have been implemented, such as botanical gardens, at the Yucatan Center for Scientific Research (CICY), which contains approximately 700 plant species; the College of the Souther Frontier (ECOSUR) which is the largest in the country with 65 ha of medium-stature semi-evergreen forest and several collections conserving more than 170 plant species; the Autonomous University of Ciudad del Carmen which protects 25 ha of mangrove; the network of medicinal gardens of the Mayas which conserve approximately 264 species of medicinal plants (Dupuy et al. 2015).

Within the framework of the global action plan for the conservation of biodiversity outlined in 1992 during the Rio de Janeiro Meeting in Brazil, in which the guidelines for the elaboration of common strategies were defined, the authors of the present contribution elaborated the project "Germplasm (Seed) bank for the conservation and management of the biodiversity found in the Mayan area" in 2009 (Fig. 2); the principal objective being to conserve the ex situ and in situ flora of native species in the Mayan area which are of medicinal agricultural, ecological, and forestry importance and which contribute to the sustainability of the south–southeast region of Mexico. Subsequently 43 expeditions were carried out in different points of the Yucatan Peninsula, in over 300 collection sites, all of which are georeferenced in the database (Fig. 3). Special attention was given to integrating accessions of seeds of endemic species and also those which are known to be in danger of extinction. The construction and establishment of the bank was achieved in 2013 within the Scientific and Technological Park of Yucatan and currently comprises 1,502 accessions of plant collections, including 352 species of 75 families.

Fig. 2 Seed bank "Germoplasm Bank," peninsula of Yucatan

6 Conclusions

The Yucatan Peninsula in Mexico is a region of great biological wealth, with a large variety of terrestrial, freshwater, and marine ecosystems which, through their connectivity, maintain its floral and faunal diversity. This diversity provides a natural resource base, allowing the development of numerous, productive activities by Peninsula's inhabitants, and also serves as a source of environmental services for society in general.

In addition, the profound knowledge acquired and developed by the Mayan people of the Yucatan regarding the multiple uses of flora and fauna has sustained their complex social and productive structures, constitute a legacy for new generations which must be rescued from the current processes of environmental and cultural deterioration.

The work of the past three decades has made the Yucatan Peninsula as one of the regions of Mexico better known, from floristic and faunal point of view, while progress has been made in understanding the functioning of natural ecosystems and forms of use by local inhabitants. This scientific research has been carried out, focusing on the diversity, origin, persistence, and exploitation of a large number of biological groups, and the impact on the ecosystems inhabited by these species (e.g., Chablé-Santos and Delfín-González 2004; Carnevali et al. 2010; Delfín et al. 2010; Calmé 2011; Dupuy et al. 2015).

Thus, the compilation and integration of reliable, up-to-date information deriving from research work in different fields of knowledge, through biodiversity

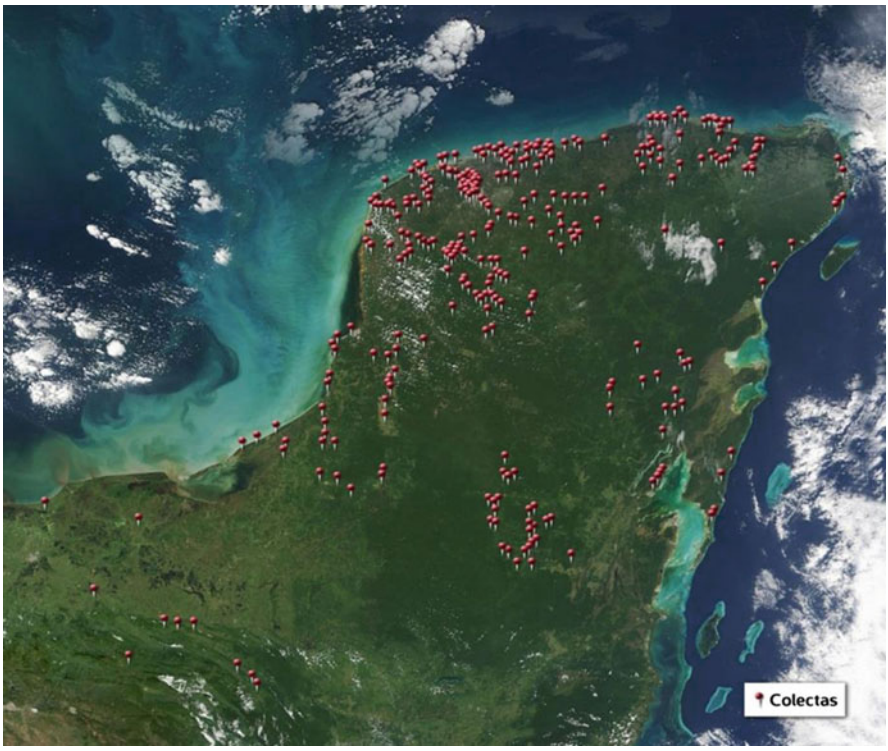

Fig. 3 Plant sites collected within the peninsula of Yucatan for seed bank "Germoplasm Bank"

studies made in Yucatán, Campeche y Quintana Roo (Durán and Méndez 2010a; Villalobos-Zapata and Mendoza 2010; Pozo et al. 2011), relating to the biological wealth and natural resources has provided a more realistic panorama of the degree of knowledge currently available regarding the biological diversity of this region and the present situation of natural resources in Yucatan. This information is fundamental in order to respond to relevant questions relating to biodiversity: What is the current situation regarding the conservation of ecosystems, communities, and floral and faunal populations? Which patterns and processes can be identified in spatial and temporal dimensions? What are the direct and indirect factors which have contributed the most to their deterioration? What possibilities of sustainable exploitation do they offer? among others.

Unfortunately, the development of a market society in the agricultural land of Yucatan has had a severe impact on the biological diversity of the region over the last two centuries (nineteenth and twentieth). The expansion of sugar cane cultivation, sisal cultivation, citrus fruit production, and extensive cattle-raising, in conjunction with cultivation in milpas (small plantations), using the system of slash and burn agriculture, has completely modified the natural landscape, by removing native vegetation.

Despite the level of transformation and deterioration that these ecosystems have suffered, significant forest lands have still been conserved and initiatives of conservation and management have been developed which allow us to surmise that it is feasible to conserve a large part of the biological diversity of the region and to revert, to some extent, ecosystem degradation.

There can be no doubt that the knowledge generated from the study of this natural laboratory will allow us to propose improved policies in environmental matters, generate proposals for the sustainable management of the local biological wealth, strengthen the governing of these resources, and integrating local wisdom and scientific knowledge in the search for a better form of interaction between humans and their environment.

Acknowledgments The authors thank to Richard Feldman who improved the last draft of this manuscript and to Ana Aguilar and Silvia Vergara for their assistance and help in the preparations of this manuscript.

References

Andrade M (2010) Transformación de los ecosistemas naturales por actividades antropogénicas. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Yucatán, Mérida, pp 316–319

Arellano-Rodríguez JA, Flores Guido JS, Tun-Garrido J, Cruz-Bojórquez MM (2003) Nomenclatura, forma de vida, uso, manejo y distribución de las especies vegetales de la península de Yucatán. Etnoflora Yucatanense UADY, Mérida

Ayala L (2010) Humedales costeros. In: Villalobos-Zapata GJ, Mendoza J (coord) La Biodiversidad en Campeche: Estudio de Estado. CONABIO/Gobierno del Estado de Campeche/UACAM/ECOSUR, Mérida, pp 116–126

Balvanera P, Cotler H et al (2009) Estado y tendencias de los servicios ecosistémicos, en Capital Natural de México, vol. II: Estado de conservación y tendencias de cambio. CONABIO, Mérida, pp 185–245

Barrera A (1962) La Península de Yucatán como Provincia Biótica. Rev Soc Mex Hist Nat 23:71–105

Bautista F (2010) El suelo. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 14–16

Beddows P, Blanchon P, Escobar E, Torres-Talamante O (2007) Los cenotes de la península de Yucatán. Arqueol Mex 16:32–35

Calmé S (2011) Uso y Manejo de Fauna Silvestre. In: Pozo C, Armijo Canto N, Calmé, S (eds) Riqueza Biológica de Quintana Roo. Un análisis para su conservación, Tomo I. ECOSUR/CONABIO/Gobierno del Estado de Quintana Roo y PPD, México, pp 165–170

Carnevali G, Tapia JL, Duno R, Ramírez I (2010) Flora Ilustrada de la Península de Yucatán: Listado Florístico. CICY, Mérida

Carnevali GC, Tapia JL, Ramírez I, Can L, Hernández S, Castillo A (2012) La flora de la Península de Yucatán Mexicana: 250 años de conocimiento florístico. Biodiversitas 101:6–10

Carricart-Ganivet JP, Horta-Puga G (1993) Arrecifes de coral de México. In: Salazar-Vallejo SI, González SE (eds) Biodiversidad Marina y Costera. CONABIO-CIQRO, México, pp 80–92

Chablé-Santos J, Delfín-González H (2004) Uso y problemática actual de la fauna silvestre en Yucatán. Universidad Autónoma de Yucatán y Secretaría de Ecología. Informe final de proyecto, México

Chablé-Santos J, Delfín-González H (2010) Uso tradicional de fauna silvestre. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 377–381

Chumba L, Barrientos R (2010) Peces dulceacuícolas. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 353–354

CONABIO-CONANP-TNC-PRONATURA-FCF, UAN L (2007) Análisis de vacíos y omisiones en conservación de la biodiversidad terrestre de México: espacios y especies. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Comisión Nacional de Áreas Naturales Protegidas, The Nature Conservancy-Programa México, Pronatura, AC, Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León, México

CONABIO-PNUD (2009) México: capacidades para la conservación y el uso sustentable de la biodiversidad. CONABIO, PNUD, México

De Groot R, Ramakrishnan PS, van de Berg A, Kulenthran T, Muller S (2005) Cultural and amenity services. In: Hassan R, Scholes R, Ash N (eds) Ecosystems and human well-being: current state and trends, vol 1 findings of the condition and trends working group of the millennium ecosystem assessment. Island Press, Washington, pp 455–476

Delfín H, Meléndez V, Manrique P, Reyes E, Chay D (2010) Insectos. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 226–228

Duch-Gary J (1991) Fisiografía del Estado de Yucatán. Universidad Autónoma de Chapingo, Texcoco

Dupuy JM, Durán R, García-Contreras G, Arellano J, Acosta E, Méndez-González M, Andrade M (2015) Conservation and use. In: Islebe G, Calmé S, Leon-Cortés J, Schmook B (eds) Biodiversity and conservation of the Yucatán Peninsula. Springer, Switzerland, pp 169–193

Durán R, Méndez M (eds) (2010a) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida

Durán R, Méndez M (2010b) Cactáeas. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 191–192

Durán R, Ramos-Pacheco L (2010) Papel de las Áreas Naturales Protegidas en la conservación de la biodiversidad. In: Durán R, Méndez M (eds) Biodiversidad y Desarrollo Humano en Yucatán. CICY, PPD-FMAM, CONABIO, SEDUMA, Mérida, pp 420–423

Durán R, Trejo-Torres JC (2010) Plantas vasculares prioritarias para la conservación. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 194–196

Durán R, Trejo-Torres JC, Ibarra-Manríquez G (1998) Endemic phytotaxa of the Peninsula of Yucatán. Harvard Pap Bot 3:263–314

Durán R, Campos G, Trejo-Torres JC, Simá P, May-Pat F, Juan-Qui M (2000) Listado Florístico de la Península de Yucatán. CICY-PNUD, Mérida

Durán R, Chi-May F, Méndez M (2012) Base de Datos de Especies Útiles de la Península de Yucatán. CICY, Mérida

Eccardi RF (2008) Biodiversidad y consumo responsable. Corredor Biológico Mesoamericano-México, CONABIO, SEMARNAT, Fomento Ecológico Banamex, Gaia Editores, México

Espadas C, Durán R, Argáez J (2003) Phytogeographic analysis of taxa endemic to the Yucatán Peninsula using geographic information systems, the domain heuristic method and parsimony analysis of endemicity. Divers Distrib 9:313–330

Espejel I (1982) Sand dunes ecology in the Peninsula of Yucatán, México. Institute of Ecological Botany/Uppsala University, Oxford

Estrada-Loera E (1991) Phytogeographic relationships of the Yucatán Peninsula. J Biogeogr 18:687–697

Ferrusquía-Villafranca I (1993) Geology of México: a synopsis. In: Ramamoorthy TP, Bye R, Lot A, Fa J (eds) Biology diversity of Mexico: origins and distribution. Oxford University Press, Oxford, pp 3–108

Flores S, Espejel I (1994) Tipos de vegetación de la Península de Yucatán. Fascículo de la Etnoflora Yucatanese. Univ Aut de Yucatán, Mérida

Flores D, Markaida U, Pérez-Jiménez JC, Ramos J (2010) Las pesquerías. In: Villalobos-Zapata GJ, Mendoza J (coord) La Biodiversidad en Campeche: Estudio de Estado. CONABIO, Gobierno del Estado de Campeche, UACAM, ECOSUR, México, pp 520–535

Gallegos ME, Merino M, Marba N, Duarte CM (1992) Flowering of Thalassia testudinum banks ex Konig in the Mexican Caribbean: age-dependence and interannual variability. Aquat Bot 43:249–255

Galletti H (1994) Actividades forestales y su desarrollo histórico. In: Dachary A (ed) Estudio integral de la frontera México-Belice, tomo I. CIQRO, Chetumal, pp 131–198

Gamboa-Pérez H (1992) Peces continentales de Quintana Roo. ln: Navarro D, Suárez E (eds) Diversidad biológica en la Reserva de la Biosfera de Sian Ka'an, Quintana Roo, México. Centro de Investigaciones de Quintana Roo/SEDESOL, México, pp 305–360

García E (2004) Modificaciones al sistema de clasificación climática de Köppen, Serie Libros No. 6. Instituto de Geografía, UNAM, México

García G, Graniel E (2010) Geología. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 4–6

García G, Méndez L, Aguilar W, Orellana (2010) This email address is being protected from spambots. You need JavaScript enabled to view it. Ambientes terrestres. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 17–20

García-Contreras G (2008) Sistema estatal sobre biodiversidad de Yucatán, bases de datos y sistema de Información geográfica. Reporte inédito del Estudio Estatal de Biodiversidad, Mérida, México

García-Contreras G (2014) Modelo geoespacial para el mapeo de las áreas con potencial para la restauración productiva del paisaje rural en la península de Yucatán. PPY - UICN/CPIC, Yucatán, México

García-Salgado M, Nava-Martinez G, Bood N, Mcfield M, Molina-Ramirez A, Yañez-Rivera B, Jacobs N, Shank B, Vasquez M, Majil I, Cubas A, Dominguez-Calderon JJ, Arrivillaga A (2008) Status of coral reefs in the Mesoamerican region. In: Wilkinson C (ed) Status of coral reefs of the world: 2008. Global Coral Reef Monitoring Network/Reef and Rainforest Research Centre, Townsville, pp 253–264

Graniel E (2010) Hidrología. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 12–13

Gutiérrez-Aguirre MA, De la Fuente-Betancourt MG, Cervantes-Martínez A (2000) Biomasa y densidad de dos especies de pastos marinos en el sur de Quintana Roo., México. Rev Biol Trop 48:313–316

Gutiérrez-Baéz C (2003) Listado florístico actualizado del estado de Campeche, México. Universidad Autónoma de Campeche, México

Hernández-Barrios JC (2002) Análisis del impacto del crecimiento de la ganadería bovina sobre el medio ambiente de la Península de Yucatán. México. Facultad de Ciencias, UNAM, México

Hernández-X E (1959) La agricultura. In: Beltrán E (ed) Los Recursos Naturales del Sureste y su Aprovechamiento, vol III. IMERNAR, México pp 3–57

Ibarra-Manríquez G, Villaseñor JL, Durán R, Meave J (2002) Biogeographical analysis of the tree flora of the Yucatán Peninsula. J Biogeogr 29:17–29

Jáuregui E, Vidal J, Cruz F (1980) Los ciclones y tormentas tropicales en Quintana Roo durante el periodo 1871–1978. Memorias del Simposio Quintana Roo: Problemática y Perspectiva, Instituto de Geografía, UNAM y CIQRO, México, pp 47–64

Jordán-Dahlgren E (1993) Atlas de los arrecifes coralinos del Caribe mexicano. Parte I, El sector Continental. CIQRO e ICML/UNAM, México

Jordán-Dahlgren E, Rodríguez-Martínez RE (2003) The Atlantic coral reefs of México. In: Cortés J (ed) Latin American coral reefs. Elsevier, Amsterdam

Jorgenson J (1992) La cacería de subsistencia practicada por la gente maya en Quintana Roo. In: Snook L, Barrera A (eds) Memorias del taller madera, Chile, caza y milpa: Contribución al manejo integral de las selvas de Quintana Roo, México. Plan de Acción Forestal Tropical. PROAFT- INIFAP, México, pp 3–64

Koleff P, Urquiza-Haas T (coords) (2011) Planeación para la conservación de la biodiversidad terrestre en México: retos en un país megadiverso. CONABIO–CONANP, México

Leopold A (1972) Wildlife of Mexico: the game birds and mammals. University of California Press, Berkeley/London

Lundell CL (1934) Preliminary sketch of the phytogeography of the Yucatán Peninsula. Contrib Am Arch 12:257–321

Méndez M, Durán R (1997) Diagnóstico del conocimiento etnobotánico actual de las plantas medicinales de la Península de Yucatán. Bol Soc Bot México 60:15–24

Millspaugh CF (1895) Contribution to the flora of Yucatán. Publ Field Columbian Museum. Bot Ser 1:1–56

Millspaugh CF (1896) Contribution II to the coastal and plain flora of Yucatán. Publ Field Museum. Bot Ser 1:279–339

Miranda F (1958) Estudios acerca de la vegetación. In: Beltrán E (ed) Los Recursos Naturales del Sureste y su Aprovechamiento. IMERNR, México, pp 215–271

Morrone JJ (2002) Presentación sintética de un nuevo esquema biogeográfico de América Latina y El Caribe. Sociedad Entomológica Aragonesa, Monografías Tercer Milenio vol 2, pp 267–275

Navarro-Mendoza M (1988) Inventario íctico de los cuerpos de agua continentales de la Reserva de la Bíosfera de Sian Ka'an, Quintana Roo. CIQRO-US FWS

Olmsted l, Durán R (1986) Aspectos ecológicos de la selva baja inundable en la reserva de la Biósfera Sian Ka'an, en Quintana Roo. Biótica 11:151–179

Olmsted I, González-Iturbe JA, Granados-Castellanos J, Durán R, Tun-Dzul F (1999) Vegetación de la Península de Yucatán. In: García de Fuentes A, Córdoba J, Chico P (eds) Atlas de Procesos Territoriales de Yucatán, pp 183–194

Orellana R, García de Miranda E, Bañuelos I, Balam M, González-Iturbe JA, Herrera F, Vidal J (1999) Climatología de la Península de Yucatán (1961–1993). In: García de Fuentes A, Córdoba J, Chico P (eds) Atlas de Procesos Territoriales de Yucatán, pp 163–182

Orellana R, Espadas C, Nava F (2010) Climas. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 10–11

Pech D, Mascaró M, Jáuregui M, Simões N, Enríquez C (2010) Ambientes marinos. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 21–23

Pozo C, Armijo N, Calmé S (eds) (2011) Riqueza Biológica de Quintana Roo. Un análisis para su conservación, Tomo I. ECOSUR/CONABIO/Gobierno del Estado de Quintana Roo/PPD, México

Ramírez-Carrillo L (2010) Las relaciones peligrosas: sociedad, naturaleza y construcción de la modernidad. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 30–34

Ramos-Fernández G, Ayala-Orozco B (2002) Population size and habitat use of spider monkeys in Punta Laguna, México. In: Marsh L (ed) Primates in fragments: ecology and conservation. Plenum/Kluwer Press, New York

Rebolledo M (2010) Hidrología. In: Villalobos-Zapata GJ, Mendoza J (coord) La Biodiversidad en Campeche: Estudio de Estado. CONABIO/Gobierno del Estado de Campeche/UACAM/ECOSUR, México, pp 2–7

Roberts CM, McClean CJ, Veron JE, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295:1280–1284

Rzedowski J (1978) La vegetación de México. LIMUSA, México

Santos JV, Mas P, López C, Snook LK (2005) El manejo forestal y la caoba en los ejidos de la Zona Maya, México. Desarrollo histórico, condiciones actuales y perspectivas. Rev Rec Nat Amb 44:27–36

Santos-Fita D, Naranjo E, Rangel-Salazar JL (2012) Wildlife uses and hunting patterns in rural communities of the Yucatan Peninsula, Mexico. J Ethnobiol Ethnomed 8:38

Schmitter-Soto JJ, Escobar-Briones E, Alcocer J, Suárez-Morales E, Elías-Gutiérrez M, Marín LE (2002) Los cenotes de la península de Yucatán. de La Lanza G, García Calderón JL (comps) Lagos y presas de México. AGT, México

Segovia A, Chablé J, Delfín H, Sosa J, Hernández S (2010) Aprovechamiento de la fauna silvestre por comunidades mayas. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 385–387

SEMARNAT (2010) Norma Oficial Mexicana NOM-059. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federación

Solís-Weiss V, Carreño S (1986) Estudio prospectivo de la macrofauna béntica asociada a las praderas de Thalassia testudinum en la Laguna de Términos, Campeche, México. An Inst Cienc Mar Limno 13:201–216

Sosa V, Flores JS, Rico-Gray V, Lira R, Ortíz JJ (1985) Etnoflora Yucatánense. Lista florística y sinonimia maya. INIREB, Xalapa, Ver. México

Sosa-Cordero E, Ramírez-González A (2011) Pesca marina. In: Pozo C, Armijo N, Calmé S (eds) Riqueza Biológica de Quintana Roo. Un análisis para su conservación, Tomo I. ECOSUR/CONABIO/Gobierno del Estado de Quintana Roo/PPD, México, pp 183–189

Sousa M, Cabrera E (1983) Listados florísticos de México II. Flora de Quintana Roo. Instituto de Biología/UNAM, México

Spalding MD, Ravilious C, Green EP (2001) World atlas of coral reefs. WCMC-UNEP/University of California Press, Berkeley

Standley PC (1930) Flora of Yucatán. Field Columbian Mus Publ Bot Ser 3:157–492

Suárez-Morales E, Rivera-Arriaga E (1998) Hidrología y Fauna Acuática de los Cenotes de la Península de Yucatán. Rev Soc Mex Hist Nat 48:37–47

Terán S, Rasmunssen C, May Cauiche O (1998) Las Plantas de la Milpa entre los Mayas. Fundación Tum Ben Kin, Mérida

Tetetla Rangel E, Durán García R, Hernández Stefanoni JL, Dupuy JM (2012) Distribución espacial de la riqueza de especies leñosas raras de la Península de Yucatán y su relación con las áreas naturales protegidas. Trop Conserv Sci 5:320–339

Trejo-Torres J, Durán R, Olmsted I (1993) Manglares de la Península de Yucatán. In: Salazar-Vallejo S, González N (eds) Biodiversidad Marina y Costera de México. CONABIO-CIQRO, México pp 660–672

Vargas MI, Yáñez-Arancibia A, Amezcua-Linares F (1981) Ecología y estructura de las comunidades de peces en áreas de Rhizophora mangle y Thalassia testudinum de la Isla del Carmen, Laguna de Términos, sur del Golfo de México. An Inst Cienc Mar Limno 8:241–266

Villa B (1959) Mamíferos de caza. In: Beltrán E (ed) Los Recursos Naturales del Sureste y su Aprovechamiento, vol III. IMERNAR, México, pp 125–148

Villalobos-Zapata GJ, Mendoza Vega J (coord) (2010) La Biodiversidad en Campeche: Estudio de Estado. CONABIO/Gobierno del Estado de Campeche/UACAM/ECOSUR, México

Yáñez-Arancibia A, Lara-Domínguez AL (1983) Dinámica ambiental de la boca de Estero Pargo y estructura de sus comunidades de peces en cambios estacionales y ciclos 24 horas (Laguna de Términos, Sur del Golfo de México). An Inst Cienc Mar Limno 10:85–116

Zaldivar A, Herrera-Silveira J, Teutli C, Herández R, Caamal J (2010) Manglares. In: Durán R, Méndez M (eds) Biodiversidad y desarrollo humano en Yucatán. CICY/PPD-FMAM/CONABIO/SEDUMA, Mérida, pp 138–139

Glacier Forelands: Lessons of Plant Population and Community Development

Brigitta Erschbamer and Marco Stefano Caccianiga

Contents

Abstract After glacier retreat, the ice-free forelands arise as easily detectable landforms where primary succession starts from the beginning onwards. Here, basic ecological lessons of colonization and community development can be learned. In this review we summarize the results of several case studies from the Austrian and Italian Alps, draw conclusions and highlight research gaps. Glacier foreland species exhibit a considerable intra-population diversity. Actual gene flow was shown to be high enough to maintain the genetic diversity throughout all successional stages. Most seeds of the glacier foreland species are light-weighted and wind-dispersed. Heavier seeds with no specific dispersal traits such as those of certain late successional species will hardly be dispersed to the pioneer stages. In most glacier forelands, a seed bank has to be developed from zero onwards. The

B. Erschbamer (✉)
Institute of Botany, University of Innsbruck, Sternwartestr. 15, Innsbruck 6020, Austria
e-mail: brigitta.erschbamer@uibk.ac.at

M.S. Caccianiga
Dipartimento di Bioscienze, University of Milano, Via Celoria, 26, Milano 20133, Italy

Progress in Botany (2017) 78: 259–284, DOI 10.1007/124_2016_4,
Published online: 8 June 2016

already established species are the ones which contribute most to the genesis of a seed bank; an input of seeds by long distance dispersal was hardly detected. A relatively high quantity of glacier foreland species has a deep physiological dormancy. Thus, they will be able to form persistent seed banks. Seedling recruitment is highly governed by drought and seed availability. Additionally, frost and heat might be essential abiotic factors for germination and seedling survival. Growth rates of the glacier foreland species vary considerably among the successional stages and seem to be phylogenetically constrained. Population growth rates are characterised by low seedling recruitment and/or high mortality rates of the seedlings; some species overcome this low reproductive success by clonal growth strategies. From seed sowing experiments we learned that facilitation and competition may occur side by side. However, this topic has to be further explored in the future. By means of plant functional types, the pioneer species were classified as fast-growing ruderal strategists. In contrast, late successional stages harbour mainly stress-tolerant species with dense leaves and low relative growth rates. Phytosociological community descriptions are a challenging topic in glacier forelands. Nevertheless, several communities were described mainly from the Italian Alps. One of the less investigated topics, although being essential in ecology, is the species interaction issue. Among the scarce studies in this context, pollination and flower-visiting insects were studied. Species interactions including different organismic levels as well as species adaptations to changing conditions are still topics to be studied along glacier forelands. Climate warming probably will enhance the speed and the pathway of colonization. If glacier forelands can act as refugia for alpine-nival species remains to be proved. Thus, glacier foreland offer ample ecological questions and further research is highly recommended.

1 Introduction

The area of the glacier forelands in the Alps increases year by year due to the ongoing retreat of the glaciers. Since the last glacier maximum at the end of the Little Ice Age (ca. 1850) the Austrian glaciers lost 50% of their area (Abermann et al. 2009) with mean longitudinal retreat rates of 10–20 m per year (Fischer and Hartl 2013). Melting processes were accelerated by the end of the last century; during the period 1969–1998, the area of the Tyrolean glaciers decreased by 17.3% and the mean ice thickness was reduced by 9.4 m (Abermann et al. 2013). Most of the newly appearing areas are bare of soil, lacking former vegetation and buried seed banks. Therefore, glacier forelands are ideal sites for studying ecological processes of successions from the very beginning onwards. Matthews (1992) called them ‘a unique type of field laboratory’, being a ‘vast natural experiment’. The spatial pattern from the youngest, recently deglaciated, to the oldest moraines represents the temporal change (chronosequence) and can be regarded as model

system for population and community development. From the end of the nineteenth century onwards, a considerable number of ecological investigations in the Swiss, Austrian and Italian Alps were published (reviews in Burga et al. 2010). The pathway of primary succession was abundantly assessed by vegetation maps and phytosociological descriptions. Most authors evidenced that glacier forelands do not have a simple chronosequence corresponding to the time of deglaciation (Matthews and Whittaker 1987; Matthews 1992; Foster and Tilman 2000) but exhibit a complex pattern depending on site factors such as grain size, moisture, snow duration, inclination, thermal conditions, solar radiation, geology, geomorphological processes (Jochimsen 1970; Crouch 1993; Fastie 1995; Vetaas 1997; Caccianiga and Andreis 2004; Raffl and Erschbamer 2004; Raffl et al. 2006a) and biotic drivers (Walker and del Moral 2003). Detecting the determining abiotic and biotic factors of glacier foreland colonization and succession was one of the major objectives of the investigations during the last decades. In this review we summarize such results and provide insights from the Alpine glacier forelands. As model systems we chose the Rotmoosferner glacier foreland (2,300–2,450 m a.s.l.) at Obergurgl, Ötztal (Tyrol, Austria, N 46.830°, E 11.041°), the Amola glacier foreland (2,400–2,560 m a.s.l., Trentino, central Italian Alps, N 46.221°, E 10.678°), the Cedec glacier foreland (2,600–2,730 m a.s.l., Lombardy, central Italian Alps, N 46.45°, E 10.583°) and the Rutor glacier foreland (2,380–3,000 m a.s.l., Valle d'Aosta, western Italian Alps, N 46.067° E 7.017°).

The cited case studies can be regarded as puzzle stones contributing to a general picture of primary succession in high altitudes to provide basic understanding of the important processes in population and community formation from successional zero onwards. The classical concepts of colonization such as facilitation, tolerance and competition (Connell and Slatyer 1977), plant strategies (Grime 1979), and functional diversity (Diaz and Cabido 2001) imply well-defined traits and interactions of the species groups involved in primary succession. We show several examples even though heterogeneity within and between glacier forelands often obliterates a clear pattern. With our review we highlight that glacier foreland colonization is a highly complex process and by no means unidirectional.

2 Dynamics and Factors Influencing Colonization

2.1 Genetic Diversity and Origin of the Species

Most glacier forelands are sites without history of plant growth. The first colonizers have to arrive to such sites from areas outside the glacier foreland. Theory suggests a low genetic diversity of the pioneer species due to founder effects; i.e. few individuals initiate colonization; thus, genetic diversity is to increase due to the consecutive gene immigration along primary succession (Giles and Goudet 1997). A second, contrasting theory suggests that the colonizers derive from a high number

of ancestral individuals from outside, and that with ongoing succession, genetic drift and/or selection might reduce genetic diversity on older successional stages (Pluess and Stöcklin 2004). To settle the debate, Raffl et al. (2006b) investigated the development of genetic diversity and the sources of the first colonizing species *Saxifraga aizoides* L. at the Rotmoos- and Gaisbergferner (Obergurgl, Tyrol, Austria). The study showed that, in contrast to the two hypotheses, the pioneer stages had the same level of genetic diversity as older successional stages. High gene flow by seeds and pollen obviously ensures a constantly high genetic diversity along the whole chronosequence. Recent gene flow was mainly assigned to the adjacent valley slope (48%, Raffl et al. 2006b) and to the glacier foreland populations (24%) whereas input from a parallel glacier valley remained rather low (4%). Similar results were obtained for populations of *Trifolium pallescens* Schreb.: gene flow occurred mainly from older populations on the glacier foreland and to a lower extent also from parallel glacier valleys (Raffl et al. 2008). For both the species, an input from far distances cannot be excluded because a considerable amount of recent gene flow could not be assigned. A sufficient gene flow from the surrounding was suggested also for other pioneer species such as *Geum reptans* L. (Pluess and Stöcklin 2004; Stöcklin et al. 2009) and *Epilobium fleischeri* Hochst. (Stöcklin et al. 2009) as well as for *Larix decidua* Mill. (Pluess 2011) on glacier forelands in Switzerland. Thus, contrary to the expectations, glacier foreland species have a high intra-population diversity due to a continuous gene flow from outside and along the chronosequence.

2.2 Seed Size, Seed Rain

Seed dispersal by wind seems to be the most important driver in glacier foreland dynamics. Warranting colonization success, the first colonizers should have abundant, tiny, wind-dispersed seeds which easily arrive to the site from outside or from older successional stages. At least for some of the pioneers the smallness of the seeds can be confirmed (Erschbamer and Mayer 2011). At the Rotmoosferner glacier foreland seed mass ranged from 41 µg (pioneer species *S. aizoides*) to 2,366 µg (late successional species *Anthyllis vulneraria* L. ssp. *alpicola*). Similarly to that of Alaskan glacier foreland colonizers (Chapin III 1993), pioneer species of Alpine glacier forelands generally have seed masses <500 µg. However, there are also exceptions such as those of the pioneers *Oxyria digyna* (L.) Hill. and *G. reptans* L. (Schwienbacher et al. 2012) with seed masses of 650 and 940 µg, respectively. They probably compensate the higher weight through adaptations of the fruits to efficient wind dispersal via wings or hairy styles. Seed mass increases towards late successional species (Erschbamer and Mayer 2011), although response to the niche position of the individual species along the successional gradient was not linear (Schwienbacher et al. 2012).

One striking question of glacier foreland dynamics is on the quantity of seeds arriving at moraines. Stöcklin and Bäumler (1996) recorded an increasing seed rain

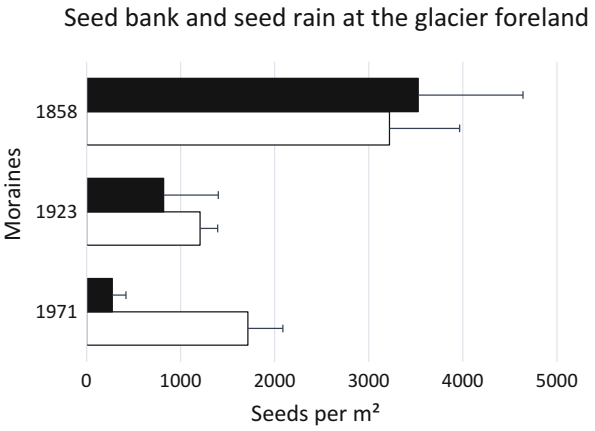

Fig. 1 Seeds per m^2 (means and standard error) in the seed bank (*black bars*) and seed rain (*white bars*) at the moraines of 1971, 1923 and 1858 of the Rotmoosferner glacier foreland (Tyrol, Austria) in 2004. Data: Marcante et al. (2009a) and Finch (2008)

from the youngest (125 seeds per m^2) to >45-year-old moraines (2,333 seeds per m^2) of the Morteratsch glacier foreland in Switzerland. In contrast, at the glacier foreland of the Rotmoosferner (Austria) a high variation was found from year to year and from moraine to moraine (Erschbamer and Mayer 2011). In 2004, the highest seed rain was assessed at the oldest moraine (3,220 seeds per m^2, Fig. 1), the lowest at the moraine of the year 1923 (1,207 seeds per m^2, Fig. 1). At the younger moraines, the highest convergence was detected between seed rain and standing vegetation with correlations of 52–61%. With increasing succession, as species diversity considerably diverged, the seed rain was less diverse than the standing vegetation (Erschbamer and Mayer 2011). Parachutists with plumes were trapped most frequently at the Morteratsch glacier foreland in Switzerland (64%, Stöcklin and Bäumler 1996), reaching a dispersal distance up to 100 m apart from the mother plants. Seeds with wings or fringe were also frequent (29%) while seeds with no apparent structure for wind dispersal were less common (6%). The latter ones were dispersed rather close to the mother plants (i.e. 0–10 m, Stöcklin and Bäumler 1996). Simulation models showed that 99.9% of the seeds will likely be dispersed <10 m (Pluess and Stöcklin 2004). Long-distance dispersal of >100 m and >1,000 m was found only in 0.015% and 0.005% of the investigated seeds, respectively. As a consequence, species with heavy seeds with no specific dispersal facilities such as those of the late successional species (i.e. *A. vulneraria* ssp. *alpicola* at the Rotmoosferner glacier foreland) can hardly be dispersed by wind to the young moraines (Erschbamer et al. 2001). All in all, morphological structures favouring dispersal but not seed mass matter for colonization processes in glacier forelands.

Flø and Hågvar (2013) showed that also plant fragments such as of mosses may be transported by wind. Thus, aerial transport is not only important for seed dispersal (and microarthropods, Flø and Hågvar 2013) but also for plant propagules of different origin. Probably also fragments of the first colonizer *S. aizoides* arrive by wind, floods or avalanches to recently deglaciated areas (Winkler et al. 2015).

2.3 Seed Bank and Seed Longevity

Seed banks are missing on recently deglaciated moraines (Chapin et al. 1994; Stöcklin and Bäumler 1996), with exception of those glacier forelands where vegetation was present in former times (Nicolussi 2009). However, from such latter sites investigations are lacking. Information is available from the Rotmoosferner glacier foreland where definitely no ancient soil is present (Marcante et al. 2009a). On the 1971 moraine 273 seeds per m^2 were found (Fig. 1), at the 1858 moraine already 3,527 seeds per m^2. Species assemblages of the seed bank and the standing vegetation gradually diverged along the chronosequence (Marcante et al. 2009a), although seed bank diversity was generally lower than that of the standing vegetation. Not all the species build a seed bank, some seeds not entering the seed bank at all or being only short-lived (transient seed bank, Thompson et al. 1993) and, with the increase in vegetation cover (oldest moraine 87% cover; outside the glacier foreland 83%, Marcante et al. 2009a), less contact to the soil prevent seed entry into the seed bank. At the other hand, some seeds may not have been detected due to a highly heterogeneous accumulation in the soil. Pioneer species may remain in the seed bank until the late successional stages. This ensures regeneration and initiates primary succession again in the case of disturbances. In plant communities outside the glacier foreland, only marginally higher numbers of seeds were detected (3,674 seeds per m^2). However, species composition was totally different from that of the glacier foreland seed bank (Marcante et al. 2009a). A distance of several hundred metres and the boulder scree of the end moraine 1858 were found to be efficient barriers for seed dispersal from outside. Only two species were in common between the seed bank of the glacier foreland and the area outside (*Poa alpina* L. and *T. pallescens* Schreb.; Marcante et al. 2009a). Glacier forelands are not following the general theory of dominant species being absent from the persistent seed bank (Fenner and Thompson 2005; Leck et al. 2008). We learned from our studies that along the whole glacier foreland right the aboveground dominants determine the prevailing species of the seed bank.

According to the presence of seeds in deeper soil layers, seed persistence has to be assumed for most of the species throughout the entire succession. The respective hypothesis was tested by a 5-year-burial experiment at the Rotmoosferner glacier foreland (Schwienbacher et al. 2010). Only 2 of 9 species, i.e. *O. digyna* and *G. reptans*, were classified as transient, the seeds of which remained viable only over one winter. Both species had high seed mass and seed dimension (calculated according to Thompson et al. 1993) in contrast to the species with persistent seeds. Seeds with a hard coat such as those of the Fabaceae (*T. pallescens* and *A. vulneraria* ssp. *alpicola*, Schwienbacher et al. 2011) or small seeds with low seed dimension (Thompson et al. 1993; Funes et al. 1999) such as those of the Saxifragaceae can probably persist in the soil for very long time periods (Schwienbacher et al. 2010). Seeds with medium weight/dimension follow an intermediate strategy, surviving two winters (*Artemisia genipi* Weber, *Achillea moschata* Jacq.). Schwienbacher et al. (2011) emphasized that in most glacier

foreland species the seeds with their deep physiological dormancies have the potential for staying viable in the soil over long times.

2.4 Germination and Environmental Stress

From the literature we know that colonization in glacier forelands often fails due to lack of seeds (Chapin et al. 1994). Conversely, pioneer species produce huge numbers of seeds: One capsule of *S. aizoides* contains 103–687 seeds (Marcante et al. 2013); and one fruit of *E. fleischeri* develops about 100 seeds (Stöcklin and Bäumler 1996). Thus, each flowering individual potentially releases several thousands or even several ten thousands of seeds. Nevertheless, recruitment remains poor on bare-ground sites. Erich Schwienbacher (2007–2009, unpublished) demonstrated bare-ground plots at the 1971 moraine of the Rotmoosferner glacier foreland to be seed limited ($p < 0.001$, Fig. 2). Aside lack of seeds, he identified drought as major obstacle for germination ($p < 0.001$, Fig. 2). In Schwienbacher's seeding experiment, 10 species (100 seeds each) were included: *S. aizoides, Linaria alpina* (L.) Hill., *Arabis caerulea* (All.) Haenke, *O. digyna, T. pallescens, P. alpina, Silene acaulis* (L.) Jacq. ssp. *exscapa, Persicaria vivipara* (L.) Ronse Decr., *Leontodon hispidus* L. and *A. vulneraria* ssp. *alpicola*. Using a factorial design, he compared plots with seed addition, irrigation, seed addition X irrigation and control plots (25 × 25 cm each). An automated sprinkling irrigation system was installed which started watering when the upper soil layers got dry. The whole system operated through a laptop and dataloggers measuring temperatures and soil water potential at the plots. Germination was highest at the treatment irrigation X seed addition with around 383 seedlings in total in August 2008 (Fig. 2), comprising

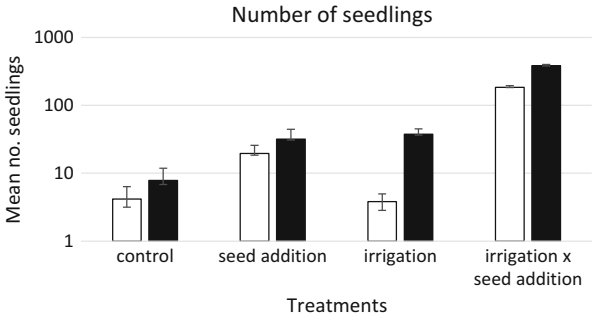

Fig. 2 Results of a multi-factorial experiment at the 1971 moraine of the Rotmoosferner glacier foreland (Tyrol, Austria): mean number of seedlings ± standard error in control plots (= plots without manipulation), in plots with seed addition, plots with automated irrigation and in plots with automated irrigation X seed addition in August 2007 (*white bars*) and 2008 (*black bars*). Seed addition was performed in 2007, automated irrigation was operating throughout the two growing seasons (data: Erich Schwienbacher, unpublished). Significant differences resulted between the treatments ($p < 0.001$). *Y*-axis: logarithmic scale

all 10 seeded species and additionally also seedlings of some naturally recruiting species such as *Saxifraga oppositifolia* L. and the pseudoviviparous *P. alpina* recruiting by bulbils. Control plots had a rather low number of seedlings (8 seedlings), seed addition plots ended up with 32 seedlings, irrigated plots with 38. Some seedlings died immediately but even one year after the experiment was terminated survival rates of the seedling cohorts were between 70% (control plots) and 79% (irrigation X seed addition plots). The lessons of these experiments are clear: (1) even in high altitudes drought can play a superior role for colonization, overriding even the factor seed availability; (2) for seedling survival, environmental conditions (including safe sites) but also chance are major drivers; however, the size of the seedling cohort is certainly the essential determinant of species establishment and persistence. High seedling numbers enhance the survival probability per species.

Especially late successional species were regarded as being seed limited: As dispersal from older stages fails, at younger successional stages those species are lacking (Niederfriniger Schlag and Erschbamer 2000; Raffl et al. 2006a; Jones and del Moral 2009) but are able to germinate in such cases when sown (Niederfriniger Schlag and Erschbamer 2000; Erschbamer et al. 2008; Erich Schwienbacher, unpublished data).

Under controlled conditions in the growth chamber, pioneer species germinated better at lower optima of temperature than late successional ones (Schwienbacher et al. 2012). This is an excellent example of species adaptation to the conditions of the mother plant, being probably a drawback with increasing temperatures in the future.

Major threats of germination are frost or heating during germination (Marcante et al. 2012, 2014). At high interspecific variability, pioneer species generally have a lower frost resistance of germinated seeds and seedlings (–3.3, –1.8°C, respectively) compared to mid (–7.3, –3.3°C, respectively) and late successional species (–3.1, –2.6°C, respectively). A similar trend in frost resistance is indicated by adult individuals along the successional gradient (Marcante et al. 2012). Frost events occur mainly at the beginning and the end, and sporadically also during the peak, of the growing season, although being sufficiently severe to kill the whole cohort of seedlings.

High temperatures might not be considered, a priori, as a grave threat in harsh alpine environments; however, advanced instrumentation and recording evidenced critical overheating and functional disturbances also at high altitudes (Neuner and Buchner 2012). At the glacier foreland, the highest temperatures occur at 0–0.5 cm above the soil surface in the absence of structured microsites (Marcante et al. 2014). Glacier foreland seedlings showed heat injuries at temperatures >43.2°C (Marcante et al. 2014). Small stones served as safe sites by reducing the temperatures at the soil surface, enabling survival of the seedlings. Overheating was particularly found during heat waves such as in 2010 when the upper soil strata dried out and as a consequence, transpiration cooling of the seedlings was not warranted anymore. With the ongoing climate warming, the number and frequency of heat waves may

increase (IPCC 2014), so that seedlings might get under risk by combined water shortage and overheating.

3 Development of Populations

A good colonizer should have a high number of seeds, an excellent seedling recruitment, fast growth and high survival rates. *S. aizoides*, a model species for initial colonization, fulfils the first prerequisite, producing a huge number of seeds per fruit (Marcante et al. 2013). The species has also a high number of seedlings at the young moraine stages (Fig. 3). Germination occurs in a rather patchy way. On sites without seedlings, sometimes few large or even very large adult individuals may occur which probably were introduced by avalanches, floods or wind from older colonized areas outside the glacier foreland (Winkler et al. 2015). Such latter colonizations may occur frequently in the case of valley glaciers with steep valley slopes adjacent to glacier forelands. Although seedlings appeared in high numbers at the younger moraines (Fig. 3), only about one half of the cohort (57%) survived at the moraine 2001. At the moraines 1971 and 1923 the percentage of survivors was even less (only 20%). Species with larger seeds such as *A. genipi* or *P. alpina* (270 µg each) had lower mortality rates at the moraine 1971 compared to the small-seeded (50 µg) *S. aizoides* (Marcante et al. 2009b). With increasing size and age of the individuals, mortality decreased. Population development largely depended on persistence of these efficiently established, larger individuals. With exception of *S. aizoides* at the pioneer stage (Winkler et al. 2015), glacier foreland populations are characterized by a highly stable population structure (Weppler et al. 2006; Marcante et al. 2009b). Demographic processes vary with moraine stage, so that pioneer species (*S. aizoides* and *A. genipi*) have progressive population growth rates at the youngest stages and decreasing ones at older stages. Species approaching the glacier tongue such as the late successional species *A. vulneraria* ssp. *alpicola* show progressive population growth rates at young moraines, decreasing towards the oldest one.

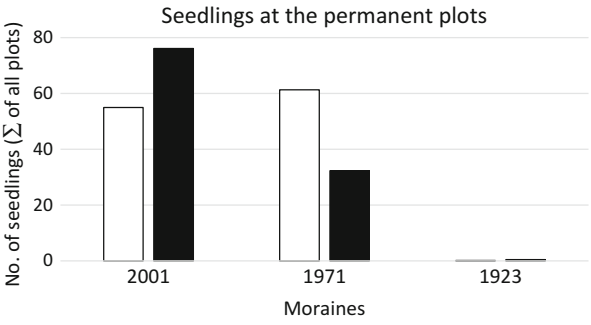

Fig. 3 Number of *Saxifraga aizoides* seedlings at the permanent plots (Σ) of the moraines 2001, 1971 and 1923 at the Rotmoosferner glacier foreland (Tyrol, Austria) in 2007 (*white bars*) and in 2008 (*black bars*). Data: Winkler and Erschbamer (2015)

Table 1 Aboveground biomass production (in mg) of glacier foreland species after 100 days growth in the growth chamber (means and standard deviation)

Successional stage	Species	Biomass (mg) mean ± st. dev
Pioneer stage	*Artemisia genipi*	86.89 ± 19.2
	Cerastium uniflorum	101.72 ± 44.9
	Saxifraga aizoides	5.47 ± 4.7
	Saxifraga oppositifolia	3.71 ± 2.3
Mid successional stage	*Artemisia mutellina*	40.67 ± 24.3
	Poa alpina	290.97 ± 45.0
	Trifolium pallescens	67.03 ± 36.5
Late successional stage	*Anthyllis vulneraria* ssp. *alpicola*	77.91 ± 13.6
	Saxifraga paniculata	10.00 ± 5.5

Data: Niederfriniger Schlag (2001)

Some glacier foreland species are able to overcome the low success of seed germination and establishment by efficient clonal growth strategies. One of the most impressive examples is that of *E. fleischeri*: Shoot runners enable the species to colonize areas which are inhospitable for seed germination such as heavily disturbed alluvial plains. Clonal propagation may be responsible for species persistence along the whole successional gradient (Stöcklin 1990). The double strategy of several glacier foreland species, having sexual and asexual reproduction (*G. reptans, P. alpina* and *P. vivipara*), has important implications for population development (Weppler et al. 2006; Winkler et al. 2010). Especially in unfavourable years, clonal reproduction of *G. reptans* was important for population development whereas sexual reproduction gained in importance during favourable years (Weppler et al. 2006).

Recruitment by seeds vs. clonal growth may be an important trade-off for population development and species persistence. Cichini et al. (2011) compared natural seedling emergence vs. clonal ingrowth from the adjacent colonized areas into artificially created gaps of 10 cm diameter. Within the gaps, the number of seedlings was significantly higher than in the already colonized surrounding areas all along the glacier foreland chronosequence; clonal ingrowth to the gaps from the surrounding vegetation was less important. This was in strong contrast to the several thousand years old grassland outside the glacier foreland, where ingrowth into the gaps was 1.5-fold higher than recruitment by seedlings. By this experiment, again it was shown that a high vegetation cover prevents seed arrival at the soil and seedling emergence. Recruitment and community dynamics evidently change towards the end of the primary succession with increasing vegetation cover.

Individual growth rates of the most important species along the chronosequence of the glacier foreland vary considerably (Table 1). Niederfriniger Schlag (2001) compared the biomass production of selected species over 100 days in the growth chamber, simulating summer day conditions (08:00–12:00, 10°C; 12:00–18:00, 20°C; 18:00–22:00, 15°C; 22:00–08:00, 5°C, 16 hours day/8 hours night, 60–80% relative humidity). The highest growth rates were recorded for *P. alpina,*

the lowest for *S. oppositifolia* (Table 1). Fertilization in the field (equivalent to 40 kg N ha^{-1} a^{-1}) was not tolerated by the pioneer species *S. aizoides*: All treated individuals (seedlings and adult individuals) died after two growing seasons (Niederfriniger Schlag 2001). Among the investigated Fabaceae, *T. pallescens* was significantly positively affected by N-fertilization: Seedlings produced 100-fold more aboveground biomass compared to controls. In contrast, *A. vulneraria* ssp. *alpicola* individuals died one year after fertilization. Obviously, not all N-fixers react and contribute most to the primary succession as suggested by the review of Walker and del Moral (2003). Notwithstanding, the presence of free-living N-fixers (such as cyanobacteria, cyanolichens) associated with biological soil crusts may play an important role for the N supply of the colonizers. However, such latter interactions have hardly been studied in glacier forelands and in alpine environments in general (Zheng et al. 2014).

4 Development of Plant Communities

Process and speed of plant community development largely depend on the factors governing recruitment such as water supply, seed limitation, and the presence of plant species that facilitate other species. Terrain age might be an additional driver: Old moraines should have a lower species turnover, i.e. less invasion by new species but frequency of established species increases, and stabilize with time. To verify this, at the Rotmoosferner glacier foreland, species number and composition changes were analysed in permanent plots in the long term at two differently aged moraines. In 1996, plots of 25×25 cm with vegetation were selected at the 1971 and the 1956 moraine according to the presence of the dominant species *S. oppositifolia* (pioneer species at the 1971 moraine) and *T. pallescens* (mid successional species at the 1956 moraine), respectively. Comparisons were made with bare-ground plots of the same size. At the 1971 moraine, species numbers per vegetated plot increased threefold during a period of 20 years (Erschbamer, unpublished data), from 3.8 ± 0.8 species in 1996 to 11.4 ± 1.7 species in 2015 ($p = 0.012$). Cover did not change significantly during that time, although high variation occurred among the plots and between the years (Fig. 4). At the 1956 moraine, species numbers increased 2.5-fold in the vegetated plots (from 6.33 ± 1.76 per 625 cm^2 in 1996 to 15.67 ± 1.45 in 2015, $p = 0.044$). Cover did not change significantly. In contrast to the initial hypothesis, species turnover was significantly higher on the older moraine with more losses ($p = 0.05$) than on the younger moraine. Some bare-ground plots remained without colonization during the whole period on both the moraines (Fig. 4). Few bare-ground plots of 1956 moraine had a considerable recruitment of up to 12 species but cover remained rather low, varying between 0 and 18.3% per plot (Fig. 4). Permanent plots prove established species to offer safe sites to newcomers (Niederfriniger Schlag and Erschbamer 2000; Erschbamer et al. 2008). Nevertheless, the recruitment at some of the bare-ground plots and the manipulative experiments of Erich Schwienbacher

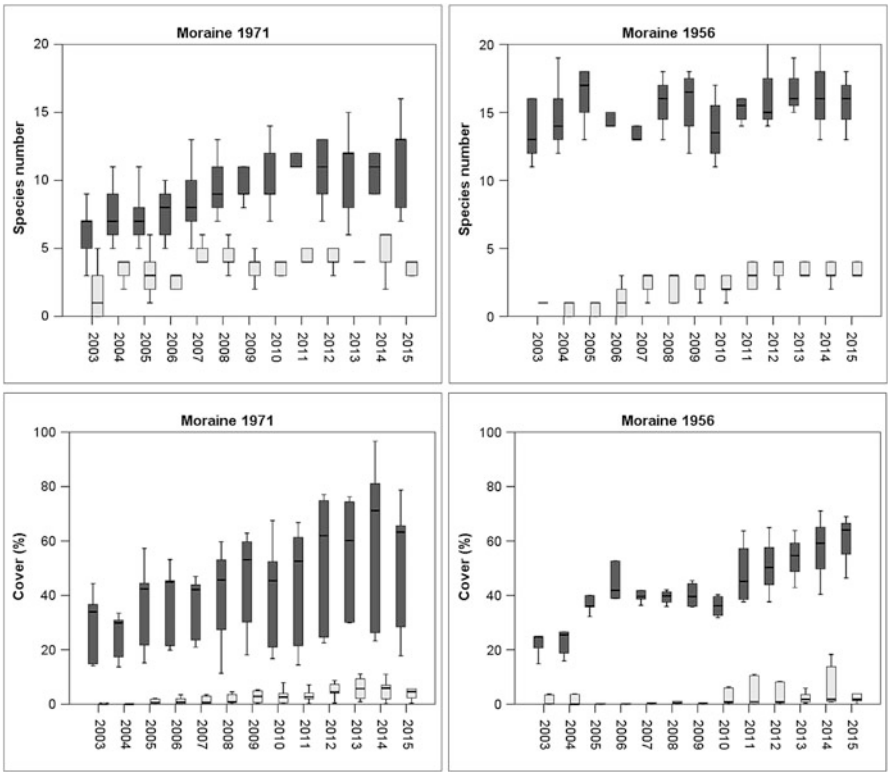

Fig. 4 Species number (*upper graphs*) and cover in % (*lower graphs*) at the permanent plots ($n = 5$) of 25 × 25 cm at the 1971 and 1956 moraines of the Rotmoosferner glacier foreland (Tyrol, Austria). *Dark grey* = plots with vegetation; *light grey* = plots without vegetation at the beginning of the investigation in 1996. Only results from 2003 onwards are shown. In 1996, at the moraine 1971 mean species number of the plots with vegetation was 3.8 ± 0.8 and mean cover amounted to $46.8 \pm 11.4\%$; at the moraine 1956 mean species number was 6.33 ± 1.76 and mean cover $64.1 \pm 10.5\%$. Data: Brigitta Erschbamer, unpublished

simulating safe sites revealed that germination does not necessarily need safe sites provided that seeds arrive at sufficiently moist sites. From a seed addition experiment we learned that facilitation and competition might occur consecutively at the same plots (Erschbamer et al. 2008), as strong competitors might replace formerly dominant species and then serve as facilitators for new seedlings. Walker and del Moral (2003) argued that facilitation might lose importance towards late successional stages as canopy closure might foster competition; however, Callaway and Walker (1997) showed that competition and facilitation may occur simultaneously and the overall prevalence of one process above the other is strongly influenced by the physical context. Specific experiments along the whole primary succession gradient could provide new insights (see Sect. 7).

5 Species Traits

Diversity in the functional traits of individual species is a key factor controlling ecosystem functioning (Diaz and Cabido 2001). A trait-based functional approach can thus provide important information about the mechanisms underpinning the development of primary succession and can give general information beyond the site-specific floristic context. Study of plant communities can focus on the identification of plant functional types (the suite of traits that shape the adaptive strategy of the species) or on the variation in individual functional traits. Both approaches have been tested on primary succession in glacier forelands. Plant functional types have been identified on Rutor glacier foreland following Grime's Competitive–Stress tolerant–Ruderal (CSR) scheme (Caccianiga et al. 2006). CSR theory predicts that the strategies of plant species are an adaptive response to a threeway trade-off in the investment of resources between the ability to compete with neighbouring plants (competitive strategy – C), tolerate limitation to primary production (stress-tolerant strategy – S) or survive disturbance (ruderal strategy – R). Morpho-functional traits of each species can be used to assess its life strategy through identifying coordinates on C, S and R axes. The pioneer community of Rutor glacier forelands hosts species with prevalent R strategy, progressively replaced by stress tolerators, eventually dominating late successional (climax s.l.) communities; the competitive component remains low, with a slight increase during mid successional stages (Fig. 5). The overall R strategies of early successional stages emphasize the role of disturbance on pioneer communities, while highlighting the occurrence of sufficient resources supporting fast-growing species with high leaf nitrogen levels such as *Cerastium uniflorum* Clariv., *O. digyna, Arabis alpina* L. Such pattern is confirmed by the trends of individual functional traits measured by Gobbi et al. (2010) along the Cedec glacier foreland. Here, average specific leaf

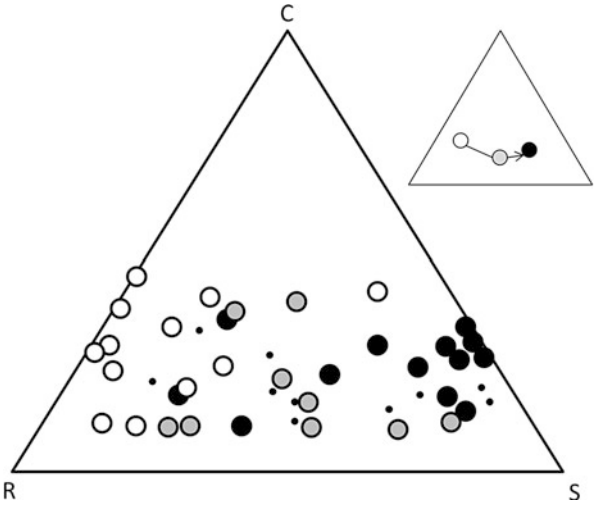

Fig. 5 CSR classification of 45 angiosperms from the foreland of the Rutor glacier, Italy. *Open circles*: early successional species; *filled grey circles*: mid successional species; *filled black circles*: late successional species and points: ubiquitous species. The *smaller triangle* (top right) shows the mean CSR strategy at early- (*open circle*), mid- (*filled grey circle*) and late-succession (*filled black circle*). Redrawn from Caccianiga et al. (2006)

area (SLA) significantly decreases and leaf dry matter content (LMDC) significantly increases during succession. As late successional communities establish, fast-growing species are replaced by such with dense leaves and low relative growth rate, associated with prevalent stress-tolerant strategy.

The shift from the mainly ruderal strategy of pioneer and mid successional stages to the stress-tolerant strategy of late successional communities is achieved through species turnover rather than species plasticity. Ricotta et al. (2015) compared functional and phylogenetic changes along the chronosequence on Rutor glacier foreland and found a significant correlation between functional and phylogenetic dissimilarity between plots, i.e. plots that were different from each other in their mean CSR strategies were also likely to be phylogenetically distinct. This means that communities with similar functional profile tend to be formed by species more related to each other than expected by chance.

6 Phytosociological Descriptions

The description of plant communities on recently deglaciated terrain has been explored since the beginning of the twentieth century (review in Caccianiga et al. 2001 and Raffl and Erschbamer 2004). Species numbers and cover increase until about 50 years after deglaciation and then stabilize (Kaufmann and Raffl 2002; Raffl et al. 2006a). Similarly, invertebrate and microbial biomass development proceeds (Kaufmann 2001, 2002; Kaufmann and Raffl 2002; Tscherko et al. 2003). Beyond 50 years, saturation effects become noticeable (Fig. 6) with negligible increase in species numbers and cover. Lichens contrast in reaching maxima towards the oldest moraine (Kaufmann and Raffl 2002).

A phytosociological description of discrete plant communities of glacier forelands is difficult because of high dynamics, scattered distribution of plant individuals and lack of discontinuities, particularly during early successional stages (Raffl et al. 2006a). The main plant community recognized in recently deglaciated

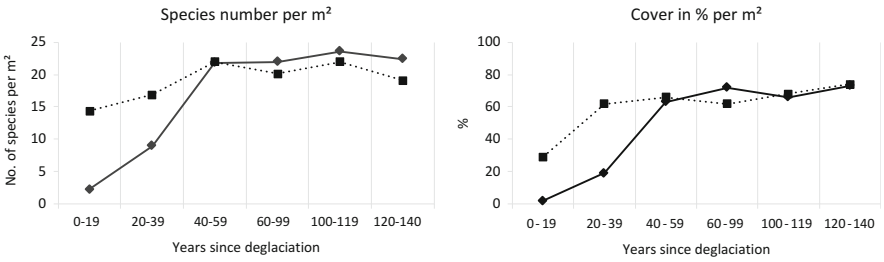

Fig. 6 *Left graph* – the number of plant species per m^2 along the chronosequence (*x*-axis: years since deglaciation) at the Rotmoosferner glacier foreland (Tyrol, Austria); *right graph* – vegetation cover in % per m^2, on the right (*solid lines*) and left (*dotted lines*) valley sides. Redrawn from Raffl et al. (2006a)

forelands on siliceous substrata is the Sieversio-Oxyrietum digynae Friedel 1956 em. Englisch et al. 1993, which includes pioneer communities at high frequency of *O. digyna, G. reptans, Poa laxa* Haenke and *C. uniflorum*. As a function of grain size distribution, water availability, substrate stability, many variants and subassociations have been described covering the variable ecological situations occurring on early successional stages where physical environmental constraints shape the plant community. Such variants include local dominance of cold stenotherm species such as *Androsace alpina* (L.) Lam. and *Ranunculus glacialis* L. at high altitude or at sites with long-lasting snow cover at the outer Alpine chains (subass. androsacetosum alpinae, Caccianiga and Andreis 2004); occurrence of *Cerastium cerastoides* (L.) Britton, *Arenaria biflora* L. or *Sagina saginoides* (L.) Karst. on level sites often soaked with meltwater; occurrence of *E. fleischeri, S. aizoides* together with low altitude species such as *Tussilago farfara* L. on highly disturbed sites particularly in inner continental Alpine sites.

A successional pathway leads from the Sieversio-Oxyrietum digynae to the Luzuletum spadiceae Rübel 1911 through the subassociation *luzuletosum* Jenny-Lips 1930 of the Sieversio-Oxyrietum (Lüdi 1921; Pirola 1959). However, the most significant successional change can be observed on stabilized Holocene moraines (nineteenth to early twentieth century moraines) and consists of the onset of high cover (up to 70%) plant communities, with an important quantitative role of grasses (*P. alpina, Agrostis rupestris* All.), Fabaceae (*T. pallescens* and *Trifolium badium* Schreb.) and other species such as *A. moschata*. These communities have been included into two associations by Caccianiga and Andreis (2004): Agrostio rupestris-Trifolietum pallescentis and Saxifrago bryoidis-Poetum alpinae, the former being linked to level, undisturbed ground and the latter occurring on moraine ridges, with many cushion species such as *Saxifraga bryoides* L. and *S. acaulis* s.l. Such associations are often difficult in view of syntaxonomic attribution, being characterized by the co-occurrence of species of screes and moraines (Thlaspietea rotundifolii) and of acidophilus grasslands (Caricetea curvulae). Becker and Dierschke (2005) published a similar syntaxonomical description for the Obersulzbachkees in the Hohe Tauern, Austria.

Plant communities occurring on calcareous glacier forelands have been much less studied. To our knowledge, no specific association has been described for carbonate glacial deposits. Observations in early successional stages of Gran Zebrù glacier (central Italian Alps, Marco Caccianiga, unpublished) indicate the occurrence of communities close to the typical Papaveretum rhaetici Wikus 1959 occurring on carbonate scree slopes. However, co-occurring are snowbed species (*Arabis coerulea, Salix reticulata* L.) indicating cool and moist microclimate, resembling conditions on carbonate rock glaciers in the same region (Tampucci et al. 2015).

The pioneer communities at the geologically heterogeneous Rotmoosferner glacier foreland on gneiss, mica schists and marble have not been classified syntaxonomically. *S. aizoides* and *S. oppositifolia* predominate the pioneer community whereas *T. pallescens* and *P. alpina* characterize the 40–70-year-old moraines (Raffl et al. 2006a; Nagl and Erschbamer 2010). At the oldest moraine

(glacier stage 1858), a *Kobresia myosuroides* (Vill.) Fiori grassland forms the latest stage of the chronosequence.

Little is known about the syntaxonomical frame of plant communities colonizing glacier forelands occurring below the treeline, which are characterized by the co-occurrence of high altitude taxa and upper montane/subalpine species on older moraines. This is particularly apparent on debris-covered glaciers, which are able to reach low altitudes; Tampucci et al. (unpublished) observed the coexistence of *Cerastium pedunculatum* Gaudin., *P. laxa, Leucanthemopsis alpina* (L.) Heywood with *Alnus alnobetula* (Ehrh.) K. Koch and *Salix appendiculata* Vill. on the moraines of Belvedere glacier (Western Italian Alps) along with overall fast successional progress.

7 Interactions

Interactions among organisms have been widely recognized as a key factor driving ecosystem functioning (Connell and Slatyer 1977; Grime 1979; Bertness and Callaway 1994; Callaway and Walker 1997; Walker and del Moral 2003; Vázquez et al. 2009) but are far from being fully investigated. According to the stress-gradient hypothesis, facilitative interactions should increase with increasing environmental stress (Bertness and Callaway 1994). However, the complexity of plant–plant interactions (Novoplansky 2009), including competition, facilitation and allelopathy, is still nearly a 'black box' in glacier foreland ecology. There is definitely a high need of experimental approaches to clarify the most important interactive processes limiting or favouring plant growth along primary succession.

In many ecosystems plant interactions with mycorrhizae predominate. Early successional stages of glacier forelands may have scarce mycorrhizal fungi available (Jumpponen et al. 2002; Cázares et al. 2005), so that pioneer plant species need to establish and grow without mycorrhization (Erschbamer et al. 2012). However, depending on plant species, for instance, *P. vivipara*, individuals are mycorrhizal, typically since the pioneer stage, as seen at the Rotmoos glacier foreland (Fleisch 2011). Old successional stages such as ca. 150 years ice-free areas can be characterized by high ectomycorrhizal diversity, as shown by Mühlmann and Peintner (2008a, b) for *Salix herbacea* L., *K. myosuroides* and *P. vivipara* (Mühlmann et al. 2008).

On glacier forelands, few studies on plant–animal interactions have been focused on pollination ecology and direct trophic relationships. Pollination was studied in *G. reptans* (Pluess and Stöcklin 2004), showing that the pollinator spectrum was mainly composed by flies (94%), followed by syrphids (4.6%) and bumblebees (1.4%). Pollen were dispersed within short reach between 4 cm and 11.5 m with '*a single rare dispersal event over 30 m*' (Pluess and Stöcklin 2004). Taking in account the harsh conditions of recently deglaciated sites, the early colonizers may suffer from pollinator limitation. Thus, self-pollination or apomixes might be more important in early compared to late successional stages. In the

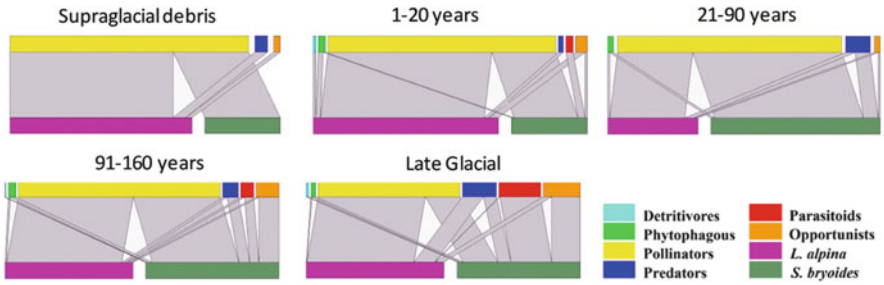

Fig. 7 Plant–anthophilous insect bipartite network along the primary succession of Amola glacier foreland, Italy, from supraglacial debris to late glacial substrata not covered by the glacier during the Holocene. Investigated plant species and insect functional groups are represented by *rectangles* with their size proportional to the relative number of visits received and made, respectively. Redrawn from Losapio et al. (2015)

Arctic, several species use a mixed mating strategy, being self-pollinated as well as outcrossing in order to reduce pollen limitation (Rydin and Borgegård 1991). Such a flexible strategy was also found for the pioneer species *S. aizoides* (Raffl et al. 2007). Nevertheless open-pollinated flowers of *S. aizoides* exhibited a higher seed number per capsule than self-pollinated flowers (Raffl et al. 2007). Similarly behaved *S. bryoides* on the Amola glacier foreland, whereas *L. alpina* turned out as self-incompatible (Losapio et al., unpublished).

Flower-visiting insects do not only consist of pollinators, but include a wide spectrum of trophic roles, including parasitoids, predators and opportunists, forming a complex network of plant–insect interactions (Fig. 7). The development of such network along the primary succession represents an example of the building of an increasingly complex community with time proceeding. On the Amola glacier foreland anthophilous insect communities were investigated along the primary succession from debris-covered glacier surface to late successional grasslands on two ubiquitous plant species, *L. alpina* and *S. bryoides* (Losapio et al. 2015). The *L. alpina* anthophilous insect community was dominated by pollinators (84%), followed by predators (9%), opportunists (5%), phytophagous and detritivores (both 1%). The *S. bryoides* insect community was composed of pollinators (59%), parasitoids (15%), opportunists (14%), predators (8%), phytophagous (3%) and detritivores (1%). Pollinators dominated the insect community in the early successional stages, while the trophic spectrum diversified throughout the succession, particularly on *S. bryoides*. From pioneer to late successional communities plant–anthophilous insect interactions changed from a network dominated by pollinators to a functionally more diversified food web; along the succession the network architecture shifts from a star-like network, where few central nodes have many connections, to a lattice-like network with no central nodes (Fig. 7, Losapio et al. 2015). The study of the development of interactions among different organisms throughout the succession could give important insight about community

robustness against local species extinction, which could have important consequences on scattered communities like those of glacier forelands.

A comparison between species assemblages of plants and trophically independent arthropods (carabid beetles) along a glacier foreland has been performed on Cedec glacier by Gobbi et al. (2010). Carabid beetles were found to be linked to quantitative parameters of plant community (i.e. plant cover and richness) rather than to its functional profile; however, both plants and carabids showed a variation in functional traits along the primary succession following a gradient both in resource availability and disturbance.

Kaufmann (2001) noticed that at recently deglaciated moraines of the Rotmoosferner glacier foreland where plant species are still absent a high number of predatory invertebrates such as carabid species, linyphiid spiders and wolf spiders prevailed. He suggested aeolian input as major predator diet. A comprehensive molecular study of the gut content revealed that the main food sources of the predators were Collembola (Raso et al. 2014). Additionally, intraguild predation was important throughout the young moraines (0–7 years ice-free and 12–20 years ice-free, respectively, Raso et al. 2014).

Interactions between plant species and herbivores are completely unexplored in Alpine glacier forelands. Few studies are available on the effects of seed predation along primary successions. Jones and del Moral (2009) found that large-seeded species such as those of the late successional species *Abies, Ribes* and *Rubus* were establishment-limited due to seed predation at the Coleman glacier foreland, USA. Weppler and Stöcklin (2006) analysed seed predation of the relatively large-seeded *G. reptans* and modelled the effects on population growth rates. Total seed mass per flower head and germination of seeds were heavily affected but all in all population growth of the species was hardly altered. Obviously, the species counteracted seed losses by clonal growth and persistence (Weppler and Stöcklin 2006). For small-seeded species seed predation seems to be negligible (Jones and del Moral 2009).

The review shows that all kinds of interactions exist in the glacier foreland and were partly studied. But further experiments are urgently required to remedy shortcomings and to understand underlying processes.

8 Climate Warming and Primary Succession

The glacier retreat in the Alps occurring since about 150 years – with some advances in between – (Fischer and Hartl 2013) is one of the striking and most visible signs of climate change in the Alps (IPCC 2014). Glacier shrinkage opens abundantly new areas for colonization. But climate change enhances also the speed of colonization: Cannone et al. (2008) found that new colonization started already one year after glacier retreat at the Sforzellina glacier foreland (Italian Alps) with up to 8 vascular plant species and mosses. At the Rotmoosferner glacier foreland the first colonizing vascular plant species arrive 3 years after deglaciation (Raffl et al. 2006a, Andrea Danler, unpublished). The increase of colonization could be

due to the direct effect of climate or to the increased availability of propagules from the nearby areas like the already colonized glacial deposits, or to a combination of the two.

Inauen et al. (2012) hypothesized that the speed of colonization on glacier forelands is enhanced by elevated atmospheric CO_2. Especially the early colonizers should be sensitive to elevated CO_2 concentrations. However, their microcosm experiment with 9 species showed no effects on total biomass production and the authors concluded that glacier foreland pioneers are not carbon limited. This is in line with numerous grassland experiments performed in high altitudes, showing that elevated CO_2 per se had no effects on species growth (review in Körner 2000, 2006) unless it was coupled with nutrient or water availability.

Experiments with open top chambers simulating +1.5°C temperature increase (= the minimum scenario of temperature enhancement till 2100, IPCC 2014) exhibited a significant increase in biomass of some glacier foreland species such as for the mid successional species *T. pallescens* or the late successional species *A. vulneraria* ssp. *alpicola* (Erschbamer 2007): Number of leaves, flowers and seeds significantly increased by warming. However, other species such as the pioneer species *A. genipi* did not change significantly or even decreased under warming such as the pseudoviviparous *P. alpina*. Early seasonal snow melt might extend the growing season but not all species might profit. Frost, drought and heat threats might also increase and the early germinating seedlings could be endangered. Thus, the ongoing climate change has to be evaluated as a complex impact on glacier foreland dynamics.

The occurrence of glacier forelands may enhance the survival of cold stenotherm species threatened by the uplift of altitudinal belts due to climate warming (Gottfried et al. 2012; Pauli et al. 2012) thanks to their microclimatic peculiarities and to their contribution to environmental heterogeneity. Glacial landforms may thus act, or have acted, as microrefugia for alpine plants during warm climatic periods (Gentili et al. 2015). This phenomenon is part of a wider role played by many geomorphological processes and landforms in increasing habitat diversity and species survival in Alpine ecosystems. Such role has been acknowledged for other glacial landforms such as debris-covered glaciers (Caccianiga et al. 2011) where cold stenotherm species can reach unusually low altitudes due to cold microclimate, and for periglacial landforms such as rock glaciers (Gobbi et al. 2014; Tampucci et al. 2015), also characterized by peculiar microclimatic features.

9 Conclusions

The points summarized in the present review emphasize that, despite marked local variability, general patterns can be depicted concerning the complex phenomenon of primary succession on glacier forelands. Lessons from glacier foreland colonization can be applied to dynamics and primary successions on artificially created

habitats, on barren areas created by catastrophes and also for predictions of climate change effects in high altitudes.

Genetic diversity is not influenced by primary succession: No founder effects and no genetic depletion were found along the chronosequence. A high number of seeds produced by pioneer species warrant gene flow over large distances.

Small seeds are a 'fundamental prerequisite' of the colonizers (Stöcklin 1990; Chapin et al. 1994; Stöcklin and Bäumler 1996; Schwienbacher et al. 2012). Larger seeds compensate the higher weight by appendages favouring wind dispersal. Species without specific dispersal structures on the seeds are dispersal limited and lack therefore on the youngest moraines. Thus, dispersal is a major driver governing colonization (Walker and del Moral 2003).

Seed dispersal is mainly of local importance; however, a low quantity of seeds may be dispersed over long distances, i.e. seed exchange from and to the glacier foreland, the latter being even more important for recent gene flow and primary succession.

Flexibility of reproduction (i.e. clonal + sexual, mixed mating) together with longevity of seeds are essential traits of successful colonizers.

Seed and establishment limitation govern population development (Chapin et al. 1994; Niederfriniger Schlag and Erschbamer 2000; Erschbamer et al. 2008; Jones and del Moral 2009). Late successional species are precluded from young moraines because they are clearly dispersal limited but they can germinate if they are sown there (Erschbamer et al. 2008).

Vegetation facilitates germination, establishment and persistence of species. Plots without vegetation are safe-site limited, seed limited and/or too dry for germination and successful recruitment: Species may appear for a short time but the overall colonization ability remains poor, the cover and diversity increase being a very slow process. Thus, large seedling cohorts of the single species are necessary to guarantee establishment and persistence.

Frost might be a problem for seedling cohorts early and late in the growing season. The heat tolerance of seedlings is not sufficient to survive summer heat waves.

A seed bank has to be built from zero onwards. Along the whole glacier foreland the seed bank composition is driven by the dominant aboveground species. Outside the glacier foreland, seed bank and standing vegetation are decoupled.

Recurrent functional patterns complete the ecological profile of the colonizers: fast growth and efficient photosynthesis testified by high SLA and high leaf N levels characterize species of early successional stages; such strategy is sustained by relatively favourable environmental conditions experienced during growing season even at high altitude (see Körner 1999, 2003). Such overall profile promotes facilitation, in contrast with the conservative/retentive strategy of late successional stages (Grime and Pierce 2012).

Changes in species composition occur extremely slowly. The development of the community involves an increase of interactions among plant and animal species with the onset of a more and more diversified network which in turn promotes ecosystem persistence and resilience.

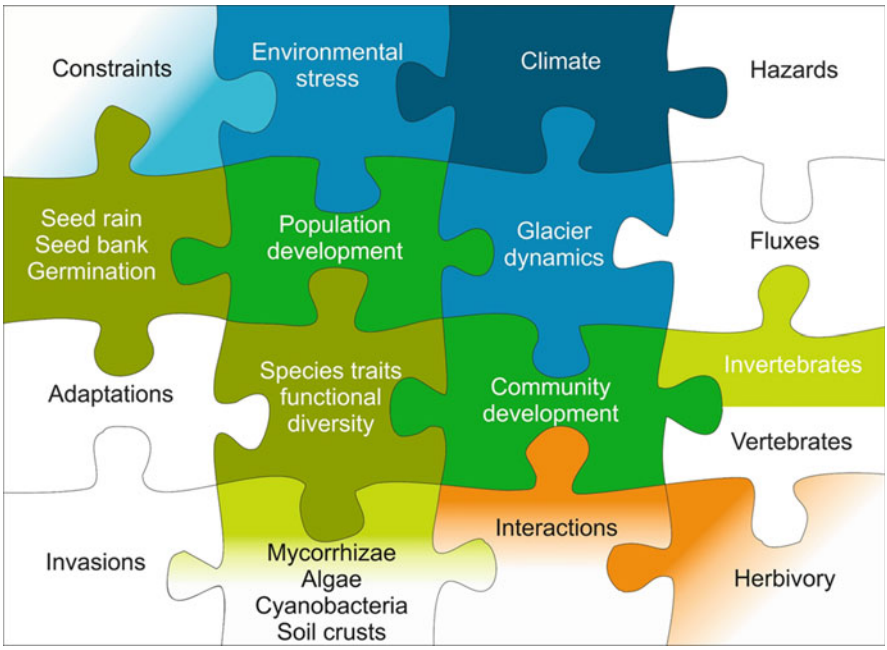

Fig. 8 Puzzle of glacier foreland research showing present knowledge (*coloured puzzle stones*) and research fields still to be explored (*white* or *partially coloured puzzle stones*). Drawings by Chiara Compostella

Climate warming may enhance the speed of colonization and change the interaction network. However, glacier forelands may also provide refugia for alpine–nival species which become increasingly threatened by the upward migration of species from lower altitudes, at least as long as glaciers will persist in the warmer mountain environment.

Primary successions in high altitude ecosystems emerge as a highly complex phenomenon, whose underlying mechanisms are still to be fully understood. Taxonomical and functional properties of ecosystems as well as interactions among organisms are expected to change throughout time. The evolution of increasingly complex communities along the primary succession, together with the differential range shifts of individual species enhanced by climate warming (Alexander et al. 2015), represents one of the most striking changes occurring on high altitude ecosystems.

Although several lessons were highlighted, glacier foreland still offers ample opportunities for ecological questions, innovative experiments and groundbreaking theories. We were able to outline particularities of population and community development, vital rates governing population growth such as seed rain and seed bank as well as species traits and functional diversity in glacier forelands (Fig. 8). Ecosystem drivers such as fluxes, the complexity of interactions or species

adaptations to changes were hardly treated. We have to admit also that not even all organismic groups were studied in glacier forelands (Fig. 8). Research is highly recommended for all these topics lacking specific insights.

References

Abermann J, Lambrecht A, Fischer A, Kuhn M (2009) Quantifying changes and trends in glacier area and volume in the Austrian Ötztal Alps (1969–1997–2006). Cryosphere 3(2):205–215

Abermann J, Kuhn M, Lambrecht A, Hartl L (2013) Gletscher in Tirol, ihre Verteilung und jüngsten Veränderungen. In: Koch EM, Erschbamer B (eds) Klima, Wetter, Gletscher im Wandel. Alpine Forschungsstelle Obergurgl 3. Innsbruck University Press, Innsbruck, pp 49–67

Alexander JM, Diez JM, Levine HM (2015) Novel competitors shape species' responses to climate change. Nature 525. doi:10.1038/nature14952

Becker T, Dierschke H (2005) Primärsukzession im Gletschervorfeld des Obersulzbachkees (Hohe Tauern, Österreich), eine Zeitreihe über fast 150 Jahre. Tuexenia 25:111–139

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends Ecol Evol 9:191–193

Burga CA, Krüsi B, Egli M, Wernli M, Elsener S, Ziefle M, Fischer T, Mavris C (2010) Plant succession and soil development on the foreland of the Morteratsch glacier (Pontresina, Switzerland): straight forward or chaotic? Flora 205:561–576

Caccianiga M, Andreis C (2004) Pioneer herbaceous vegetation on glacier forelands from the Italian Alps. Phytocoenologia 34(1):55–89

Caccianiga M, Andreis C, Cerabolini B (2001) Vegetation and environmental factors during primary succession on glacier forelands: some outlines from the Italian Alps. Plant Biosyst 135:295–310

Caccianiga M, Luzzaro A, Pierce S, Cerabolini B, Ceriani RM (2006) The functional basis of a primary succession resolved by CSR classification. Oikos 112:10–20

Caccianiga M, Andreis C, Diolaiuti G, D'Agata C, Mihalcea C, Smiraglia C (2011) Debris-covered glaciers as a habitat for plant life. Holocene 21(6):1011–1023

Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958–1965

Cannone N, Diolaiuti G, Guglielmin M, Smiraglia C (2008) Accelerating climate change impacts on alpine glacier forefield ecosystems in the European Alps. Ecol Appl 18:637–648

Cázares E, Trappe JM, Jumpponen A (2005) Mycorrhiza-plant colonization patterns on a subalpine glacier forefront as a model system of primary succession. Mycorrhiza 15:405–416

Chapin FS III (1993) Physiological controls over plant establishment in primary succession. In: Miles J, Walton DWH (eds) Primary succession on land. Blackwell Science Publishing, Oxford, pp 161–178

Chapin FS III, Walker LR, Fastie CL, Sharman LC (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecol Monogr 64:149–175

Cichini K, Schwienbacher E, Marcante S, Seeber GUH, Erschbamer B (2011) Colonization of experimentally created gaps along an alpine successional gradient. Plant Ecol 212:1613–1627

Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. Am Nat 111:1119–1144

Crouch HJ (1993) Plant distribution patterns and primary succession on a glacier foreland: a comparative study of cryptogams and higher plants. In: Miles J, Walton DWH (eds) Primary succession on land. Blackwell Science Publishing, Oxford, pp 133–145

Diaz S, Cabido M (2001) Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 11:646–655

Erschbamer B (2007) Winners and losers of climate change in a central alpine glacier foreland. Arct Antarct Alp Res 39:237–244
Erschbamer B, Mayer R (2011) Can successional species groups be discriminated based on their life history traits? A study from a glacier foreland in the Central Alps. Plant Ecol Div 4:341–351
Erschbamer B, Kneringer E, Niederfriniger Schlag R (2001) Seed rain, soil seed bank, seedling recruitment, and survival of seedlings on a glacier foreland in the Central Alps. Flora 196:304–312
Erschbamer B, Niederfriniger Schlag R, Winkler E (2008) Colonization processes on a central Alpine glacier foreland. J Veg Sci 19:855–862
Erschbamer B, Bösch D, Fleisch M, Peintner U, Schwienbacher E (2012) Keimung und Etablierung im Gletschervorfeld in Abhängigkeit von abiotischen und biotischen Faktoren. Ber d Reinh-Tüxen-Ges 24:89–102
Fastie CL (1995) Causes and ecosystem consequences of multiple pathways on primary succession on Glacier Bay, Alaska. Ecology 76:1899–1916
Fenner M, Thompson K (2005) The ecology of seeds. Cambridge Universisty Press, Cambridge, p 250
Finch KR (2008) Diasporenregen im Gletschervorfeld des Rotmoosferners, Obergurgl, Ötztal. Diplomarbeit Univ, Innsbuck, 82 pp
Fischer A, Hartl L (2013) Langzeitmonitoring von Gletschermassenbilanzen und –längenänderungen in Tirol. In: Koch EM, Erschbamer B (eds) Klima, Wetter, Gletscher im Wandel. Alpine Forschungsstelle Obergurgl 3. Innsbruck University Press, Innsbruck, pp 31–48
Fleisch M (2011) Mykorrhizastatus von Pionierpflanzen und viviparen Pflanzen aus Primärsukzessionsstandorten des Rotmoosferner Gletschervorfeldes. Diplomarbeit Univ, Innsbruck, 46 pp
Fløø D, Hågvar S (2013) Aerial dispersal of invertebrates and mosses close to a receding alpine glacier in southern Norway. Arct Antarct Alp Res 45:481–490
Foster BL, Tilman D (2000) Dynamic and static views of succession: testing the descriptive power of the chronosequence approach. Plant Ecol 146:1–10
Funes G, Basconcelo S, Díaz S, Cabido M (1999) Seed size and shape are good predictors of seed persistence in soil in temperate mountain grasslands of Argentina. Seed Sci Res 9:341–345
Gentili R, Baroni C, Caccianiga M, Armiraglio S, Ghiani A, Citterio S (2015) Warm-stage microrefugia for alpine plants: feedback between geomorphological and biological processes. Ecol Complexity 21:87–99
Giles BE, Goudet J (1997) Genetic differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. Am Nat 149:507–526
Gobbi M, Caccianiga M, Cerabolini B, Luzzaro A, Pierce S, De Bernardi F (2010) Plant adaptive responses during primary succession are associated with functional adaptations in ground beetles on deglaciated terrain. Community Ecol 11(2):223–231
Gobbi M, Ballarin F, Compostella C, Lencioni V, Seppi R, Tampucci D, Caccianiga M (2014) Physical and biological features of an active rock glacier of the Italian Alps. Holocene 24 (11):1624–1631
Gottfried M, Pauli H, Futschik A, Akhaltkatsi M, Barančok P, Benito Alonso JL, Coldea G, Dick J, Erschbamer B, Fernández Calzado MR, Kazakis G, Krajči J, Larsson P, Mallaun M, Michelsen O, Moissev D, Moissev P, Molau U, Merzouki A, Nagy L, Nakhutsrishvili G, Pedersen B, Pelino G, Puscas M, Rossi G, Stanisci A, Theurillat J-P, Tomaselli M, Villar L, Vittoz P, Vogiatzakis I, Grabherr G (2012) Continent-wide response of mountain vegetation to climate change. Nat Climate Change 2:111–115
Grime JP (1979) Plant strategies and vegetation processes. Wiley, Chichester, 222 pp
Grime JP, Pierce S (2012) The evolutionary strategies that shape ecosystems., Wiley-Blackwell, 244 pp

Inauen N, Körner C, Hiltbrunner E (2012) No growth stimulation by CO_2 enrichment in alpine glacier forefield plants. Glob Change Biol 18:985–999
IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Pachauri R, Meyer L (eds) Geneva, 151 pp
Jochimsen M (1970) Die Vegetationsentwicklung auf Moränenboden in Abhängigkeit von einigen Umweltfaktoren. Alpin-Biol. Stud., Veröff. Univ. Innsbruck 46
Jones CC, del Moral (2009) Dispersal and establishment both limit colonization during primary succession on a glacier foreland. Plant Ecol 204:217–230
Jumpponen A, Trappe JM, Cázares E (2002) Occurrence of ectomycorrhizal fungi on the forefront of retreating Lyman Glacier (Washington, USA) in relation to time since deglaciation. Mycorrhiza 12:43–49
Kaufmann R (2001) Invertebrate succession on an alpine glacier foreland. Ecology 82:2261–2278
Kaufmann R (2002) Glacier foreland colonisation: distinguishing between short-term and long-term effects of climate change. Oecologia 130:470–475
Kaufmann R, Raffl C (2002) Diversity in primary succession: the chronosequence of a glacier foreland. In: Körner C, Spehn EM (eds) Mountain biodiversity. A global assessment. The Parthenon Publishing Group, London, pp 177–190
Körner C (1999) Alpine plants: stressed or adapted? In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell Scientific Publications, pp 297–311
Körner C (2000) Biosphere responses to CO_2 enrichment. Ecol Appl 10:1590–1619
Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer
Körner C (2006) Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol 172:393–411
Leck MA, Parker VT, Simpson RL (2008) Seedling ecology and evolution. Cambridge University Press, Cambridge
Losapio G, Jordan F, Caccianiga M, Gobbi M (2015) Structure-dynamic relationship of plant–insect networks along a primary succession gradient on a glacier foreland. Ecol Mod 314:73–79
Lüdi W (1921) Die Pflanzengesellschaften des Lauterbrunnentales und ihre Sukzession. Beitr. Geobot. Landesaufn. Schweiz, Zürich 9:1–364
Marcante S, Schwienbacher E, Erschbamer B (2009a) Genesis of a soil seed bank on a primary succession in the Central Alps (Ötztal, Austria). Flora 204:434–444
Marcante S, Winkler E, Erschbamer B (2009b) Population dynamics along a primary succession gradient: do alpine species fit into demographic succession theory? Ann Bot 103:1129–1143
Marcante S, Sierra-Almeida A, Spindelböck JP, Erschbamer B, Neuner G (2012) Frost as a limiting factor for recruitment and establishment of early development stages in an alpine glacier foreland. J Veg Sci 23:858–868
Marcante S, Kiebacher T, Erschbamer B (2013) Reproductive responses of glacier foreland species to simulated climate change. Coll Phytosociol XXIX:361–373
Marcante S, Erschbamer B, Buchner O, Neuner G (2014) Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field. Plant Ecol 215:747–758
Matthews JA (1992) The ecology of recently-deglaciated terrain. A geoecological approach to glacier forelands and primary succession. Cambridge University Press, Cambridge, 386 pp
Matthews JA, Whittaker RJ (1987) Vegetation succession on the Storbreen glacier foreland, Jotunheimen, Norway: a review. Arct Alp Res 19:385–395
Mühlmann O, Peintner U (2008a) Mycobionts of *Salix herbacea* on a glacier forefront in the Austrian Alps. Mycorrhiza 18:171–180
Mühlmann O, Peintner U (2008b) Ectomycorrhiza of *Kobresia myosuroides* at a primary successional Glacier forefront. Mycorrhiza 18:355–362
Mühlmann O, Bacher M, Peintner U (2008) *Polygonum viviparum* mycobionts on an alpine primary successional glacier forefront. Mycorrhiza 18:87–95

Nagl F, Erschbamer B (2010) Vegetation und Besiedlungsstrategien. In: Koch EM, Erschbamer B (eds) Glaziale und periglaziale Lebensräume im Raum Obergurgl. Alpine Forschungsstelle Obergurgl 1. Innsbruck University Press, Innsbruck, pp 121–143

Neuner G, Buchner O (2012) Dynamics of tissue heat tolerance and thermotolerance of PS II in alpine plants. In: Lütz C (ed) Plants in alpine regions. Cell physiology of adaption and survival strategies. Springer, New York, pp 61–74

Nicolussi K (2009) Alpine Dendrochronologie – Untersuchungen zur Kenntnis der holozänen Umwelt- und Klimaentwicklung. In: Schmidt R, Matulla C, Psenner R (eds) Klimawandel in Österreich. Alpine space – man & environment. Innsbruck University Press, Innsbruck, pp 41–54

Niederfriniger Schlag R (2001) Primärsukzession im Gletschervorfeld. Keimung, Etablierung, Wachstum und Interaktionen im Gletschervorfeld des Rotmoosferners (Ötztal, Tirol). Dissertation, University Innsbruck, 111 pp

Niederfriniger Schlag R, Erschbamer B (2000) Germination and establishment of seedlings on a glacier foreland in the Central Alps, Austria. Arct Antarct Alp Res 32:270–277

Novoplansky A (2009) Picking battles wisely: plant behaviour under competition. Plant Cell Environ 32:726–741

Pauli H, Gottfried M, Dullinger S, Abdaladze O, Akhaltkatsi M, Benito Alonso JL, Coldea G, Dick J, Erschbamer B, Fernández Calzado MR, Ghosn D, Holten J, Kanka R, Kazakis G, Kollár J, Larsson P, Moiseev D, Moiseev P, Molau U, Molero Mesa J, Nagy L, Pelino G, Puscas M, Rossi G, Stanisci A, Syverhuset AO, Theurillat J-P, Tomaselli M, Unterluggauer P, Villar L, Vittoz P, Grabherr G (2012) Recent plant diversity changes on Europe's mountain summits. Science 336:353–355

Pirola A (1959) Flora e vegetazione periglaciale sul versante meridionale del Bernina. Flora et Vegetatio Italica, mem. 1, Gianasso ed., Sondrio, 114 pp

Pluess AR (2011) Pursuing glacier retreat: genetic structure of a rapidly expanding *Larix decidua* population. Mol Ecol 20:473–485

Pluess AR, Stöcklin J (2004) Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. Am J Bot 91:2013–2021

Raffl C, Erschbamer B (2004) Comparative vegetation analyses of two transects crossing a characteristic glacier valley in the Central Alps. Phytocoenologia 34:225–240

Raffl C, Mallaun M, Mayer R, Erschbamer B (2006a) Vegetation succession pattern and diversity changes in a glacier valley, Central Alps, Austria. Arct Antarct Alpi Res 38:421–428

Raffl C, Schönswetter P, Erschbamer B (2006b) 'Sax-sess' – genetics of primary succession in a pioneer species on two parallel glacier forelands. Mol Ecol 15:2433–2440

Raffl C, Marcante S, Erschbamer B (2007) The role of spontaneous selfing in the pioneer species *Saxifraga aizoides*. Flora 202:128–132

Raffl C, Holderegger R, Parson W, Erschbamer B (2008) Patterns in genetic diversity of *Trifolium pallescens* populations do not reflect chronosequence on alpine glacier forelands. Heredity 100:526–532

Raso L, Sint D, Mayer R, Plangg S, Recheis T, Brunner S, Kaufmann R, Traugott M (2014) Intraguild predation in pioneer predator communities of alpine glacier forelands. Mol Ecol 23:3744–3754

Ricotta C, Bacaro G, Caccianiga M, Cerabolini B, Moretti M (2015) A classical measure of phylogenetic dissimilarity and its relationship with beta diversity. Basic Appl Ecol 16:10–18

Rydin H, Borgegård S-O (1991) Plant characteristics over a century of primary succession on islands: Lake Hjälmaren. Ecology 72:1089–1101

Schwienbacher E, Marcante S, Erschbamer B (2010) Alpine species seed longevity in the soil in relation to seed size and shape – a 5-year burial experiment in the Central Alps. Flora 205:19–25

Schwienbacher E, Navarro-Cano JA, Neuner G, Erschbamer B (2011) Seed dormancy in alpine species. Flora 206:845–856

Schwienbacher E, Navarro-Cano JA, Neuner G, Erschbamer B (2012) Correspondence of seed traits with niche position in glacier foreland succession. Plant Ecol 213:371–382

Stöcklin J (1990) Populationsstruktur des Rohbodenpioniers *Epilobium fleischeri* Hochst. (Onagraceae) auf dem Morteratsch-Gletschervorfeld. Verh Ges Ökol XIX/II:30–43

Stöcklin J, Bäumler E (1996) Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. J Veg Sci 7:45–56

Stöcklin J, Kuss P, Pluess AR (2009) Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with alpine plant species. Bot Helv 119:125–133

Tampucci D, Boffa G, Mangili F, Gobbi M, Caccianiga M (2015) Vegetation outlines of two active rock glaciers with contrasting lithology. Plant Sociol 52(1):9–18

Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. Funct Ecol 7:236–241

Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E (2003) Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. Eur J Soil Sci 54:685–696

Vázquez DP, Blüthgen N, Cagnolo L, Chacoff NP (2009) Uniting pattern and process in plant-animal mutualistic networks: a review. Ann Bot 103:1445–1457

Vetaas OR (1997) Relationship between floristic gradients in a primary succession. J Veg Sci 8:665–676

Walker LR, del Moral R (2003) Primary succession and ecosystem rehabilitation. Cambridge University Press, Cambridge, 442 pp

Weppler T, Stöcklin J (2006) Does pre-dispersal seed predation limit reproduction and population growth in the alpine clonal plant Geum reptans? Plant Ecol 187:277–287

Weppler T, Stoll P, Stöcklin J (2006) The relative importance of sexual and clonal reproduction for population growth in the long-lived alpine plant *Geum reptans*. J Ecol 94:869–879

Winkler E, Marcante S, Erschbamer B (2015) Demography of the alpine pioneer species *Saxifraga aizoides* in different successional stages at the glacier foreland of the Rotmoosferner (Obergurgl, Ötztal, Austria). Tuexenia 35:267–283

Winkler E, Marcante S, Erschbamer B (2010) Demographic consequences of the two reproductive modes in *Poa alpina* L. along a primary succession gradient in the Central Alps. Arct Antarct Alp Res 42:227–235

Zheng L-J, Maier S, Grube M, Türk R, Gruber JP, Peer T (2014) Alpine biological soil crusts on the Hochtor (Grossglockner high alpine route, Hohe Tauern, Austria): soils function and biodiversity. Acta ZooBot Austria 150(151):175–196

The "Forgotten" Ecology Behind Ecological Status Evaluation: Re-Assessing the Roles of Aquatic Plants and Benthic Algae in Ecosystem Functioning

Susanne C. Schneider, Sabine Hilt, Jan E. Vermaat, and Martyn Kelly

Contents

Abstract Aquatic plants and benthic algae have long been used as indicators for nutrient enrichment in lakes and streams. Evaluations of the performance of indices calculated from species assemblages of aquatic plants and algae are generally based on correlations with water nutrient concentrations. We argue that this is a misinterpretation, because water chemistry is both cause and effect: higher nutrient concentrations may cause enhanced plant and algal growth and change their assemblages, but plants and benthic algae also remove nutrients from the water. Additionally, biotic interactions blur water chemistry – aquatic plant relationships. We suggest that indices can be improved by relating biotic responses to quantifiable

S.C. Schneider (✉)
Norwegian Institute for Water Research, Gaustadalleen 21, 0349 Oslo, Norway
e-mail: susi.schneider@niva.no

S. Hilt
Leibniz Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany

J.E. Vermaat
Institute of Environmental Sciences, Norwegian University of Life Sciences, 1430 Ås, Norway

M. Kelly
Bowburn Consultancy, 11 Monteigne Drive, Bowburn, Durham DH6 5QB, UK

Progress in Botany (2017) 78: 285–304, DOI 10.1007/124_2016_7,
Published online: 28 May 2016

causal stressors, such as nutrient loading, instead of using water chemistry for performance evaluation of the indices. In addition, a tiered approach, i.e., the use of simpler indices for getting an overview and of sophisticated methods in doubtful cases, could avoid unnecessary costs and efforts while giving important monitoring and management information.

1 Introduction

Clean waters ensure the provision of safe drinking water, protect human health, support economic and recreational activities, and provide healthy habitats for flora and fauna. Regular monitoring of the condition of water is, therefore, a prerequisite to its safe and sustainable use. Proponents of ecological assessment of rivers and lakes argue that this complements chemical monitoring because the biota provide a longer-term insight into prevailing conditions than chemical measurements, and because living elements may respond to all stressors within an ecosystem (Karr 1999). Aquatic ecologists have long been aware that macrophytes and benthic algae are affected by, but also shape their chemical, physical, and hydrological environment (Butcher 1933). Aquatic plants are considered useful as indicators for what has been termed "ecological status" in Europe (EC 2000) and "ecosystem health" or "biotic integrity" elsewhere (Karr 1991, 1999). In Europe, the Water Framework Directive (WFD; EC 2000) has boosted the interest of scientists and water managers in aquatic plants and algae, because phytoplankton and phytobenthos are mandatory elements in status assessment of rivers and lakes, along with benthic invertebrates and fish. The WFD was welcomed by many for putting aquatic ecology, rather than chemistry alone, at the base of management decisions (Hering et al. 2010). But are we using the biological indicators well? Do present-day biological indicators perform better than their predecessors, some of which were developed more than 100 years ago? Does the way we use water plants and algae as indicators for the WFD constitute a "Progress in Botany"? In this review, we argue that a large part of the potential information aquatic plants could provide is ignored. This is due mainly to the lack of well-defined cause-effect-relationships. In addition, the imprecise use of the term "eutrophication," which has continued over the last century, and the unfortunate use of "hydrochemistry-response" correlations for performance evaluation of biological indicators have introduced considerable confusion.

In order to explain our reasoning, we briefly review the history of ecological assessment in freshwater, and point to the underlying ecological interactions, which appear to have been "forgotten" in the use of benthic floral indicators for the WFD. We then argue that an index that is based on well-defined stressor-response

relationships indeed would have the potential to become a tool that is useful for overall status assessment, for identifying and quantifying stressors that likely are responsible for the deterioration of a water body and for planning suitable management measures to improve ecosystem health. In this review we focus on the benthic flora in rivers, though many of our examples are from lakes (when no data exist from rivers), and many of our arguments are also valid for other organism groups (= "biological quality elements" in the terminology of the WFD).

2 A Short History of Ecological Status Assessment in Rivers

The first methods for ecological assessment were not all based on strictly scientific evidence, but instead were rooted in a sound and often lifelong practical experience. The modern history of biological monitoring in rivers began in Europe, at a time when the human population was sufficiently large to produce both well-educated scientists and spectacularly polluted rivers. The most widely known example is probably the river Thames in London, which – in the nineteenth century, produced such a horrible smell that sheets soaked in vinegar were hung in the Parliament in the hope of offsetting the noxious air wafting in from the river (Cairns and Pratt 1993). Hassall (1850) used evidence of algae and other microscopic life present in reservoirs around London as a means of raising awareness of the potential link between water quality and health, some 30 years before the discovery of the actual causal agents. At these times, organic pollution and associated diseases were the most widespread impact on rivers due to human population increase and industrial activities combined with lack of advanced sewage treatment (Billen et al. 1999). It is therefore not surprising that the first assessment systems targeted organic pollution.

The idea of using biological indicators as a means of assessing river water quality probably originated with the work of Kolkwitz and Marsson (1902). These authors observed that different benthic taxa occurred sequentially downstream of a source of organic pollution, and changed in a predictable way along the course of the river. Based on these observations they developed a list of organisms which would indicate "saprobity" (the degree of organic pollution) in rivers. The presence of these indicator organisms at a river or stream site could then be used as a measure of the degree of contamination by organic matter (primarily sewage) and the resulting decrease in dissolved oxygen. This first list of indicator organisms was based on empirical observations, combined with deductions of possibly causal relationships. Pantle and Buck (1955) were the first to propose a means by which the list of indicator organisms present at a site could be converted to a quantitative measure of the "saprobity" at a river or stream location (the "Saprobienindex").

The first lists of indicator organisms for the Saprobienindex contained both macroinvertebrates and benthic algae (Kolkwitz and Marsson 1908). However,

primary producers were generally believed to relate more directly to inorganic nutrients, rather than to organic pollution (Schmetje and Kohmann 1987), such that later revisions of the Saprobienindex (Friedrich 1990) used heterotrophic organisms exclusively as indicators. During the second half of the twentieth century, due to the increasing standard of wastewater treatment, the degradation of organic matter was moved more and more from the river into the wastewater treatment facilities, whilst inorganic nutrients such as nitrogen and phosphorus continued to be released into the rivers. Thus, a need developed to differentiate heterotrophic processes, which are related to organic pollution ("Saprobie"), from autotrophic processes, which are related to inorganic nutrients ("Trophie"). In parallel to improvements in wastewater treatment and the increased importance of inorganic nutrients relative to organic pollution, trophic rankings of macrophyte species were developed for rivers (e.g., Kohler et al. 1974; Newbold and Palmer 1979). They paved the way for the development of various macrophyte indices (Holmes et al. 1999; Schneider and Melzer 2003; Haury et al. 2006). The main advantage of such indices compared to hydrochemistry was their simplicity (Tremp and Kohler 1995), and because they provide information about the **effects** of nutrient discharges rather than merely quantifying their load (Holmes et al. 1999). This is important because sensitivity and resilience to nutrient enrichment may vary substantially across ecosystems (Janse et al. 2008). Nevertheless, the validity of macrophyte indices was generally shown by relating them to water nutrient concentrations. This introduced a logical inconsistency: on the one hand, indices were "validated" against hydrochemistry, whilst at the same time proponents argued that these biological indices do not indicate hydrochemistry but, rather, the effects of nutrient loading.

The evolution of algal-based methods followed a slightly different trajectory, with early methods (Descy 1979; Lange-Bertalot 1979; Coste in CEMAGREF 1982) not differentiating between organic and inorganic pollution for monitoring river quality. Much of the work subsequently has focused on one group: the diatoms, to the exclusion of other groups of algae (Kelly 2013; Kelly et al. 2015). In particular, Coste in CEMAGREF (1982) proposed the diatom-based Indice de Polluosensibilité Specifique (IPS) which was adapted and adopted by the Agence de l'Eau Artois-Picardie in northern France for routine environmental assessments in a region where invertebrate analyses proved to be insufficiently sensitive (Prygiel and Coste 1993). A second generation of methods did attempt to differentiate between inorganic and organic pollution (Kelly and Whitton 1995; Rott et al. 1999) in response to new European Union legislation. However, the IPS, which is calibrated against a "general degradation" gradient, continues to be popular throughout Europe (see Kelly 2013).

There is evidence that diatoms do act as good proxies for the entire phytobenthos (Kelly 2006; Kelly et al. 2008; Schneider et al. 2013b), though a lot of photosynthetic diversity is overlooked by adherence to a diatom-only system. Some national assessment systems do include larger algae within their macrophyte survey methods (see Kelly 2013, for details) whilst a few have developed methods based on soft-bodied algae that are used either in conjunction with diatoms (Schaumburg et al. 2004) or alone (Schneider and Lindstrøm 2011). Diatom assessment systems

generally have strong correlations with water nutrient concentrations (Hering et al. 2006a), although such correlations are mostly based on spatial associations, and little reliable experimental data exist that could underpin these relationships.

3 Aquatic Plants, Benthic Algae, and the Water Framework Directive

The WFD (EC 2000) did not introduce an entirely new concept, but it did put the importance of biological monitoring into a common legal framework relevant for all member states of the European Union. Now deterioration and improvement of ecological quality were defined by the response of the biota, rather than by physical or chemical variables, and the benthic flora became a mandatory element for river status assessment. However, in spite of this fundamental change, many methods eventually adopted for WFD assessment were largely modifications of metrics that had been in use before (Kelly et al. 2009; Bennett et al. 2011; Birk et al. 2012). There are several possible reasons: (1) a reluctance amongst policy makers and managers to spend money for developing new assessment methods, (2) a desire among scientists and managers to continue using existing time series, or (3) the conclusion that existing methods actually were well-suited for the WFD.

While each of these reasons is understandable, one consequence is that many "new" WFD-compliant ecological assessment methods using aquatic plants and benthic algae were still based on correlations with measured water chemical parameters. This was not seen as a disadvantage; on the contrary. Hering et al. (2006b) pointed out that correlating the results of a metric to the stressor gradient is a central part of developing an index for ecological assessment of aquatic ecosystems. They recommended data on biochemical oxygen demand (BOD) or oxygen content to describe the impact of organic pollution, or concentrations of phosphorus and nitrogen to describe the trophic status of a sampling site. Indeed, a large number of studies have been published in recent years, testing different WFD metrics based on correlations between the metrics and measured water total phosphorus concentrations (e.g., Penning et al. 2008; del Pozo et al. 2010; Timm and Moels 2012; Lyche-Solheim et al. 2013). Such studies are usually based on the underlying assumption that the metric having the strongest correlation with measured phosphorus concentration is "best," and consequently this is the one that should be used for future monitoring of eutrophication.

While it can hardly be doubted that well-explained stressor-response relationships should underpin ecological assessment methods, this also leaves us with a conundrum: if it is necessary for an ecological metric (e.g., species composition of benthic flora) to correlate closely with a measured chemical variable (e.g., water phosphorus concentration), then what is gained by putting ecology rather than chemistry at the base of management decisions? A possible answer could be that the correlation between measured variable and ecological response may have

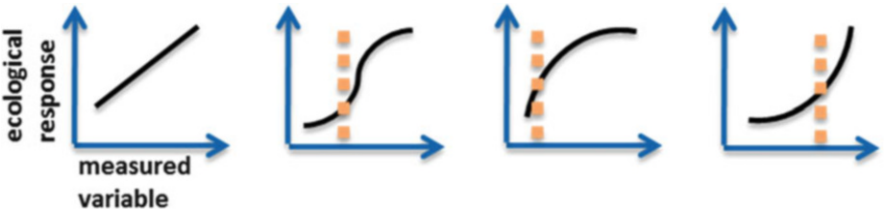

Fig. 1 Potential relationships between a measured variable (e.g. water total phosphorus concentration) and an ecological response (e.g. species composition of aquatic flora); the figures exemplify a linear, threshold, asymptotic and exponential response (from *left* to *right*); *vertical dashed lines* exemplify where critical values of the measured variable may be set, such that they lie before or after steep parts of the ecological response

various shapes (Fig. 1). In case of threshold, asymptotic or exponential responses, critical values for the measured variable may be set to match ecological response (Fig. 1). Indeed, sudden shifts from macrophyte to phytoplankton dominance have been reported in response to nutrient loading for rivers as well as lakes (Scheffer et al. 1993; Hilt et al. 2011). However, apart from the fact that **linear** correlations would not be appropriate for comparing response sensitivity of different ecological metrics (Penning et al. 2008; Lyche-Solheim et al. 2013), this also would mean that ecological monitoring is no longer necessary once the relationship between the measured variable (e.g., water total phosphorus concentration) and ecological response (e.g., species composition of aquatic flora) has been established. In all cases depicted in Fig. 1, the ecological response could easily be calculated from the measured variable, so there would be no need for water managers to spend money for additional monitoring of the ecological response. In other words: the "fundamental change" introduced by the WFD would cease to exist.

The solution to the conundrum is to recognize that water chemistry – ecological response relationships are purely descriptive tools that rank data more or less correctly along a gradient from unimpacted to the most impacted water bodies, rather than being causal dose–response relationships. Although water phosphorus concentration has been widely used as a general proxy for the stressor "eutrophication," neither phosphorus concentration nor eutrophication actually is the stressor.

4 The "Forgotten Ecology": Nutrient Uptake by Plants – Nutrient Cycling

Water chemistry is both cause and effect, although testing of WFD indices generally only assumes the former. On the one hand, enhanced nutrient concentrations may cause enhanced plant and algal growth and lead to changes in assemblage composition. On the other hand, however, plants and benthic algae also remove nutrients from the water, directly by incorporating them into their biomass, and

indirectly through their effects on biogeochemical processes. For example, aquatic macrophytes can create biochemical conditions that favor phosphorus (P) deposition (Chambers et al. 1989; Dodds 2003 and the references therein; Blindow et al. 2014). CO_2 assimilation during photosynthesis results in increased pH and a lowered solubility of $CaCO_3$ and consequently calcite precipitation on the surface of macrophytes. Most photosynthetic aquatic plants in hard water are capable of precipitating calcite. The charophytes, in particular, can be heavily calcified and more than 50% of the total plant dry weight has been reported to originate from $CaCO_3$. Phosphorus co-precipitates with calcite and can constitute up to 23% of total P in calcified charophytes (Siong and Asaeda 2006 and the references therein). In addition, root oxygen release of macrophytes can form iron crusts in anaerobic sediment leading to an enhanced sorptive P fixation (Dollan and Hupfer 2003). Decomposing plants, in turn, may lead to sudden increases in dissolved nutrient concentrations (Barko and Smart 1981; Twilley et al. 1986). Macrophyte beds can also affect nutrient retention by trapping suspended particulate matter from the turbulent overlying water (Vermaat et al. 2000; Schulz et al. 2003).

However, while aquatic plants may remove nutrients from the water, these may nevertheless still be available to them. Indeed, most rooted aquatic plants take up the majority of their nutrients from sediments (Carignan and Kalff 1980; Barko and Smart 1981; Chambers et al. 1989) and sediment nutrient concentrations are by no means always correlated with water nutrient concentrations (Schneider and Melzer 2004). Aquatic plants and benthic algae can reduce water exchange across the sediment-water boundary thus decreasing advective transport of P away from sediments (James et al. 2004). They may also use groundwater-born nutrients (Perillon and Hilt 2016).

As a consequence of these processes, the ecological status indicated by benthic algae and macrophytes in the littoral zone of shallow lakes is not necessarily consistent with open-water concentrations of phosphorus and/or nitrogen, e.g., in Lake Tahoe (Loeb 1986), Lake Taupo (Hawes and Smith 1993), Lake Huron (Barton et al. 2013), and Lake Ohrid (Schneider et al. 2014). In Norway, mass development of macrophytes can occur in streams with extremely low water nutrient concentrations (Schneider et al. 2013a). This phenomenon also applies to water bodies that recently underwent restoration measures aiming at the reduction of nutrient loading. Phytoplankton has been found to respond rapidly to external nutrient loading reduction in lakes, whereas a significant delay was observed for submerged macrophytes colonizing the littoral areas as lake sediments still stored nutrients from earlier periods with higher loading (Hilt et al. 2010, 2013). This delayed response of macrophytes compared to phytoplankton is partly due to their use of nutrients stored in sediments, to which phytoplankton has no access. In addition, a number of biological interactions may prevent a recolonization with species indicating less eutrophic conditions in water bodies that underwent a strong decline in nutrient loading (Hilt et al. 2013; Eigemann et al. 2016). The shading effect of periphyton (a complex matrix of algae and microbes growing on underwater surfaces such as stones or plants) on macrophytes might be one of the most

common of these interactions (Phillips et al. 1978; Köhler et al. 2010). In contrast to earlier assumptions, periphyton density is often not controlled by nutrient loading but top-down by a fish-grazer-periphyton cascade (a high number of fish feeding on grazing macroinvertebrates results in high periphyton biomass, whilst a low number of fish results in greater grazing activity by macroinvertebrates, leading to a lower periphyton biomass; Jones and Sayer 2003). In addition, herbivory by birds and fish might play a significant role in preventing macrophyte reestablishment (Bakker et al. 2013), particularly when combined with periphyton shading (Hidding et al. 2016). All these interactions blur a simple correlation between water chemistry and assemblages of aquatic plants and benthic algae.

But then, if water nutrient concentration can be both cause and effect of changes in aquatic plant and algal assemblages and therefore cannot simply be "the stressor," what "stressor" should we measure instead? In the early days of ecological assessment, managers accepted indicator lists inferred from expert judgment also without reliable data as to what the indicators actually indicate. Now we have to provide evidence that a metric indeed "responds" to a stressor (Birk et al. 2012) and scientists search for easily quantifiable parameters in order to provide this evidence. This resulted in the use of water chemistry (often total phosphorus concentrations) as a proxy for "eutrophication". However, it has been known for a long time (Ohle 1955) that water nutrient concentrations alone are not sufficient to determine eutrophication. We have explained above why water phosphorus concentrations may not be useful as a proxy for the stressor, and we will now show why "eutrophication" is not a stressor either.

5 Wanted: The Stressor!

The principle behind ecological assessment is straightforward: if a stressor affects biota, then the condition of the biota can be used to assess the intensity of the stressor (Fig. 2). Most metrics based on aquatic flora have been developed to assess "eutrophication" (e.g., Kelly and Whitton 1995; Fisher et al. 2010; Kolada et al. 2014). Unfortunately, ever since their coining (Naumann 1929), the terms "eutrophication", "oligotrophic", and "eutrophic" have variously and confusingly been used to describe ecosystem processes (e.g., increased plant growth) or ecosystem characteristics (e.g., water nutrient concentrations; Rodhe 1969). The inconsistent use of the term "eutrophication" has repeatedly been pointed out (e.g., Rodhe 1969; Hutchinson 1973; Wetzel 2001). Attempting to reach a common understanding, the OECD defined eutrophication as "response in water to overenrichment by nutrients", resulting in "symptoms such as algal blooms" or the "heavy growth of certain rooted aquatic plants" (Vollenweider and Kerekes 1982). Similar definitions, i.e., describing an enrichment of water by nutrients that causes an accelerated growth of algae and plants, were used in national and international legislation (e.g., DIN 4049-2 1990; European Court of Justice 2009; European Commission 2009).

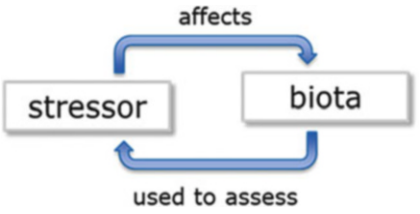

Fig. 2 The principle of ecological assessment: if a stressor affects biota, then the condition of the biota may be used to assess the intensity of the stressor

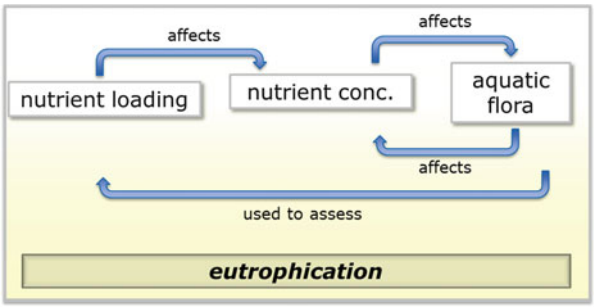

Fig. 3 Eutrophication is a process in which increased nutrient loading leads to increased growth of macrophytes and algae and changes in their species composition. Since the stressor (nutrient loading) is difficult to quantify, water chemistry (mainly total phosphorus) is used as a proxy, and water chemistry – biota relationships were used to develop metrics for aquatic flora

This means that eutrophication is a **process**, and its meaning includes several linked "cause and effect" relations from nutrient enrichment through to accelerated plant and algal growth, rather than merely the **cause** of this process (Fig. 3). Eutrophication is caused by nutrients entering the ecosystem via different internal and external sources that are used by aquatic plants and algae. We therefore argue that the stressor which benthic plants and algae react to, and consequently against which benthic floral indices should be regressed, is nutrient loading (from external and internal sources) rather than "eutrophication" (= the process that leads from nutrient enrichment to accelerated plant and algal growth) or "nutrient concentration" (= cause and effect of specific aquatic flora assemblages). In rivers and streams, nutrient loading should be expressed relative to stream discharge, because benthic plants generally are not exposed to the entire water column. Using "loading relative to discharge" instead of concentration would prevent the confusion of cause and effect. It would circumvent the problem that is caused by the uptake of nutrients by benthic algae and plants, leading simultaneously to reduced water nutrient concentrations and enhanced plant and algal growth at a site. It would also take into account the temporal variability in water nutrient concentrations that cause uncertainty in average concentrations. We hypothesize that average nutrient concentrations should reflect nutrient loading in anthropogenically unimpacted

headwater streams, as well as in eutrophic rivers receiving a more or less continuous nutrient input. In these systems, nutrient concentrations may well be useful for understanding benthic floral responses. However, in systems with variable or steadily declining nutrient concentrations, e.g., because measures have been taken to reduce external nutrient loading, any relationship between spot-measured nutrient concentration and benthic floral indices will be blurred due to nutrient uptake by plants and benthic algae, nutrient storage in sediments and temporal variability in nutrient inputs from various sources. Therefore we have to question the perception that the biological metric with the strongest correlation with measured water phosphorus concentration is, automatically, the one which best indicates "the stressor".

The WFD added an additional layer of complexity: the biota present at a site have to be compared with the biota at anthropogenically unimpacted reference sites: the greater the difference, the poorer the ecological status. Accepting the possible complications of identifying true reference sites (Pardo et al. 2012; Bouleau and Pont 2015), this approach has the advantage that it is comparable across countries and ecoregions, because a relative difference is quantified instead of absolute indicator values. It comes, however, with a drawback: biota are affected by a multitude of stressors including over-enrichment with nutrients, acidification, habitat degradation, siltation, changes in hydrological regime, increased water temperature, toxic substances, competition, or interference from invasive alien species (Von der Ohe et al. 2014). Many rivers are subject to multiple stressors, and these often have interactive effects on the biota, including the benthic flora (Schneider et al. 2013b; Piggott et al. 2015). Just quantifying the difference in species composition and abundance of aquatic flora between impacted sites and the (presumed) reference state for those sites fulfills the demands of the WFD by indicating whether one (or several) stressors are affecting the flora at the sampling site (Fig. 4). It does, however, not necessarily determine which of the stressors

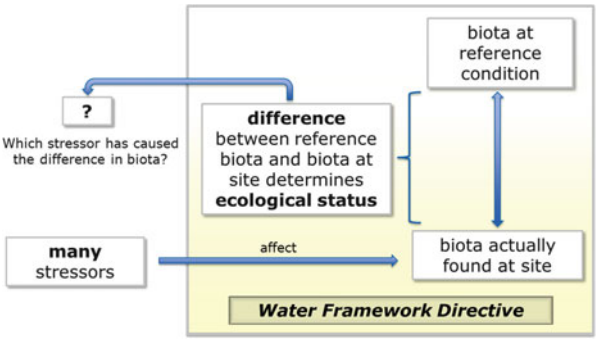

Fig. 4 Assessment according to the Water Framework Directive (WFD) is based on the difference between the biota at the sampling site, and those at unimpacted reference sites. While this approach has many advantages, such as comparability across ecoregions, it also has the drawback that many different stressors may impact the aquatic flora. Water managers can thus not easily infer which stressor caused degradation

actually caused the difference. For a water manager, however, this is highly relevant: s/he needs to understand which measures are required to restore a degraded ecosystem.

The countries in the European community have adopted different approaches to deal with the challenges posed by the WFD. Some researchers developed new indices and related them to "general degradation" (e.g., Hering et al. 2004; Gabriels et al. 2010). Although such an approach fulfills the demands of the WFD, it is of limited use to water managers since the indices may not diagnose the cause of degradation (Friberg 2014). Others adjusted "pre-WFD-indices" by re-calculating the index values relative to reference conditions (Kelly 2013); these indices also fulfill the demands of the WFD, but since they were designed to correlate closely with water chemistry, their additional value to hydrochemical measurements remains unclear. So how should we progress between Scylla and Charybdis?

6 What Information Can We Get from Benthic Flora?

We do not question the principles of the WFD, which has brought many achievements, among them the re-organization of water management by hydrological catchments rather than by administrative borders, the harmonization of classification and monitoring tools across Europe, the focus on ecosystem integrity instead of mere pollution control (Hering et al. 2010; Birk et al. 2012), and active engagement with stakeholders (Steyaert and Ollivier 2007). However, we argue that there is room for improvement of the ecological tools. Ecological assessment should be able to:

- quantify degradation,
- diagnose causes of degradation: identify the main stressor(s),
- pick up warning signals of unknown or underestimated stressors,
- identify management priorities by differentiating heavily impacted from less impacted sites,
- document improvements following restoration/rehabilitation, and
- communicate key information to non-specialist stakeholders.

Multi-metric indices have been recommended before as a highly reliable ecological assessment tool (Hering et al. 2006b). A multi-metric index combines individual measurements into a single metric, which can be used to assess a site's overall condition. If each component that constitutes the multi-metric index is related to a specific stressor, information about both type and magnitude of the stressor that causes the overall degradation can easily be extracted by tracing each individual metric. The benthic flora has mainly been used to assess nutrient enrichment (Birk et al. 2012), but is sensitive to a number of additional stressors, among them acidification (Arts et al. 1990; Schneider and Lindstrøm 2009; Juggins et al. 2016), salinization (Smith et al. 2009), hydromorphological alterations (Mjelde et al. 2013), siltation (Wagenhoff et al. 2013), increased dissolved organic

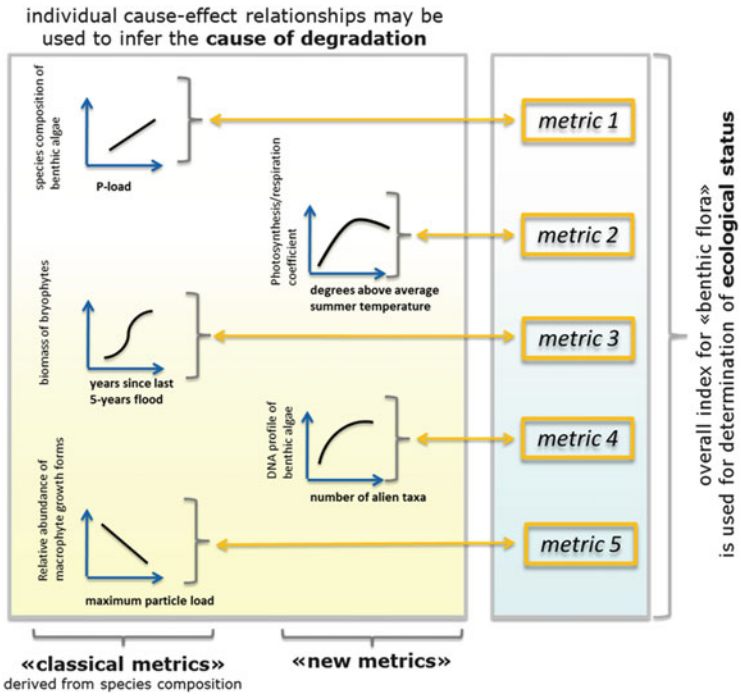

Fig. 5 Hypothetical construction of a multimetric index for status assessment based on aquatic flora. Note that individual relationships are hypothetical. Each metric that constitutes the multimetric index must be based on a cause-effect relationship. Additional metrics may readily be added (e.g. with respect to the effect of different pollutants); Different metrics may be combined, for example by following the "worst case" principle, into a single value that indicates ecological status. By tracing each individual metric, the type and magnitude of the stressor that caused degradation can be diagnosed. Individual metrics may include "classical" metrics that are based on species composition and abundance of aquatic flora, but also "new metrics" that may e.g. be based on physiological measurements. Note that the individual elements that constitute the multimetric index should be independent of each other (i.e. indicate different stressors)

carbon concentrations (Brothers et al. 2014), exotic herbivores (Krupska et al. 2012), and contaminants (Ricart et al. 2010). It should therefore be possible to develop a multi-metric index that can do both overall status assessment (by combining the individual metrics, for example, by following the "worst case" principle), and diagnose different causes of degradation (by tracing each individual metric; Fig. 5). The value of each individual metric can be calculated relative to its value at reference sites, such that the demands of the WFD are fulfilled. Individual metrics may include "classical" metrics that are based on species composition and abundance of aquatic flora. In some cases, different metrics that infer different stressors may even be calculated separately from the same species list. This is done in Norway, where metrics for nutrient enrichment and acidification are calculated from a list of benthic algal taxa present at a river site (Schneider et al. 2013b). However, it is important that the individual elements that constitute the multi-

metric index are **independent** of each other (e.g., because they indicate different and independent stressors). If they just use the same data to calculate a number of metrics that all indicate more or less the same stressor (and whose performance is "evaluated" by their correlation with water total phosphorus concentration), information about causal relationships is difficult to infer. In addition, the risk of failing to achieve "good ecological status" will increase with the number of constituent metrics (when the worst case principle is used for combining the individual metrics)!

This problem arises, however, partly because the constituents of existing multimetrics are organized in parallel (i.e., the index value results from many individual metrics that all have to be calculated). Were they to be organized, at least partially, in series then it would be possible to tailor the "package" of metrics closely to individual circumstances (Kelly 2013; DeNicola and Kelly 2014), thereby avoiding unnecessary expense and effort. We suggest that this may be addressed by using more general, comparatively simple and cheap methods like the Trophic Diatom Index, Trophic Index, or Periphyton Index of Trophic Status (TDI, TI, or PIT; Kelly and Whitton 1995; Rott et al. 1999; Schneider and Lindstrøm 2011) for ecological "triage" to sort out the "clearly very good" and "clearly degraded" sites (Kelly 2013; Kelly et al. 2015), and only use sophisticated methods

1. at sites which are close to the boundary between good and moderate status,
2. when small or slow improvements in ecological status (for example, after measures have been taken) need to be demonstrated,
3. in cases where there is doubt about which stressor may have caused degradation, or
4. when there is reason to suspect a slow degradation where sophisticated methods may give an early warning signal that would be overlooked with the simpler methods.

Such an approach may be compared with the daily work of a family doctor, who uses simple "indicators" such as body temperature, blood pressure, presence of spots or tender areas, or heart rate patterns to obtain an overview. Only the more "complicated" cases are sent to specialists who have access to sophisticated and expensive methods such as magnetic resonance imaging, to diagnose causes, quantify the severity of the problem, or monitor its development.

Such a tiered approach also opens the way for new metrics, e.g., based on physiological processes and functional ecology that provide more powerful diagnostic capabilities than is possible from analysis of assemblage composition and abundance. Although to our knowledge no ready-to-use methods exist yet, new tools based on, e.g., molecular biological data, ecosystem functioning, or physiological measurements may well add important information to the "classical" methods. New methods may, for example, be more sensitive to a given stressor, or react to different or previously ignored stressors (e.g., an increase in water temperature). If water managers make clear statements about the stressors which need to be addressed, then ecologists should be able to design a suite of useful tools.

Hill et al. (2000) combined metrics based on periphyton taxonomy, biomass, and phosphatase activity into an index of biotic integrity, and the different constituent metrics were related to different chemical, physical habitat, and landscape variables. Our approach is similar to Hill et al. (2000), but we suggest organizing the constituent metrics at least partially in series instead of in parallel, and we suggest putting a stronger focus on inferring the causes of ecosystem degradation from the constituent metrics. In that way, unnecessary expense and effort can be avoided, and causes of degradation can be inferred, which provides important information to managers. In interpreting these indices, however, we should take the "classical" ecological interactions between biota and their environment into account: if a scientifically soundly developed ecological metric indicates high nutrient load at a site where water phosphorus concentrations are low, then this is a clear sign for (1) internal nutrient supply via the sediment, (2) discontinuous nutrient supply at times when water chemistry was not measured, and/or (3) significant uptake of nutrients into plant and benthic algal biomass. In any case, scientists and water managers should start searching for the source of nutrient supply instead of criticizing a "poor" index that does not adequately mirror water chemistry.

7 Conclusions

The aquatic benthic flora is an integral part of well-functioning aquatic ecosystems. It is important that catchment managers have access to effective means of assessing the "health" of the benthic flora in order to ensure delivery of essential ecosystem services. This must move beyond the approach that has been used so far, where the biota are regarded as simplistic "mirrors" of water chemistry. For developing and interpreting assessment tools, we should

- use water chemistry – biotic response relationships as descriptive tools only, and not confuse them with quantitative stressor – response relationships
- should make sure stressor-response relationships are based on experimental evidence, instead of on diffuse associations between biota and hydrochemistry where the uncertainty in quantifying the intensity of the stressor is blamed on a poor performance of the ecological metric
- consider a tiered approach, i.e., using more general and comparatively "simple" indices (which nevertheless must be firmly based on scientific evidence) for an overview and more sophisticated methods in doubtful or complicated cases; this could avoid unnecessary costs and efforts while giving important ecological and management information.

The next generation of biotic indices must take into account the underlying ecological processes. If we make sure to not "forget" the ecology behind ecological status evaluation, then aquatic plants and benthic algae do have the potential to become progressive assessment tools that meet future challenges in water

management and will aid our understanding of ecosystem responses to a variety of stressors.

Acknowledgements This manuscript resulted from >15 years of work on ecological assessment and the Water Framework Directive. Numerous colleagues have participated in discussions, and many organizations provided funding for a multitude of different projects. We thank the authors of the Water Framework Directive for putting ecology into focus, the editor and a reviewer for constructive feedback, and a countless number of colleagues for inspiring discussions.

References

Arts GHP, Roelofs JGM, De Lyon MJH (1990) Differential tolerances among soft-water macrophyte species to acidification. Can J Bot 68:2127–2134

Bakker ES, Sarneel JM, Gulati RD, Liu ZW, van Donk E (2013) Restoring macrophyte diversity in shallow temperate lakes: biotic versus abiotic constraints. Hydrobiologia 710:23–37

Barko JW, Smart RM (1981) Sediment-based nutrition of submersed macrophytes. Aquat Bot 10:339–352

Barton DR, Howell ET, Fietsch CL (2013) Ecosystem changes and nuisance benthic algae on the southeast shores of Lake Huron. J Great Lakes Res 39:602–611

Bennett C, Owen R, Birk S, Buffagni A, Erba S, Mengin N et al (2011) Bringing European river quality into line: an exercise to intercalibrate macro-invertebrate classification methods. Hydrobiologia 667:31–48

Billen G, Garnier J, Deligne C, Billen C (1999) Estimates of early-industrial inputs of nutrients to river systems: implication for coastal eutrophication. Sci Total Environ 243(244):43–52

Birk S, Bonne W, Borja A, Brucet S, Courrat A, Poikane S, Solimini A, van de Bund WV, Zampoukas N, Hering D (2012) Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol Indic 18:31–41

Blindow I, Hargeby A, Hilt S (2014) Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. Hydrobiologia 737:99–110

Bouleau G, Pont D (2015) Did you say reference conditions? Ecological and socio-economic perspectives on the European Water Framework Directive. Environ Sci Policy 47:32–41

Brothers S, Köhler J, Meyer N, Attermeyer K, Grossart HP, Mehner T, Scharnweber K, Hilt S (2014) A feedback loop links brownification and anoxia in a temperate, shallow lake. Limnol Oceanogr 59:1388–1398

Butcher RW (1933) Studies on the ecology of rivers: I. On the distribution of macrophytic vegetation in the rivers of Britain. J Ecol 21:58–91

Cairns J, Pratt JR (1993) A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg DM, Resh VH (eds) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York

Carignan R, Kalff J (1980) Phosphorus sources for aquatic weeds: water or sediments? Science 207:987–988

CEMAGREF (1982) Etude de méthodes biologiques quantitatives d'appreciation de la qualité des eaux. Rapport Q.E. Lyon-A.F.B. Rhône-Mediterranée-Corse

Chambers PA, Prepas EE, Bothwell ML, Hamilton HR (1989) Roots versus shoots in nutrient uptake by aquatic macrophytes in flowing waters. Can J Fish Aquat Sci 46:435–439

del Pozo R, Fernandez-Alaez C, Fernandez-Alaez M (2010) An assessment of macrophyte community metrics in the determination of the ecological condition and total phosphorus concentration of Mediterranean ponds. Aquat Bot 92:55–62

DeNicola DM, Kelly MG (2014) Role of periphyton in ecological assessment of lakes. Freshw Sci 33:619–638

Descy JP (1979) A new approach to water quality estimation using diatoms. Nova Hedwigia 64:305–323

DIN 4049-2 (1990) Hydrologie; Begriffe der Gewässerbeschaffenheit. Deutsches Institut fuer Normung, Berlin, p 25

Dodds WK (2003) The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J Phycol 39:840–849

Dollan A, Hupfer M (2003) Immobilisation of phosphorus by iron-coated roots of submerged macrophytes. Hydrobiologia 506–509:635–640

Eigemann F, Mischke U, Hupfer M, Schaumburg J, Hilt S (2016) Biological indicators track differential responses of pelagic and littoral areas to nutrient load reductions in German lakes. Ecol Indic 61:905–910

European Commission (2000) Directive 2000/60/EC. Establishing a framework for community action in the field of water policy. European Commission PE-CONS 3639/1/100 Rev 1, Luxembourg

European Commission (2009) Guidance document on eutrophication assessment in the context of European water policies. Technical Report - 2009 – 030, Luxembourg

European Court of Justice (2009) Judgment of the Court (Third Chamber) of 10 December 2009 (Case C-390/07). European Commission v United Kingdom of Great Britain and Northern Ireland. Failure of a Member State to fulfill obligations – Environment – Directive 91/271/EEC – Urban waste water treatment – Article 3(1) and (2), Article 5(1) to (3) and (5) and Annexes I and II – Initial failure to identify sensitive areas – Concept of 'eutrophication' – Criteria – Burden of proof – Relevant date when considering the evidence – Implementation of collection obligations – Implementation of more stringent treatment of discharges into sensitive areas. Official Journal of the European Union, C 24/4

Fisher J, Deflandre-Vlandas A, Coste M, Delmas F, Jarvie HP (2010) Assemblage grouping of European benthic diatoms as indicators of trophic status of rivers. Fundam Appl Limnol 176:89–100

Friberg N (2014) Impacts and indicators of change in lotic ecosystems. WIREs Water 1:513–531

Friedrich G (1990) Eine Revision des Saprobiensystems. Zeitschrift fur Wasser- und Abwasserforschung 23:141–152

Gabriels W, Lock K, De Pauw N, Goethals PLM (2010) Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). Limnologica 40:199–207

Hassall AH (1850) Memoir on the organic analysis or microscopic examination of water supplied to the inhabitants of London and the suburban districts. Lancet 1:230–235

Haury J, Peltre MC, Tremolieres M, Barbe J, Thiebaut G, Bernez I et al (2006) A new method to assess water trophy and organic pollution – the Macrophyte biological index for rivers (IBMR): its application to different types of river and pollution. Hydrobiologia 570:153–158

Hawes I, Smith R (1993) Effect of localised nutrient enrichment on the shallow epilithic periphyton of oligotrophic Lake Taupo, New Zealand. N Z J Mar Freshw Res 27:365–372

Hering D, Meier C, Rawer-Jost C, Feld CK, Biss R, Zenker A, Sundermann A, Lohse S, Bohmer J (2004) Assessing streams in Germany with benthic invertebrates: selection of candidate metrics. Limnologica 34:398–415

Hering D, Johnson RK, Kramm S, Schmutz S, Szoszkiewicz K, Verdonschot PFM (2006a) Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response due to stress. Freshw Biol 51:1757–1785

Hering D, Feld CK, Moog O, Ofenbock T (2006b) Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566:311–324

Hering D, Borja A, Carstensen J, Carvalho L, Elliott M, Feld CK, Heiskanen AS, Johnson RK, Moe J, Pont D, Lyche Solheim A, van de Bund W (2010) The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci Total Environ 408:4007–4019

Hidding B, Bakker ES, Hootsmans MJM, Hilt S (2016) Synergy between shading and herbivory triggers plant loss and regime shifts in aquatic systems. Oikos (in press). doi:10.1111/oik.03104

Hill BH, Herlihy AT, Kaufmann PR, Stevenson RJ, McCormick FH, Johnson CB (2000) Use of periphyton assemblage data as an index of biotic integrity. J N Am Benthol Soc 19:50–67

Hilt S, Van de Weyer K, Köhler A, Chorus I (2010) Submerged macrophyte responses to reduced phosphorus concentrations in two peri-urban lakes. Restor Ecol 18(S2):452–461

Hilt S, Köhler J, Kozerski HP, Scheffer M, Van Nes E (2011) Abrupt regime shifts in space and time along rivers and connected lakes systems. Oikos 120:766–775

Hilt S, Adrian R, Köhler J, Monaghan MT, Sayer C (2013) Clear, crashing, turbid and back – long-term changes of macrophyte assemblages in a shallow lake. Freshw Biol 58:2027–2036

Holmes NTH, Newman JR, Chadd S, Rouen KJ, Saint L, Dawson FH (1999) Mean trophic rank: a user's manual. R&D Technical Report E38. Environment Agency, Bristol

Hutchinson GE (1973) Eutrophication – the scientific background of a contemporary practical problem. Am Sci 61:269–279

James WF, Barko JW, Butler MG (2004) Shear stress and sediment resuspension in relation to submersed macrophyte biomass. Hydrobiologia 515:181–191

Janse JH, De Senerpont Domis LN, Scheffer M, Lijklema L, Van Liere L, Klinge M, Mooij W (2008) Critical phosphorus loading of different types of shallow lakes and the consequences for management estimated with the ecosystem model PCLAKE. Limnologica 38:203–219

Jones JI, Sayer CE (2003) Does a fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? Ecology 84:2155–2167

Juggins S, Kelly M, Allott T, Kelly-Quinn M, Monteith D (2016) A Water Framework Directive-compatible metric for assessing acidification in UK and Irish rivers using diatoms. Science of the total environment (in press)

Karr JR (1991) Biological integrity: a long-neglected aspect of water resource management. Ecol Appl 1:66–84

Karr JR (1999) Defining and measuring river health. Freshw Biol 41:221–234

Kelly MG (2006) A comparison of diatoms with other phytobenthos as indicators of ecological status in streams in northern England. In: Witkowski A (ed) Proceedings of the 18th international diatom symposium 2004. Biopress, Bristol, pp 139–151

Kelly MG (2013) Data rich, information poor? Phytobenthos assessment and the Water Framework Directive. Eur J Phycol 48:437–450

Kelly MG, Whitton BA (1995) The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. J Appl Phycol 7:433–444

Kelly MG, King L, Jones RI, Barker PA, Jamieson BJ (2008) Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. Hydrobiologia 610:125–129

Kelly MG, Bennett C, Coste M, Delgado C, Delmas F, Denys L et al (2009) A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. Hydrobiologia 621:169–182

Kelly MG, Schneider SC, King L (2015) Customs, habits and traditions: the role of non-scientific factors in the development of ecological assessment methods. WIREs Water 2:159–165

Kohler A, Brinkmeier R, Vollrath H (1974) Verbreitung und Indikatorwert der submsersen Makrophyten in den Fliessgewässern der Friedberger Au. Ber Bay Bot Ges 45:5–36

Köhler J, Hachoł J, Hilt S (2010) Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquat Bot 92:129–136

Kolada A, Willby N, Dudley B, Noges P, Sondergaard M, Hellsten S, Mjelde M, Penning E, van Geest G, Bertrin V, Ecke F, Maemets H, Karus K (2014) The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecol Indic 45:407–415

Kolkwitz R, Marsson M (1902) Grundsätze für die biologische Beurteilung des Wassers nach seiner Flora und Fauna. Mitteilungen der königlichen Prüfanstalt für Wasserversorgung und Abwasserbeseitigung 1:33–72

Kolkwitz R, Marsson M (1908) Ökologie der pflanzlichen Saprobien. Mitteilung aus der Königlichen Prüfungsanstalt für Wasserversorgung und Abwässerbeseitigung Heft 1:508–519

Krupska J, Pełechaty M, Pukacz A, Ossowski P (2012) Effects of grass carp introduction on macrophyte communities in a shallow lake. Oceanol Hydrobiol Stud 41:35–40

Lange-Bertalot H (1979) Pollution tolerance as a criterion for water quality estimation. Nova Hedwigia 64:285–304

Loeb SL (1986) Algal biofouling of oligotrophic Lake Tahoe: causal factors affecting production. In: Evans LV, Hoagland KD (eds) Algal biofouling. Elsevier Science Publishers, Amsterdam, pp 159–173

Lyche-Solheim A, Feld CK, Birk S, Phillips G, Carvalho L, Morabito G, Mischke U, Willby N, Søndergaard M, Hellsten S, Kolada A, Mjelde M, Böhmer J, Miler O, Pusch MT, Argillier C, Jeppesen E, Lauridsen TL, Poikane S (2013) Ecological status assessment of European lakes: a comparison of metrics for phytoplankton, macrophytes, benthic invertebrates and fish. Hydrobiologia 704:57–74

Mjelde M, Hellsten S, Ecke F (2013) A water level drawdown index for aquatic macrophytes in Nordic lakes. Hydrobiologia 704:141–151

Naumann E (1929) Einige neue Gesichtspunkte zur Systematik der Gewässertypen. Arch Hydrobiol 20:191–198

Newbold C, Palmer M (1979) Trophic adaptations of aquatic plants. NCC CST Notes number 18. Nature Conservancy Council, Peterborough

Ohle W (1955) Beiträge zur Produktionsbiologie der Gewässer. Arch Hydrobiol XXII (Suppl):456–479

Pantle K, Buck H (1955) Die biologische Überwachung der Gewässer und die Darstellung der Ergebnisse. Gas- und Wasserfach Wasser/Abwasser 96:609–620

Pardo I, Gomez-Rodriguez C, Wasson JG, Owen R, van de Bund W, Kelly M, Bennett C, Birk S, Buffagni A, Erba S, Mengin N, Murray-Bligh J, Ofenboeck G (2012) The European reference condition concept: a scientific and technical approach to identify minimally-impacted river ecosystems. Sci Total Environ 420:33–42

Penning WE, Dudley B, Mjelde M, Hellsten S, Hanganu J, Kolada A, van den Berg M, Poikane S, Phillips G, Willby N, Ecke F (2008) Using aquatic macrophyte community indices to define the ecological status of European lakes. Aquat Ecol 42:253–264

Perillon C, Hilt S (2016) Groundwater influence differentially affects periphyton and macrophyte production in lakes. Hydrobiologia (in press). doi:10.1007/s10750-015-2485-9

Phillips GL, Eminson DF, Moss B (1978) A mechanism to account for macrophyte decline in progressively eutrophicated waters. Aquat Bot 4:103–125

Piggott JJ, Salis RK, Lear G, Townsend CR, Matthaei CD (2015) Climate warming and agricultural stressors interact to determine stream periphyton community composition. Glob Chang Biol 21:206–222

Prygiel J, Coste M (1993) The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom indices. Hydrobiologia 269(270):343–349

Ricart M, Guasch H, Alberch M, Barcelo D, Bonnineau C, Geiszinger A, Farre M, Ferrer J, Ricciardi F, Romani AM, Morin S, Proia L, Sala L, Sureda D, Sabater S (2010) Triclosan persistence through wastewater treatment plants and its potential toxic effects on river biofilms. Aquat Toxicol 100:346–353

Rodhe W (1969) Crystallization of eutrophication concepts in Northern Europe. In: Proceedings of a symposium on eutrophication: causes, consequences, correctives. National Academy of Sciences, Washington, pp 50–64

Rott E, Pipp E, Pfister P, van Dam H, Ortler K, Binder N, Pall K (1999) Indikationslisten für Aufwuchsalgen in Österreichischen Fliessgewassern. Teil 2: Trophieindikation. Bundesministerium für Land- und Forstwirtschaft, Vienna, Austria

Schaumburg J, Schranz C, Foerster J, Gutowski A, Hofmann G, Meilinger P, Schneider S, Schmedtje U (2004) Ecological classification of macrophytes and phytobenthos for rivers in Germany according to the Water Framework Directive. Limnologica 34:283–301

Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E (1993) Alternative equilibria in shallow lakes. Trends Ecol Evol 8:275–279

Schmetje U, Kohmann F (1987) Bioindikation durch Makrophyten – indizieren Makrophyten Saprobie? Arch Hydrobiol 199:455–469

Schneider S, Lindstrøm EA (2009) Bioindication in Norwegian rivers using non-diatomaceous benthic algae: the acidification index periphyton (AIP). Ecol Indic 9:1206–1211

Schneider S, Lindstrøm EA (2011) The periphyton index of trophic status PIT: a new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers. Hydrobiologia 665:143–155

Schneider S, Melzer A (2003) The Trophic Index of Macrophytes (TIM) – a new tool for indicating the trophic state of running waters. Int Rev Hydrobiol 88:49–67

Schneider S, Melzer A (2004) Sediment and water nutrient characteristics in patches of submerged macrophytes in running waters. Hydrobiologia 527:195–207

Schneider SC, Moe TF, Hessen DO, Kaste O (2013a) Juncus bulbosus nuisance growth in oligotrophic freshwater ecosystems: different triggers for the same phenomenon in rivers and lakes? Aquat Bot 104:15–24

Schneider SC, Kahlert M, Kelly MG (2013b) Interactions between pH and nutrients on benthic algae in streams and consequences for ecological status assessment and species richness patterns. Sci Total Environ 444:73–84

Schneider SC, Cara M, Eriksen TE, Budzakoska Goreska B, Imeri A, Kupe L, Lokoska T, Patceva S, Trajanovska S, Trajanovski S, Talevska M, Veljanoska Sarafilovska E (2014) Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica 44:90–97

Schulz M, Kozerski HP, Pluntke T, Rinke K (2003) The influence of macrophytes on sedimentation and nutrient retention in the lower River Spree (Germany). Water Res 37:569–578

Siong K, Asaeda T (2006) Does calcite encrustation in Chara provide a phosphorus nutrient sink? J Environ Qual 35:490–494

Smith MJ, Ough KM, Scroggie MP, Schreiber ESG, Kohout M (2009) Assessing changes in macrophyte assemblages with salinity in non-riverine wetlands: a Bayesian approach. Aquat Bot 90:137–142

Steyaert P, Ollivier G (2007) The European Water Framework Directive: how ecological assumptions frame technical and social change. Ecol Soc 12(1):25

Timm H, Moels T (2012) Littoral macroinvertebrates in Estonian lowland lakes: the effects of habitat, season, eutrophication and land use on some metrics of biological quality. Fundam Appl Limnol 180:145–156

Tremp H, Kohler A (1995) The usefulness of macrophyte monitoring-systems, exemplified on eutrophication and acidification of running waters. Acta Botanica Gallica 142:541–550

Twilley RR, Ejdung G, Romare P, Kemp WM (1986) A comparative study of decomposition, oxygen consumption and nutrient release for selected aquatic plants occurring in an estuarine environment. Oikos 47:190–198

Vermaat JE, Santamaria L, Roos PJ (2000) Water flow across and sediment trapping in submerged macrophyte beds of contrasting growth form. Arch Hydrobiol 148:549–562

Vollenweider RA, Kerekes J (1982) Eutrophication of waters. Monitoring, assessment and control. OECD cooperative programme on monitoring of inland waters (Eutrophication control), Environment Directorate, OECD, Paris, p 154

von der Ohe PC, Apitz S, Arbaciauskas K, Beketov MA, Borchardt D, de Zwart D, Goedkoop W, Hein M, Hellsten S, Hering D, Kefford BJ, Panov VE, Schafer RB, Segner H, van Gils J,

Vegter JJ, Wetzel MA, Brack W (2014) Status and causal pathway assessments supporting River Basin management. In: Brils J, Brack W, Müller-Grabherr D, Négrel P, Vermaat JE (eds) Risk-informed management of European River Basins. Springer, Heidelberg, p 395

Wagenhoff A, Lange K, Townsend CR, Matthaei CD (2013) Patterns of benthic algae and cyanobacteria along twin-stressor gradients of nutrients and fine sediment: a stream mesocosm experiment. Freshw Biol 58:1849–1863

Wetzel RG (2001) Limnology – lake and river ecosystems, 3rd edn. Academic, San Diego

Beneficial Soil Microbiota as Mediators of the Plant Defensive Phenotype and Aboveground Plant-Herbivore Interactions

Martin Schädler and Daniel J. Ballhorn

Contents

M. Schädler (✉)
Department Community Ecology, Helmholtz-Centre for Environmental Research (UFZ), Theodor-Lieser-Str. 4, 06120 Halle, Germany

iDiv – German Centre for Integrative Biodiversity Research, Deutscher Platz 5e, 04103 Leipzig, Germany
e-mail: martin.schaedler@ufz.de

D.J. Ballhorn
Department of Biology, Portland State University, 1719 SW 10th Ave, Portland, OR 97201, USA
e-mail: ballhorn@pdx.edu

Progress in Botany (2017) 78: 305–344, DOI 10.1007/124_2016_13,
Published online: 15 June 2016

Abstract The symbiosis with beneficial soil microbiota importantly affects plant physiology, growth and community structure. These effects are known to translate into changes of aboveground plant-herbivore interactions, and there is increasing evidence that microbial symbioses alter the defensive plant phenotype far beyond the primary plant metabolism. Microbe-mediated changes in plant defensive traits have been reported for various plant-microbe systems including both bacterial and fungal mutualists. Microbial mutualists not only affect the expression of direct plant defences, but also alter indirect defences like volatile production and extrafloral nectaries and thus have cascading effects on higher trophic levels. By simultaneously affecting a suite of plant defensive traits, they may modulate the benefits and costs of alternative defence strategies. Our understanding of the impact of plant-associated microbial mutualists in food webs is critical to elucidate their functional role in ecosystems. However, it is still limited by a lack of integration of natural complexity and evolutionary context into concepts and studies of microbe-plant-herbivore interactions.

1 Introduction

Plants growing in natural environments continually interact with a variety of other co-occuring species. Amongst the most common partners are those that exhibit beneficial interactions belowground like mycorrhizal fungi and nitrogen-fixing bacteria. Mycorrhizal associations are traditionally classified as endomycorrhizae and ectomycorrhizae. The most common endomycorrhizal fungi are obligate biotrophic arbuscular (AMF) from the phylum *Glomeromycota* which are associated with the roots of more than 80% of plant species (Smith and Read 2008). In contrast, ectomycorrhizal fungi (EMF) are associated with about only 3% of all seed plants, but this spectrum involves the majority of trees. Both types of mycorrhizae provide nutrients (e.g. phosphorous, nitrogen) and water to the plant in exchange to host photosynthates and are known to have important consequences for the plants' performance as well as the dynamics of plant populations and communities (e.g. mycorrhizae: Smith and Read 2008; van der Heijden et al. 1998b). Another important group of plant mutualists is the nitrogen-fixing bacteria. Over 15,000 plant species from more than 12 families have the ability to form associations with these microorganisms and therefore have access to the atmospheric nitrogen pool (Corby 1981; Sprent 2001). Amongst them, the legumes (Fabaceae) are associated with nitrogen-fixing rhizobia and have enormous economic (e.g. soybean, common bean, alfalfa, clover, pea) and ecological value. The contribution of legumes to productivity and the nitrogen pool of natural and

agricultural ecosystems are considerable (Hector et al. 1999; Herridge et al. 2008; Spehn et al. 2002; Waughman et al. 1981). Furthermore, mutualistic nitrogen fixation influences plant community structure and diversity (van der Heijden et al. 2006). Because of these effects, rhizobia are considered keystone species (Wardle 2002) and ecosystem engineers (Crooks 2002). Other nitrogen-fixing bacteria are actinomycetes from the genus *Frankia* which live in symbiosis with plants from eight families (mainly shrubs and trees; see Wall 2000). Two other diverse groups of beneficial soil organisms are free-living plant growth-promoting bacteria (PGPB) and plant growth-promoting fungi (PGPF) which stimulate plant growth in various ways, e.g. by increasing the availability of nutrients in the rhizosphere, positively affecting root growth, promoting mutualistic relationships and antagonizing pathogenic organisms (Glick 1995; Jogaiah et al. 2013; Pii et al. 2015; Vessey 2003).

The mutualism with soil microbiota is seen as an important factor influencing the colonization of land by plants and their evolution (Selosse et al. 2015). There is increasing recognition that besides the impact on plant growth, beneficial soil microbes further influence the relationship between plants and other associated organisms. Virtually all terrestrial plants are associated with invertebrate herbivores which affect plant growth and reproduction and shape the structure and dynamics of plant communities (Crawley 1997; Schädler et al. 2004). Especially for mycorrhizal fungi, it has been shown repeatedly that this mutualism affects plant tolerance, resistance and defence against insect herbivores aboveground (Gehring and Whitham 2002; Hartley and Gange 2009; Koricheva et al. 2009). However, both types of interaction often simultaneously affect the same traits of plant growth and physiology under natural conditions, and recently it has become evident that the underlying genetic and physiological pathways induced by and involved in the different interactions overlap substantially (Schenk et al. 2008). Belowground mutualists can be thus seen as an integral part of the plant-herbivore relationship with consequences for speciation processes (Gange et al. 2002b). Recent experimental work shows that the link between root mutualists and herbivores not only bases on the conventional concept of this relationship of an increased availability of nutrients which change the nutritional value of plant tissue and may increase the ability of plants to produce secondary compounds (Fig. 1). Further, the establishment and maintenance of mutualism is associated with considerable physiological costs due to the high demand of photosynthates (Kaschuk et al. 2009). Additionally, the induction of specific defence traits by mutualists may further raise ecological costs caused by the deterrence of potentially beneficial biotic partners (pollinators, parasitoids) by direct and indirect defence mechanisms (Godschalx et al. 2015). This implies complex links between root mutualists and defensive traits of plants. In this review, we aim to discuss the ways how beneficial belowground microbes affect the complete defensive phenotype of plants. We will further assess which factors contribute to the variability of the observed effects and put this in the context of the complexity of natural systems.

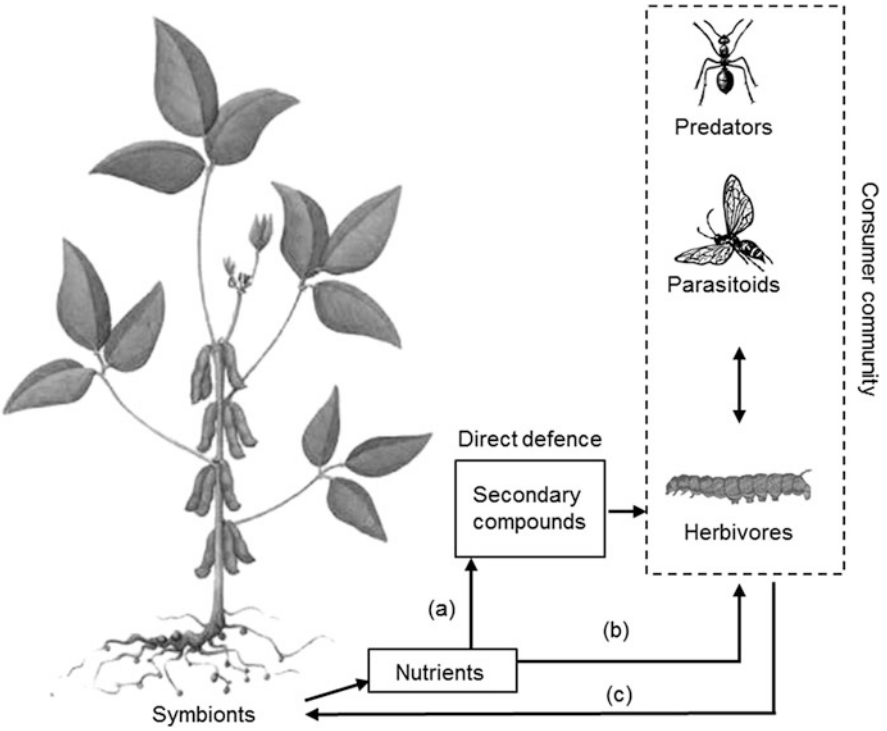

Fig. 1 Conventional view of the influence of root symbionts on the interactions between plants and herbivores. The symbiosis with root mutualists stimulates the production of secondary compounds as a direct consequence of additional nutritional resources (*a*). The combined effects of an enhanced direct defence and the increased level of nutrients in plant tissue (*b*) further depend on the herbivore feeding mode. Secondary consumers are indirectly affected via changes in herbivore performance and abundance. Feedback effects of herbivores on the mutualists are the result of a reduced ability of plants to allocate resources below ground

2 Effects of Root Mutualists on Resistance and Direct Defence Mechanisms

2.1 Plant Resistance Against Invertebrate Herbivores

The resistance of plants against herbivores may be modified by changes in the plant nutritive value, plant tolerance and plant defence. Mutualistic relationships with nutrient-providing microbes may have the potential to affect these traits. For mycorrhizal fungi, effects on insect herbivores have been recognized for decades. Firstly, by providing nutrients mycorrhizal fungi may increase the vigour of host plants with positive effects on the performance of insect herbivores as well as positive effects for the plant's ability to tolerate herbivory (Borowicz 1997; Gehring and Whitham 1994; Halldorsson et al. 2000). Secondly, the resources

supplied by mycorrhizal fungi may allow plants to increase investment into plant defence by upregulating secondary compounds against generalist insect herbivores (Bi et al. 2007; Gange and West 1994; Jones et al. 1991; Yao et al. 2007). In contrast, specialist and sucking herbivores, which are less influenced by specific defence compounds (but see Ballhorn et al. 2007), tend to be generally positively affected due to mycorrhization (Babikova et al. 2014a; Borowicz 2013; Demirözer et al. 2014; Gehring and Whitham 2002; Hartley and Gange 2009; Koricheva et al. 2009; Ueda et al. 2013). This is in accordance with observations of Ueda et al. (2013) who found that the abundance of phloem feeders on soybean was increased by mycorrhization even though chewing herbivores were not affected. However, species which have a very similar feeding mode may also show different responses to mycorrhization, e.g. parenchyma cell feeders (Borowicz 1997; Hoffmann et al. 2009; Koschier et al. 2007). Even for specific herbivores, it has been shown that the effects may differ between sampling dates (Ueda et al. 2013) or depend on soil conditions (Murrell et al. 2015). Moreover, different AMF species may have different effects on a given herbivore, but the net effect of an AMF community may largely reflect the effects of one of the component AMF species (i.e. "super fungus", see Bennett and Bever 2007). According to the high overall variability of effects between and within feeding groups and species, Yang et al. (2014) found no significant overall effect of AMF on aboveground herbivores (but did find a negative effect on belowground herbivores) in a meta-analysis.

The variability of effects may also be explained by differences between mycorrhized and non-mycorrhized plants in their responses to prior feeding by herbivores. Using a number of grass and herb species, Kempel et al. (2010) showed that a leaf-chewing herbivore (cotton leafworm, *Spodoptera littoralis*) may profit in terms of biomass from mycorrhization but not if the plants experienced leaf damage by a herbivore before. The observation that the increase of plant biomass caused by mycorrhization was only evident for non-damaged plants indicates that the allocation of resources to plant growth and herbivore resistance is mediated by mycorrhization. Barber (2013) demonstrated increased feeding by the closely related *Spodoptera exigua* on mycorrhized plants only after prior damage but observed no changes in herbivore biomass. This implies that in this case, a lowered nutritional quality of leaf tissue was counterbalanced by compensatory feeding. Obviously, the induction of resistance or tolerance mechanisms by previous herbivore attack is an important mediator of the impact of mycorrhization on herbivore performance and may be especially important for natural systems where plants are commonly affected by multiple herbivory events. Again, the feeding style of the herbivore may importantly affect the induction of defence responses.

AMF also can influence herbivore behaviour without immediate effects on herbivory intensity. For instance, herbivores may spend more time or enhance oviposition on leaves of mycorrhized plants without causing greater feeding damage (Cosme et al. 2011; Kula et al. 2005).

The influence of EMF on tree-aboveground herbivore interactions has received much less attention by ecologists, probably due to the difficulty of using tree-EMF systems for field or lab experiments (Hartley and Gange 2009). The few available

studies produced contradictory results so far which do not yet allow for generalizations (Manninen 1999; Manninen et al. 1998, 1999, 2000; Rieske 2001). Moreover, insect herbivores of tree species which are able to form mutualisms with both AMF and EMF may show different responses to the degree of colonization by both types of mycorrhiza (Gange et al. 2005; Mueller et al. 2005).

For rhizobia, the effects on aboveground herbivores were only recently investigated by ecologists, though effects on belowground herbivores (Johnson et al. 2006) and defence traits (Johnson et al. 1991) have been occasionally documented. Traditionally, the potential influence of rhizobia has been suggested to be positive for aboveground herbivores due to an increased level of N in plant tissues (Wilson and Stinner 1984). In an experiment Kempel et al. (2009) demonstrated positive effects of rhizobia on the performance of a leaf-chewing insect on an acyanogenic strain of white clover (*Trifolium repens*), whereas on a cyanogenic strain, this effect was not evident. This implies that the effect of increased nutritional quality might be alleviated by increased production of secondary compounds. In contrast aphid reproduction showed no consistent pattern based on rhizobial mutualism. Accordingly, Thamer et al. (2011) documented an increased production of cyanogenic compounds by lima beans growing with rhizobia and an increased resistance against a specialized leaf-feeding beetle (Mexican bean beetle; *Epilachna varivestis*), but no changes in the amount of soluble protein in leaf tissue or leaf mass per area. In the – to our knowledge – only study on the effects of *Frankia* on herbivores, Hendrickson et al. (1993) showed that an inoculation with a mixture of *Frankia* isolates increased the occurrence of aphids on seedlings of different *Alnus* species.

N-fertilization reduces the dependency of plant growth on nitrogen made available by rhizobial mutualism, and the use of N-fertilized plants in experiments is a suitable way to disentangle the effects of N-availability from other effects of rhizobia. Whilst some studies showed higher aphid reproduction on rhizobial plants (Whitaker et al. 2014), other studies demonstrated reduced reproduction of aphids on rhizobial plants compared to N-fertilized plants (Brunner et al. 2015; Dean et al. 2009; but see Dean et al. 2014), indicating that nodulation may have different effects on phloem-feeding herbivores depending on the plant's dependency on rhizobia-derived N. In contrast Katayama et al. (2010) found that a herbivorous spider mite on nodulated soybeans showed higher reproduction rates also at higher soil N levels. Thus, the different form of N derived from rhizobial symbiosis may favour herbivores compared to the form of N derived from soil. Again, this effect may differ between feeding guilds. Dean et al. (2014) showed that the performance of a leaf chewer was higher on rhizobial plants than on fertilized plants with similar total N levels in plant tissue but found no changes in the performance of aphids.

Further, abiotic conditions may mediate the link between beneficial soil microbes and herbivores. Pangesti et al. (2015a) observed the influence of soil composition on the effects of the rhizobacterium *Pseudomonas fluorescens* on a leaf herbivore and the expression of defence-related genes of plants. This is in line with the knowledge that the efficiency of N-fixation by rhizobia also varies across soil types and climatic conditions (Gopalakrishnan et al. 2015). Since differences in

light conditions may also affect carbon availability for plants, a mediating effect of light intensity on the link between symbionts and herbivores seems to be plausible. However, Heath and McGhee (2012) found little evidence for such an effect.

Most experimental studies, however, consider root symbionts as a presence/absence-treatment but ignore the fact that in natural systems plants do not grow with or without mutualists, but instead may differ in the degree of colonization. Whilst it is known that the abundance of mutualists importantly mediates plant performance (Gange and Ayres 1999), its consequences for plant-herbivore interactions are poorly understood. The degree of colonization may affect the plant's ability to cope with herbivory on the one hand, but on the other microbial mutualists and herbivores depend on plant resources and likely compete especially under resource-limited conditions. Garrido et al. (2010) demonstrated that plant tolerance to herbivores was negatively related to AMF colonization, and Vannette and Hunter (2013) showed that the performance of a specialist herbivore was increased with increasing colonization by AMF. These different patterns can be caused by the nonlinearity of the cost-benefit ratio of mutualistic relationships. For instance, the physiological costs of maintaining a mutualism should increase linearly with the degree of mycorrhization or nodulation, whereas the nutritional benefits should – depending on the level of nutrient availability in the environment – not exceed above a certain threshold (Vannette and Hunter 2011). These authors further found a quadratic response to the intensity of mycorrhization by the production of secondary compounds indicating that the cost-benefit ratio of mutualisms may feedback on defence strategies.

2.2 Production of Secondary Plant Compounds

Changes in both plant nutritional quality and defence chemistry have been suggested to be the basis of the effects of root mutualists on herbivore performance (Hartley and Gange 2009, Fig. 1). The differential responses of specialist versus generalist herbivores and leaf chewers versus sap feeders further imply that both mechanisms work simultaneously. Whilst it was initially suggested that additional resources provided by mutualists may make plants more attractive to herbivores on the one hand, but also relax the trade-off between plant growth, tolerance and defence (Herms and Mattson 1992), some studies have shown that the effects on resistance cannot be exclusively explained by a higher availability of nutrients (Fritz et al. 2006; Katayama et al. 2014; Liu et al. 2003). Whilst the effects of root mutualists on plant nutrition and nutritional quality of plant tissue are common knowledge, changes in plant secondary chemistry have become increasingly acknowledged in the last years.

Mutualism with AMF affects the biosynthesis of a wide range of secondary plant compounds that function as anti-herbivore defences (Bi et al. 2007), possibly a relic of the parasitic nature of their evolutionary history (see Sect. 4). Stimulation of the production of a wide range of compounds like terpenes and terpenoids (Demirözer

et al. 2015; Gerlach et al. 2015; Kapoor et al. 2007; Karst et al. 2015; Shrivastava et al. 2015; Tao et al. 2016; Wang et al. 2015), alkaloids (Abu-Zeyad et al. 1999; Andrade et al. 2013), flavonoids (Zubek et al. 2015) and phenolics (Araim et al. 2009; Ceccarelli et al. 2010; Toussaint et al. 2007) is reported in the literature for herbaceous plant species as well as for trees (Mechri et al. 2015). The mutualism with AMF has been repeatedly shown to affect the production of phenolic compounds both quantitatively and qualitatively. This has been found to be especially relevant for soluble phenolic compounds, whereas cell wall-bound phenolics are less affected (Yao et al. 2007). Again, evidence for the effects of EMF on defence compounds is scarce. However, mutualisms with EMF have been shown to increase levels of phytoalexins in roots of trees (Morandi 1996).

The effects of species interactions on the production of plant secondary metabolites seem to be context dependent. For instance, several studies demonstrated the presence and absence of these effects using the same mutualistic partners (Fontana et al. 2009). Moreover, plant ontogeny has been shown to modify the effects of AMF on the cyanogenic potential of plants (Miller et al. 2014). Similarly, for EMF it was shown that different fungus x tree species combinations may either decrease or increase phenol concentrations in willows (Baum et al. 2009).

The ability to increase the production of defence compounds upon herbivore attack (induced defence) is a common way for plants to overcome the high physiological and ecological costs that would occur if maintaining chemical defence under herbivore-free conditions (Ballhorn et al. 2014a). Surprisingly, the research on the effects of root mutualists on secondary chemistry has focused on the expression of constitutive levels of resistance and defence. The few studies which consider induced defence, however, produced conflicting results and demonstrated decreased (Bennett et al. 2009) as well as increased induction (Kempel et al. 2010) of resistance against herbivores or even both patterns across a range of plant species (Kempel et al. 2013). The basis of this altered ability of the plant to respond to herbivore attack seems to be less related to an increase in available resources due to mutualism, but rather is driven by influencing the crosstalk between the salicylic acid (SA)- and jasmonate (JA)-dependent defence pathways (see Sect. 4). However, Bennett et al. (2009) found that the inoculation with a mix of AMF may also suppress inducible direct defence in *Plantago lanceolata* in terms of iridoid glycoside production, whereas Pankoke et al. (2015) found no effect of mycorrhization by *Rhizophagus intraradices* on the metabolome of the same plant species. In contrast and again for *P. lanceolata,* Wang et al. (2015) showed increased levels of catalpol (an iridoid glycoside) due to mycorrhization by *Funneliformis mosseae* already prior to exposure to herbivory and no further increase due to herbivory and therefore suggest a systemic induction rather than priming. It might be speculated that different species of mycorrhizal fungi (or any other variability in experimental conditions) may induce different plant responses. Fontana et al. (2009) demonstrated for the same plant species that mycorrhization by *R. intraradices* also did not increase direct defence via iridoid glycosides but did induce changes in the emission of volatile compounds (see Sect. 3.3). This implies that mycorrhization does not generally enhance the plant's ability to increase defence responses but may

cause a shift of defence strategies. This suggestion was also supported for oaks growing with EMF which showed a decreased expression of genes related to direct defence mechanisms and plant regrowth due to herbivore attack in inoculated oak trees, but a stronger expression of genes related to indirect defence via volatile production and the attraction of natural enemies of herbivores (Bacht 2015).

Rhizobial symbioses have been found to be especially stimulating for the synthesis of nitrogen-intensive compounds (HCN: Thamer et al. 2011, alkaloids: Irmer et al. 2015; Johnson et al. 1987) and also indole as a volatile compound with direct functionality as a repellent against herbivores (Ballhorn et al. 2013). This suggests that in fact changes in the N-availability trigger these effects. In the case of pyrrolizidine alkaloids, nodules are obviously the site of biosynthesis and the source from which these compounds are transported throughout the plant (Irmer et al. 2015). Interestingly, Thamer et al. (2011) showed that nodulation increased the production of cyanogenic defence compounds but not the amount of soluble proteins in tissue. Protein content is an important measure of the plant's nutritional quality, and some proteins are also required for enzymatic detoxification of cyanide (Ballhorn et al. 2009). Thus, the increase of cyanogenic potential of plants was not compensated for by the use of additional N from the rhizobial mutualism for the synthesis of proteins, suggesting a selective channelling to specific physiological pathways. However, this selective channelling might be the result of herbivore feeding since in another study using the same study system but without an attacking herbivore, the levels of both cyanogenic compounds and proteins were increased with rhizobial mutualism (Godschalx et al. 2015). In some cases the contribution of rhizobia to defence compound production seems to be even more complex. Valdez Barillas et al. (2007) have found that rhizobia contribute to the synthesis of alkaloids produced by a fungal endophyte in locoweed (*Oxytropis sericea*), possibly also by supplying nitrogen in the form of specific amino acids, which are precursors of alkaloids.

3 Effects of Root Mutualists on Indirect Plant Defences

3.1 Effects on Herbivore-Natural Enemies Relationships

Since insect herbivores are often strongly controlled by their natural enemies in natural systems, direct or indirect effects of belowground mutualists on parasitoids and predators may importantly mediate the influence of symbionts on plant-herbivore interactions. This idea, however, has received comparatively little attention until now. Gange et al. (2003) found lower infestation rates of leaf miners on plants in relation to AMF colonization in a field assay, but showed under controlled conditions that different AMF species may have varying effects. Likewise, other authors found higher infestation rates and parasitoid performance on mycorrhized plants (Guerrieri et al. 2004; Hempel et al. 2009; Hoffmann et al. 2011a, c, d;

Wooley and Paine 2011). The effects of mycorrhization on the third trophic level are not necessarily a reflection of the effects on herbivores. Moon et al. (2013) demonstrated the positive effects of AMF on different gall makers and leaf miners but observed inconsistent effects on their parasitoids. Similarly, Ueda et al. (2013) showed that the effects of AMF on a predator differed between sampling dates.

The influence of root mutualists on parasitic infections of herbivores has been a rather unexplored area of research until now. In a study system consisting of different milkweed species and commercial AMF inoculation treatments, the monarch butterfly and its protozoan parasite, Tao et al. (2015) found that resistance and tolerance of the caterpillars to the parasite were affected by mycorrhiza via plant species-specific effects on primary (phosphorous) and secondary plant compounds (cardenolides). To our knowledge, this is the only study until now demonstrating effects of root mutualists on herbivore-parasite interactions. Likewise, only few studies have demonstrated that the effects of root mutualists on the third trophic level have the potential to alleviate the more detrimental effects of herbivores on mycorrhized plants (Hoffmann et al. 2011b, c).

3.2 Production of Extrafloral Nectar

One widespread strategy of plants to reduce herbivory is the production of extrafloral nectar (EFN) to attract mutualistic predators, particularly ants (Weber and Keeler 2012). Plants may be obligate ant mutualists that constitutively produce EFN or facultatively ant-defended plants that express EFN upon herbivore attack (Kost and Heil 2008; Wagner 1997). The production of EFN is associated with metabolic costs (Heil 2011), and its production may therefore be constrained by C-allocation within the plant (Ballhorn et al. 2014b). Additional C sinks like root mutualists thus may reduce the plant's ability for EFN production. Accordingly, inoculation with AMF has been found to cause a reduction of the production of EFN by *Vicia faba* (Laird and Addicott 2007). EFN production may not only have physiological but also ecological costs since the attraction of ants has been shown to deter beneficial pollinators (Ness 2006). Thus, since mycorrhized plants often show increased levels of secondary compounds (see Sect. 2.2), the reduced production of EFN might not be due to C limitation but as an option to save unnecessary expenditure on defence.

The first study investigating the influence of rhizobial mutualism on EFN showed that rhizobia decreased EFN production only upon induction by herbivore feeding whilst non-induced plants showed no effect (Summers and Mondor 2011). This implies that nodulated plants favour other resistance mechanisms than indirect defence via predator attraction. Interestingly, the only study so far which has investigated the effects of rhizobial nodulation on direct and indirect plant defence demonstrated that the decrease of EFN production in rhizobial plants was counteracted by an increased production of N-based cyanogenic compounds (Godschalx et al. 2015, Fig. 2). Thus, additional resources from root mutualists

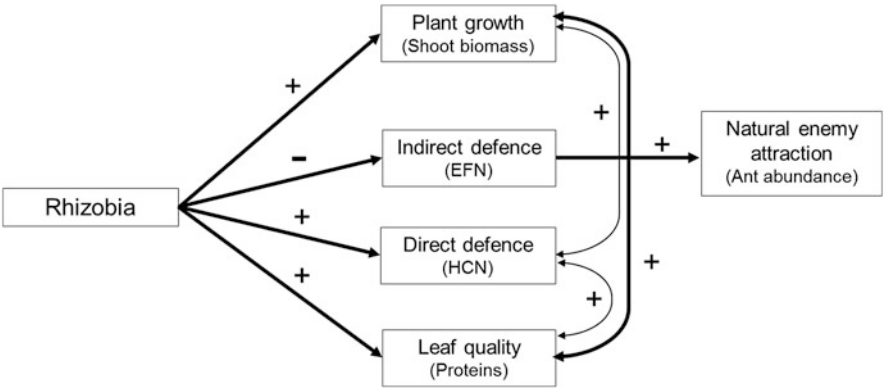

Fig. 2 Relationship between rhizobial symbiosis and plant traits related to growth and defence in lima bean *(Phaseolus lunatus)*. The potentially positive effects of rhizobia on herbivores by increasing plant biomass and leaf quality (concentration of soluble proteins) are counteracted by an increase of cyanogenesis in leaves. Plant growth and leaf quality showed significant positive covariation with each other but also marginally significant positive covariation (*thin lines*) with direct defence. Thus, root mutualists can relax growth-defence trade-offs in plants instead of favouring one of these processes as alternative strategies. Moreover, rhizobia induced a switch in the defence strategies. Plants growing without rhizobia showed a higher production of extrafloral nectar (EFN) which showed no covariation with other plant traits. Higher production of EFN attracted more natural enemies of herbivores (ants). The high carbon demand of both rhizobia and EFN can be interpreted as the physiological basis of the switch in defence strategies. Modified from Godschalx et al. (2015)

might induce a shift towards direct defences as a way to avoid physiological and ecological costs associated with indirect defences and the maintenance of the mutualism.

3.3 Production of Volatile Organic Compounds

Whilst in some studies it has been concluded that mycorrhizal fungi attract parasitoids via changes in plant architecture (Gange et al. 2003), others demonstrated that mycorrhized plants are clearly more olfactory attractive to parasitoids than un-mycorrhized plants (Guerrieri et al. 2004). Plants produce a wide range of volatile organic compounds (VOCs) upon herbivore attack some of which are known to attract carnivores, mainly parasitoid wasps, and therefore act as an indirect defence (e.g. Rostás and Turlings 2008). VOCs can also have a function in intra- and interspecific defence-associated plant-plant signalling (Heil and Bueno 2007) and may serve as a direct defence by repelling herbivores (Heil 2004).

The first evidence of mycorrhizal effects on the production of VOCs comes from observations of the mutualism with EMF, which has been found to increase the production of several volatile compounds in roots of trees which are known to

impede the growth of pathogenic fungi (Karst et al. 2015; Krupa et al. 1973; Krupa and Fries 1971). Some of these compounds have also been found to be more abundant in the aboveground parts of mycorrhized trees and are effective as direct and indirect defences via predator attraction (Karst et al. 2015). In contrast, Manninen et al. (1998) found no changes in volatile compounds emitted from the shoots and roots of pine growing with or without EMF. The mycorrhiza-induced production of VOCs seems to be not only triggered by herbivore feeding but also other stress factors. For instance, pine seedlings showed a pronounced production of volatiles when grown in caesium-contaminated soil together with mycorrhizal fungi (Henke et al. 2015).

The colonization of AMF is also known to affect volatile production in plants. For *Satureja macrostema*, it was shown that the presence of AMF caused a higher production of volatile terpenes (Carreon-Abud et al. 2015). In contrast, upon induction by an herbivore, AMF-mycorrhized plants showed a weaker production of volatile sesquiterpenes (Fontana et al. 2009). This was also demonstrated by Babikova et al. (2014a) together with an increased attractiveness of mycorrhized plants for aphids. Interestingly, this effect was independent of a previous infestation by aphids. Again, rather than a general reduction or increase of volatile production, the composition of the volatile blend was found to be differentially altered by AMF colonization (Leitner et al. 2010; Rapparini et al. 2008; Schausberger et al. 2012). Schausberger et al. (2012) demonstrated that the responsiveness of a predatory mite to mycorrhiza-mediated changes in volatile blend depends on the duration of feeding by an herbivorous mite, with strong effects observed only if the plants were infested for at least 6 days. Thus, differences in intensity, duration and possibly also the feeding mode of herbivores may contribute to the variability of the observed effects. To complicate things further, different fungal species may have different effects on volatile blends of belowground (Sun and Tang 2013) or aboveground parts of the plant (Hart et al. 2015).

The importance of rhizobia for volatile production was investigated for the first time in the study of Ballhorn et al. (2013). In their experiment, rhizobial lima bean plants produced less VOCs overall following induction by jasmonic acid application but showed strong changes in the composition of the volatile blend compared to rhizobia-free controls. Rhizobia-colonized plants emitted lower amounts of compounds produced via the octadecanoid, mevalonate and non-mevalonate pathways, whereas the same plants released higher amounts of the shikimic acid-derived compounds. In particular, the amount of the N-containing indole was enhanced by nodulation, suggesting a role for the additional N derived from rhizobia. In an olfactometer trial, Ballhorn et al. (2013) sowed that a specialist herbivorous beetle preferred non-rhizobial plants and that indole acts as a direct repellent compound (see also Veyrath et al. 2016). Moreover, recent studies revealed an even more complex role for indole in plant-herbivore interactions. Erb et al. (2015) showed that herbivore-induced indole enhances the induction of defensive volatiles in neighbouring plants and primes defence reactions in leaves of the attacked plant.

Changes in herbivore-induced production of VOCs by plants have also been observed for other beneficial soil-borne microbes. For instance, the volatile blend of rhizobacteria (*P. fluorescens*)-treated *Arabidopsis* plants was less attractive to parasitoids (Pineda et al. 2013b) but more attractive in other studies using different strains of the same bacterium (Pangesti et al. 2015b). Further, a non-mycorrhizal plant growth-promoting fungus caused an increased attractiveness of tomato plants for an aphid parasitoid (Battaglia et al. 2013). Clarification is required of whether there is a general pattern of a reduction or increase of certain volatile compounds from specific metabolic pathways and with specific functions in communication between plants, herbivores and parasitoids in response to beneficial soil microbiota. Plant growth-promoting soil bacteria may further produce VOCs themselves (reviewed in Choudhary et al. 2016) which may serve as signal molecules for plant defence. A role of these compounds for anti-herbivore defence seems to be possible but has not been demonstrated yet.

4 Molecular Background of Plant-Mediated Mutualist-Herbivore Interactions

First explanations of the increased resistance of mycorrhized plants against herbivores favour the role of increased availability of nutrients and the accompanying changes in the C:N ratio (Jones et al. 1991), P-content (Sampedro et al. 2011) or the relaxation of the defence-growth trade-off (Bennett et al. 2006). However, several experiments have demonstrated that a higher availability of nutrients does not mimic or alleviate the effects of root mutualists on plant resistance (e.g. Brunner et al. 2015; Dean et al. 2009, 2014; Fritz et al. 2006; Katayama et al. 2014; Liu et al. 2007). Together with the fact that many of the changes in plant resistance and defence depend on previous induction by herbivore feeding (see above), this indicates that the mutualism causes modifications within the host plant. Ultimately, the interactions between fungi, plants and herbivores depend on the regulation of plant gene activities. Recent developments in genomics allow the analysis of the genetic basis of biotic interactions (e.g. Cartieaux et al. 2008; Colebatch et al. 2002a, b; Michel et al. 2006). These patterns are the first step for the identification of genes involved in biotic interactions including interactions with the mycorrhizal fungi and herbivores (Wullschleger et al. 2007). Herbivores influence the transcription for hundreds of target plant genes (Baldwin et al. 2001; Hermsmeier et al. 2001; Roda and Baldwin 2003; Schmidt et al. 2005), perhaps leading to an entire metabolic reorganization (Hui et al. 2003). Similarly, interactions between plants and root symbionts lead to changes in the expression of genes in the host plant (Wiemken and Boller 2002). Some of these genes are relevant for the allocation of resources as well as reactions to stress and defence (Herrmann and Buscot 2007; Liu et al. 2007). Therefore, the interactions between mycorrhizal

fungi and herbivory are strongly accompanied by changes in genetic expression processes in host plants.

Several publications have extensively reviewed the molecular background of root mutualist-mediated induced resistance of plants to herbivores and pathogens (e.g. Cameron et al. 2013; Jung et al. 2012; Pangesti et al. 2013; Pieterse et al. 2013; Pozo and Azcon-Aguilar 2007; Zamioudis and Pieterse 2012); thus we will only give a short outline here. The establishment and maintenance of symbiosis with beneficial root microbiota are dependent upon a modulation of molecular pathways related to the synthesis of the plant hormones jasmonic acid (JA), salicylic acid (SA) and others. This modulation can be interpreted as a result of the possible evolution of mycorrhizal mutualism from a parasitic interaction (Remy et al. 1994) which is obviously still reflected by an initial recognition of mutualists as pathogenic invaders (Pozo and Azcon-Aguilar 2007). The resulting suppression of SA production in plants is necessary for the establishment of both rhizobial and mycorrhizal mutualism (Zamioudis and Pieterse 2012) and is compensated by an increased JA production (reviewed in Pozo and Azcon-Aguilar 2007). Accordingly, increased JA levels have been observed in mycorrhized roots of several plants (see Bi et al. 2007 for review), but also free-living plant growth-promoting bacteria are known to induce anti-herbivore defence via the expression of the JA pathway and suppression of the SA pathway (Pangesti et al. 2015a; Pineda et al. 2012; Planchamp et al. 2015). In the study of Pineda et al. (2012), transcriptomic analyses revealed an expression of the JA pathway only upon attack by a generalist aphid but not by a specialized aphid. Thus, the degree of specialization may not only determine the responsiveness of defence reactions but also their induction. The pathways of both hormones are activated by different plant enemies and induce different defence responses. Whilst the SA pathway induces plant responses upon attack by biotrophic pathogens, JA coordinates defence responses against necrotrophic pathogens and herbivores (Glazebrook 2005; Pieterse et al. 2008). The trade-off between the synthesis and the corresponding defence pathways is the result of the depression of both the sensitivity and biosynthesis of JA by SA (reviewed by Ballare 2011). The stimulated JA pathway and accompanying expression of defence-related genes lead to a primed plant state which allows for a quick and efficient activation of defence mechanisms like the production of toxic secondary compounds (Jung et al. 2012; Pozo and Azcon-Aguilar 2007). This phenomenon is referred to as induced systemic defence and has been described for mycorrhizal fungi and rhizobacteria (Conrath 2009; Pineda et al. 2010; van Loon et al. 1998). In accordance with the finding of Kempel et al. (2010) who demonstrated interacting effects of induction and mycorrhization on an increased resistance against herbivores in different herb and grass species, JA is known to induce defence responses in both monocotyledonous and dicotyledonous plants (Okada et al. 2015). The cross-communication of JA- and SA-related signalling pathways (including other plant hormones like ethylene, abscisic acid, etc.) regulates plant resistance against both pathogens and herbivores (Koorneef and Pieterse 2008; Pozo et al. 2004) and results in a trade-off between the resistance against these two groups of organisms (Beckers and Spoel 2006).

This makes the possible role of root mutualists in the overall resistance of plants against biotic antagonists even more complex. In addition to the effects of defence expression, an increased level of JA may lead to rapid photosynthate export from leaves to stems and roots thus decreasing nutritional quality of leaf tissue for herbivores, but may also lead to greater plant tolerance by shielding resources (Babst et al. 2005). Further, defence-related signalling may also operate via mycorrhizal networks and may therefore not only induce defences in the attacked plants but also in neighbouring plants (Babikova et al. 2014b).

Pineda et al. (2013a) demonstrated that stressful environmental conditions (e.g. nutrient deficiency, drought, salt and ozone) intensify the impact of beneficial soil microbiota on herbivores. This suggests a mediation of the crosstalk of plant signalling pathways by abiotic conditions and provides a conceptual framework for placing microbe-plant-herbivore interactions in the context of a changing environment.

5 Digging Deeper into Complexity

5.1 Effects on the Consumer Community

Since root mutualists are known to not only affect plant performance and defence against herbivores but also the structure and dynamics of plant communities (van der Heijden et al. 1998a, b, 2006, 2008), it might be a trivial assumption that they also affect the structure of consumer communities. However, empirical evidence for such effects is scarce, especially for natural systems where experimental manipulations are difficult.

Fungicides are sometimes applied to reduce mycorrhization in plant communities in order to investigate accompanying changes of the consumer community. For the invasive plant *Deinandra fasciculata*, it was shown that the suppression of soil fungi in the new range does not affect herbivore density and community composition but increased predator density and altered predator community composition (Schreck et al. 2013). Kula and Hartnett (2015) interpreted the negative effects of AMF reduction by fungicides on herbivory in a tallgrass plant community as a result of the increased plant diversity in this treatment. However, in natural plant communities, mycelia of mycorrhizal fungi produce so-called common mycelial networks which connect the roots of individuals within and across plant species (Simard and Durall 2004). These networks may serve as paths for the exchange of herbivory-induced defence molecules. Babikova et al. (2013) demonstrated that the composition of volatiles of noninfested plants changes if they are connected to aphid-infested plants via mycorrhizal hyphae causing lower attractiveness of plants for aphids and higher attractiveness for aphid parasitoids. Thus, in addition to the nutritional benefit to plants involved in these hyphal networks (Simard and Durall

2004), they also may have community wide consequences for associated consumer species even if only single plants are attacked by herbivores.

In general, the rhizobial symbiosis seems to favour the abundance and to change the community composition of herbivores (and their natural enemies) in plant communities, whereas there seem to be no general effects on species richness or evenness (Katayama et al. 2011b; Simonsen and Stinchcombe 2014).Katayama et al. (2011a) found an increased abundance of both sap-feeding and chewing insects on soybeans growing with rhizobia under field conditions; thus, the herbivore feeding mode seems to be of minor importance in this case. Unfortunately, from the data provided by the authors, it is not clear if this increase is caused by the build-up of higher population densities of herbivore species or by an increase of species numbers.

Nodulation by rhizobia has been shown to change the composition of honeydew produced by aphids feeding on the host plants. Whitaker et al. (2014) showed that aphids on plants with rhizobia produced honeydew with higher contents of sugar and lower contents of nitrogen, probably due to the fact that the nitrogen form derived from the mutualism can be better assimilated by the aphids. This qualitative change in honeydew composition may not only affect mutualistic relationships between aphids and ants but also nutrient dynamics in the soil and food webs (Milcu et al. 2015; Stadler et al. 2001).

5.2 Interacting Effects in the Rhizosphere

The taxonomic and functional diversity of organisms in soil is known to be extremely high, and soil food webs are characterized by a multitude of species interactions with potential effects on plants and ecosystem processes. Our perception of the effects of soil biota on the performance of plants and their associated organisms is predominantly based on studies investigating separate effects of specific biota but only rarely considers the role of potential interactions between soil biota. However, these interactions are often an implicit part of many experiments which manipulate one group but neglect the potentially mediating role of other organisms. This is especially evident in field studies or even experiments with non-sterilized soils where one group is manipulated (excluded or added), and any effects have to be interpreted as the combined effects of the specific player and its interactions. There are only few studies which investigated how interactions in the soil community involving mutualistic organisms cascade up to aboveground herbivores.

Multiple mutualist effects are a common phenomenon in natural systems, and their consequences for the partners are affected by a number of factors in complex ways (Afkhami et al. 2014). In essence, such effects are mediated by the balance between costs and benefits between partners and the control by plants over these interactions. However, these relationships are constrained by the overlap and crosstalk between shared genetic pathways of the host that control the different

mutualists (Marx 2004; Genre and Russo 2016). In the case of two nutrient-provisioning mutualistic partners, the possibility that one mutualist may reduce the benefit of the other partner, along with the prospect of competition for colonization sites of plant roots between mycorrhiza and rhizobia, has been discussed (Kiers and Denison 2008; Larimer et al. 2010). However, positive feedbacks between mycorrhization and nodulation have been also observed (Antunes et al. 2006). Depending on resource conditions both in terms of soil nutrients and photosynthates produced by the plant (e.g. depending on light conditions) synergistic, neutral and antagonistic consequences of these tripartite mutualisms on plant growth can be expected. However, synergistic effects of co-inoculations of rhizobia and mycorrhizal fungi on plant performance seem to be the prevailing pattern (Ossler et al. 2015). The combination of field studies and greenhouse experiments suggests that inoculation with one mutualistic partner may limit the ability to colonize the plant root by the other partner on the one hand but that colonization by both rhizobia and AMF is positively genetically correlated and no evidence for a colonization trade-off under field conditions on the other (Ossler et al. 2015).

Mycorrhizal fungi and rhizobia do not only interact with each other but are also known to interact with a multitude of other microbiota in soil with consequences on plant growth (e.g. Chandanie et al. 2009; Frey-Klett et al. 2007; Medina et al. 2003). The co-inoculation of rhizobia with a wide range of free-living plant growth-promoting bacteria was found to enhance nodulation, N-fixation, the production of phytohormones and consequently the performance of several legumes (see Gopalakrishnan et al. 2015 for review). It seems to be a reasonable assumption that such effects will translate into changes of the relationship between plants and herbivores. The co-inoculation with AMF and PGPB can have positive effects on plant performance (Medina et al. 2003). Synergistic effects of AMF and free-living N-fixing bacteria on the production of secondary defence compounds have also been demonstrated (Awasthi et al. 2011). Interacting effects of AMF and PGPF are more rarely investigated but may also have synergistic effects on plant growth and resistance against soil-borne pathogens (Chandanie et al. 2009). Only very few studies investigated consequences of interactions between different soil microbiota on aboveground herbivores. The interaction of AMF and free-living N-fixing bacteria was shown to have independent and additive effects on host plant choice and performance of a herbivorous mite (Khaitov et al. 2015), but synergistic effects on the production of secondary compounds have also been described (Awasthi et al. 2011). Martinuz et al. (2012) showed that rhizobia and a mutualistic strain of the root-colonizing endophyte *Fusarium oxysporum* independently induced systemic resistance against an aphid on squashes but had no interacting effects. Moreover, some free-living bacteria (mycorrhization helper bacteria) are known to be closely associated with mycorrhizal fungi and are now seen as an integral component of the mycorrhizal mutualism (Bonfante and Anca 2009). Gram-positive actinomycetes represent a widespread group of such helper bacteria (Frey-Klett et al. 2007) that have a number of effects on plant growth, enzyme production and disease resistance (Lehr et al. 2007; Tarkka and Hampp 2008).

In general, the multitude of responses arising from interacting effects of rhizosphere mutualists on plant growth and herbivore performance are in line with the current recognition of the whole soil microbiome as a complex mediator of plant growth and ecosystem functions (Berendsen et al. 2012; Hol et al. 2010). Recent studies do not show a clear relationship between microbiome complexity and effects on plant-insect interactions (Pangesti et al. 2013). Future studies should focus on patterns and principles in the complexity of systemic effects of the soil community on plants and aboveground interactions. An important aspect within this framework is the ability of the plant to actively modulate the soil community (Bezemer et al. 2010). Recently, it has been discussed that the stimulation of the plant's defence system cannot be assigned to the exclusive influence of mutualists like mycorrhizal fungi but has to be interpreted as a cumulative effect of the microbial community in the mycorrhizosphere including nonpathogenic microbiota (Cameron et al. 2013). This is supported by a number of studies which shows interacting effects of root mutualists and free-living bacteria and fungi on plant performance and plant resistance (see above). Similarly, Santhanam et al. (2015) showed that a consortium of five root-associated bacteria was necessary to induce resistance against sudden-wilt disease in *Nicotiana attenuata*, whereas single bacteria species did not show such effect (see also Sarma et al. 2015 for the enhanced efficiency of microbial consortia). Taken together, this orchestrated action of plants and their associated herbivores and microorganisms above- and belowground strongly concurs with the concept of a *holobiont-like* system (Castell et al. 2015). Such systems are evolving systems of interacting biological units (in this case individuals and populations) with the ability to adapt to changing environments. The close physiological and genetic interplay between the mycorrhizosphere microbiome and the plant can thus be interpreted as integral part of the coevolution with plant enemies (herbivores, pathogens).

5.3 *Feedback Effects of Herbivores on Root Mutualists*

Since herbivory may limit the ability of the plant to allocate resources to their mutualists, colonization rates and abundance of beneficial microbiota may be affected by herbivore damage. For instance, EMF may use between 10 and 50% of the photosynthates of host plants (Hobbie and Hobbie 2006; Simard et al. 2002) and may therefore be very sensitive to herbivory-induced changes in C-allocation (Markkola et al. 2004). For seedlings of red oak, Frost and Hunter (2008) observed a 63% decrease in allocation of carbon belowground and an increased allocation to new foliage following herbivory. Similarly, herbivory by caterpillars of the gypsy moth (*Lymantria dispar*) decreased C-partitioning towards belowground parts with mature leaves being the carbon sink (Babst et al. 2008). Accordingly, several studies demonstrated detrimental effects of herbivory on mycorrhizal colonization (Gange et al. 2002a; Gehring and Whitham 1994; Markkola et al. 2004; Saravesi et al. 2014; Trocha et al. 2016). Other studies, however, showed no effects (Varga

et al. 2009) or an increase of AMF colonization upon vertebrate or invertebrate grazing but also changes in the diversity and composition of the AMF community (Eom et al. 2001; Kula et al. 2005; Mikola et al. 2005; Tawaraya et al. 2012; Techau et al. 2004). However, Wearn and Gange (2007) found that the intensity of grazing might play a role, and compared to no or intensive grazing, moderate herbivory by rabbits increased mycorrhization of grasses under field conditions. This is in line with the observation that moderate levels of feeding may increase the allocation of carbon belowground (Babst et al. 2005). Of course, soil conditions may shape such effects since the allocation of C to the mutualist is dependent on the benefit of the interaction for the plant. In a recent meta-analysis, Barto and Rillig (2010) found that biologically meaningful reductions of mycorrhizal colonization due to herbivory are rather rare. Moreover, herbivory-induced changes in mycorrhizal colonization seem to be transient and may disappear within a few days after the absence of herbivory (Nishida et al. 2009). Further, the reduction of photosynthetic capacity by defoliation may change the strategy of mutualists to invest resources. For instance, Garcia and Mendoza (2012) found that defoliation did not change root length colonized by AMF and spore density but decreased vesicular colonization and increased hyphal density. Accordingly, with these controversial findings, a recent meta-analysis showed a general but nonsignificant trend towards negative effects of herbivory on AMF (Yang et al. 2014). Similarly, defoliation has been shown to have no (Pastore and Russell 2012; Techau et al. 2004), positive (Heath and Lau 2011) or negative effects (Kempel et al. 2009) on the number of nodules. However, at least in some cases, this effect is solely attributable to changes in root biomass but not to relative nodulation intensity (Kempel et al. 2009).

Alternatively to changes in C-allocation patterns, colonization rates of root mutualists might be affected by herbivory via changes in signalling networks. For instance, the herbivory-induced increase of jasmonic acid levels has been found to increase both nodulation (Hause and Schaarschmidt 2009) and mycorrhization rates (Kiers et al. 2010). Landgraf et al. (2012) found that repeated wounding of leaves of *Medicago truncatula* locally and systemically increased JA levels and the expression of JA-induced genes with positive effects on AMF colonization, but no effects on nodulation. Using a field quantitative genetic approach, Heath and McGhee (2012) found that the negative phenotypic correlation between nodulation and herbivory was mediated by plant biomass allocation patterns. There was, however, a positive genetic correlation between nodulation and herbivory indicating shared genetic pathways for both interactions. The exact underlying basis for this relationship remains still elusive, but a central role for the regulation of JA-dependent plant responses seems to be plausible (Heath and McGhee 2012). This again points towards the role of a common evolutionary background of nodulation (and probably other forms of symbiosis) and resistance against herbivores in plants. This common background again may account for the parasitic origin of the mutualism with both mycorrhizal fungi (Remy et al. 1994) and rhizobia (Dresler-Nurmi et al. 2007) which can be interpreted as the basis for the induction of plant defence responses by these mutualists (see Sect. 4).

Beside effects of herbivores on root symbionts via changes in plant physiology, herbivores may directly affect plant-symbiont interactions. For instance, aphid honey dew production may not only influence nitrogen availability in soil but also the relative importance of the direct uptake via plant roots and biological nitrogen fixation via rhizobia (Katayama et al. 2014).

5.4 The Role of Genotypic Variability of Microbiota and Plants

Both the interaction between plants and their mutualists and the interactions between plants and herbivores are the result of a coevolutionary history which may show a high spatial variability (Thompson 2005). Thus, ecologically differing environments (selection mosaics) may lead to genotypes with locally adapted interaction traits. In the case of root mutualists, variation of abiotic resources is known to affect the benefit/cost relationship of this interaction both by affecting the beneficial role of mutualists as providers of nutrients (Neuhauser and Fargione 2004; Weese et al. 2015) and by affecting the ability of plants to provide photosynthates under different light conditions (Ballhorn et al. 2016). Similarly, plant defence and resistance are highly variable amongst populations and genotypes (Castillo et al. 2014; Schädler et al. 2010). In a tritrophic interaction between plants, root mutualists and consumers, this leads to *genotype x genotype x genotype x environment* interaction and consequently to a high variability of possible outcomes of this interaction especially along gradients of environmental variability or experimental conditions. However, evidence for selection mosaics of plant-root mutualist relationships is still limited and contradictory (Barrett et al. 2012; Heath et al. 2010; Hoeksema et al. 2009; Piculell et al. 2008). Nevertheless, genotypic effects on the outcome of mutualisms are known both for the microbial mutualist and the plant, and plants growing in the field with locally adapted mycorrhizal fungi have been shown to grow better and suffer less herbivore damage than plants growing with a commercial inoculum (Middleton et al. 2015).

It has been shown that the effects of AMF colonization on the enhancement of alkaloid content in the roots of *P. lanceolata* and on volatile blends released upon herbivore feeding by *M. truncatula* (Leitner et al. 2010) are plant genotype dependent. Also, the effect of leaf herbivory on nodulation was found to be variable amongst plant populations (Heath and Lau 2011). In comparison, the genotype of AM fungi has been found to have rather minor effects on the herbivores in some cases (Wooley and Paine 2007) but important effects in others (Roger et al. 2013). Further, different AMF genotypes differentially attracted a parasitoid wasp (Wooley and Paine 2011), whilst several studies showed contrasting effects of different strains of the same free-living PGPB on defence traits, e.g. of *P. fluorescens* (e.g. Pineda et al. 2013b; Pangesti et al. 2015b, see also Pangesti et al. 2013). Moreover, some genotypes of mutualists may be potentially

exploitative partners of plants (Douglas 2008) that can be interpreted as a relic of their parasitic evolutionary origin (Remy et al. 1994). These genotypes gain fitness benefits from the plants without providing resources to the plant (Bronstein 2001). The effects of such "cheating" genotypes for rhizobia-plant-insect interactions have been investigated by Simonsen and Stinchcombe (2014). They found that herbivore damage in the field was higher on plants with beneficial rhizobia than on plants with inefficient rhizobia. Thus, herbivory may help to stabilize the coexistence of beneficial and exploitative mutualists by causing a higher damage to plants with beneficial mutualists.

6 Conclusions and Future Directions

6.1 Root Mutualists and the Defensive Phenotype of Plants

Traditionally, the main role of root mutualists for plant performance has been discussed as the result of increased nutrient availability with positive effects on plant growth and nutrition. Accordingly, the impact of these mutualists on herbivores was initially interpreted as an effect of this increased availability of resources on the herbivores directly or as a mediator of trade-offs between growth, defence and tolerance in plants (Bennett et al. 2006; growth-differentiation balance hypothesis, see below). Here, we show that root mutualists like mycorrhizal fungi and rhizobia can be seen as an integral part of the plant's defence system and mediate the link between herbivores and plants in multiple ways (Fig. 3). In recent years it became obvious that virtually all aspects of plant defence are affected by root mutualists which can therefore be seen as important mediator of the plant's defensive phenotype. Root mutualists are part of the soil community, and their effects on plants depend on synergistic and antagonistic interactions with other soil biota. This review focussed on interactions with microbiota but interactions with soil fauna are also common (Alguacil et al. 2011; Johnson and Rasmann 2015; Koller et al. 2013a; Koller et al. 2013b; Li et al. 2013; but see Eisenhauer et al. 2009). The resulting changes in nutritional quality of the plant and the activation of signalling pathways are often modulated by an induction through herbivore feeding which may determine the production of compounds and substances related to direct or indirect defence, but also the allocation of nutrients and photosynthates to growth or resistance responses of the plant. The direct impact of such changes on the herbivore is the net effect of changes in nutrient content and production of secondary compounds and depends on the herbivore feeding mode and the resulting sensitivity to plant defence responses. Recently, there has been an increasing awareness of a mediation of indirect defence mechanisms by root mutualists via a changed production of volatile organic compounds and a reduced production of extrafloral nectar with consequences for the attraction of natural enemies of the herbivores. Direct and indirect defences seem to be used by plants

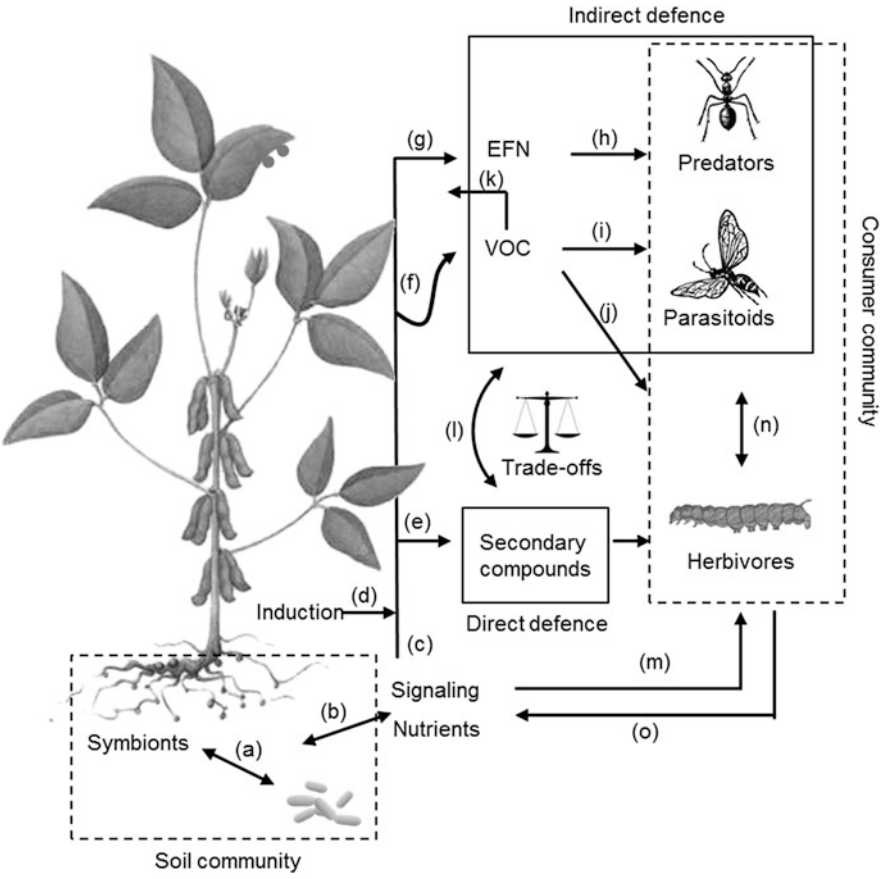

Fig. 3 Conceptual model of the influence of root symbionts on the defensive genotype of plants. The activity of root symbionts and their interactions with other biota of the soil community (*a*) cause an increase of plant nutrition and priming of plants via modulation of signalling pathways (*b*). This leads to modification of the plant's defence system (*c*) which is often triggered by an induction by stresses like herbivore feeding (*d*). As a result, the production of secondary defence compounds is often increased (*e*). Root symbionts do also affect indirect defence traits. The composition of volatile organic compounds (VOC) has been observed to change due to symbiosis (*f*) with consequences for parasitoid attraction (*i*), repellence of herbivores (*j*) and probably also for plant–plant communication (*k*). Plants growing with root symbionts have been shown to produce less extrafloral nectar (EFN) (*g*) resulting in a reduced attractiveness for predators (*h*). Therefore, root symbionts interfere with trade-offs between several defence mechanisms (*l*), e.g. by shifting resources to direct defence and tuning down indirect defences which may compete with symbionts for the same resources (photosynthates) like EFN. The interplay between increased nutritional quality (*m*) and plant defence syndromes affects the performance of herbivores depending on their feeding mode and specialization. Finally, due to the plant-mediated effects of symbionts on the performance and behaviour of herbivores and their natural enemies as well as due to consequently changed top-down and bottom-up effects (*n*), root symbionts are mediators of the structure of aboveground consumer communities. By reducing the amount of available photosynthates and inducing responses in signalling pathways (*o*), herbivory may feedback on root symbionts and interacting biota in the soil (*b*)

to a certain degree as alternative strategies (Ballhorn et al. 2008) with given costs and benefits which can be mediated by mutualists. Trophic linkages between herbivores and their predators and parasitoids further lead to changes in the consumer community associated with plants. Whilst this review focusses on the linkages between belowground mutualists and aboveground enemies, there is increasing evidence that also the linkages involving aboveground mutualists like pollinators and seed dispersers are further important in this context (Rodriguez-Echeverria and Traveset 2015). Finally, even if there is evidence that the establishment of mutualisms and herbivory have a joined genetic basis, herbivory-induced changes in C-allocation and defence responses may lead to feedback responses of mutualists to herbivory.

6.2 *Root Mutualists in the Context of the Rhizosphere Microbiome*

The stimulation of the defence system of plants leading to priming and systemic induced resistance seems to be a key process regulating the plant-mediated interactions between belowground mutualists and aboveground herbivores. However, given the *holobiont-like* nature (Castell et al. 2015; see Sect. 5.4) of the plant-soil microbiome system, interactions with functionally diverse mycorrhizosphere organisms may importantly mediate these effects and may also have shaped the coevolutionary dynamics within this system. This aspect has been largely neglected in many experiments using inoculation with a given root symbiont without controlling for the (interacting) effects of other microbiota. Future studies should experimentally disentangle the effects of specific biota in the rhizosphere. A further possible way to assess the importance of mycorrhizal fungi without affecting the root-associated microbiome might be the use of transformed plant lines with silenced key genes of the symbiosis pathway (Groten et al. 2015). However, it has to be taken into account that there might also be indirect or side effects upon transformation within the metabolic response network. In a further step, it would be experimentally and conceptually challenging to scale up from controlled experiments under artificial and sometimes axenic conditions to the patterns which can be observed in natural systems with a functionally complex microbiome.

6.3 *Linking Mechanisms to Defence Strategies*

Again, special attention has to be turned on the crosstalk between differentially expressed signalling pathways and phytohormones as well as the underlying genetic mechanisms (e.g. transcriptional changes) and element allocation patterns. Despite the considerable progress regarding molecular techniques and the

generation of transcriptomes in the last years, our understanding of the relationship between changes in the regulation of genes as well as gene families (related to certain metabolic pathways) due to biotic interactions and observed physiological effects and ecological effects is still in its infancy. Tarkka et al. (2013) assessed transcriptional changes in oak RNA in response to seven functionally different biotic interactors including EMF and herbivores and found highly diverse patterns of differentially expressed contigs related to different metabolic pathways like growth, defence, nutrient and sugar transport as well as the production of different hormones. This supports the view of Pangesti et al. (2013) that the outcome of microbe-plant-insect interactions depends on the orchestrated interplay of different hormonal pathways regulating not only defence but also growth and development of plants. The relative importance of growth and defence as functions of the primary and secondary metabolism, respectively, is hypothesized to vary across levels of resource availability (growth-differentiation balance hypothesis, see Herms and Mattson 1992). The decrease of sink strength of growth which may lead to a higher availability of carbon to differentiation processes including defence is usually related to low levels of nutrients which are provided by root mutualists like nitrogen and phosphorous (Matyssek et al. 2012). Thus, root mutualists can be expected to be important mediators of this relationship since they do not only provide additional resources but also modulate metabolic pathways. By doing so, however, root mutualists may not necessarily favour growth or defence as alternative strategies (see also Fig. 2). Accordingly, Tao et al. (2016) demonstrated that the effects of AMF on tolerance and chemical defence in milkweeds are correlated with their effects on plant nutrition and growth, suggesting that an increased resource acquisition due to mutualism compensates costs of chemical defence. Similarly, in lima bean nodulation has been shown to favour both plant growth and anti-herbivore defence by cyanogenesis (Thamer et al. 2011), a kind of defence for which evidence for the validity of the growth-differentiation hypothesis has been demonstrated (Ballhorn et al. 2014a).

6.4 Cultivated Plant Species: Application or Special Case?

A large part of studies have been conducted with crop plants or other cultivated plant species (especially in the case of legumes and rhizobia). This might have consequences for any findings regarding the influence of root mutualists on defence strategies of plants. Kempel et al. (2011) demonstrated that certain trade-offs between defence strategies can be found in wild plants but not in cultivated plants. Cultivated plants are the result of artificial selection without any selection pressure by herbivores, and therefore these plants are released from evolutionary forces that shape trade-offs in nature (Leimu and Koricheva 2006). In contrast, secondary compounds have sometimes been selectively eliminated in cultivated plants (Wink 1988), resulting in a greater resistance of wild ancestors against various pests than their cultivated counterpart (de Lange et al. 2014). The loss of defence trade-offs in

cultivated plants indicates that evolutionary rather than physiological forces shape these trade-offs (Kempel et al. 2011). Thus, the transfer of findings from studies with cultivated plants to natural systems has to be questioned. The importance of beneficial soil microbiota for plant resistance and defence syndromes in wild versus cultivated plants deserves further experimental comparisons. This might be a precondition for the application of beneficial microbiota to pest-related problems in agriculture and the enhancement of plant productivity (Crotti et al. 2012; Tkacz and Poole 2015; Vannette and Hunter 2009). Moreover, breeding crop plants for traits that promote beneficial linkages between root mutualists and plant resistance and the use of mutualists have been suggested to be a potential contributor of food security (Orrelland and Bennett 2013). This of course requires in-depth knowledge of the underlying evolutionary and genetic basis of these interactions.

6.5 Considering Multilevel Complexity

The challenge of future studies will be to integrate natural complexity into the research on root symbiont-plant-herbivore interactions. This applies to all levels of organization and ranges from the complexity in the rhizosphere community (including genotypic variability) to complex crosstalking networks of genetic and physiological plant responses, the within- and between-species diversity at the level of plant populations to multiple herbivory and their integration in aboveground food webs. This review demonstrates that there are different ways in which belowground mutualists may modulate the plant response to herbivory. Some of these responses may act as alternative strategies, e.g. the expression of direct and indirect defence mechanisms. Thus, in natural systems the decreased performance of herbivores due to higher contents of secondary compounds in plants growing with beneficial soil microbes may be alleviated by a reduced control by predators due to a lower investment in indirect defence mechanisms. The benefit for the plant is then not determined in terms of reduced herbivore pressure but rather by the relative physiological and ecological costs of the different strategies on the background of, e.g. resource availability, plant competition and more. These complex situations can only hardly be mimicked in manipulative experiments, and a deeper understanding of the role of belowground mutualists requires a combination of experimental, observational and theoretical approaches. Further, the plant-mediated linkages between soil microbes and herbivores can be assumed to be the basis for selection pressures and eco-evolutionary feedbacks between the communities, organismal traits and species interactions (Biere and Tack 2013). Genotypic variability and locally adapted interaction traits in mutualist-plant-herbivore interactions (see above) have to be interpreted in this context and are a promising area of future research. Global change phenomena are of increasing relevance in this context, since plant-mediated linkages between below- and aboveground biota have been shown to be affected by the abiotic environment (see Sect. 2.1) which

also may serve as selection pressure for the genetic and evolutionary architecture of this relationship.

Acknowledgements We thank Mehmet A. Balkan (Portland State University) for commenting on a former version of the manuscript. Funding by the National Science Foundation (NSF) to DJB (IOS grant #1457369) is gratefully acknowledged.

References

Abu-Zeyad R, Khan AG, Khoo C (1999) Occurrence of arbuscular mycorrhiza in *Castanospermum australe* A. Cunn & C Fraser and effects on growth and production of castanospermine. Mycorrhiza 9:111–117. doi:10.1007/s005720050008

Afkhami ME, Rudgers JA, Stachowicz JJ (2014) Multiple mutualist effects: conflict and synergy in multispecies mutualisms. Ecology 95:833–844. doi:10.1890/13-1010.1

Alguacil MD, Torrecillas E, Lozano Z, Roldan A (2011) Evidence of differences between the communities of arbuscular mycorrhizal fungi colonizing galls and roots of Prunus persica Infected by the root-knot nematode *Meloidogyne incognita*. Appl Environ Microbiol 77:8656–8661. doi:10.1128/aem.05577-11

Andrade SAL, Malik S, Sawaya A, Bottcher A, Mazzafera P (2013) Association with arbuscular mycorrhizal fungi influences alkaloid synthesis and accumulation in *Catharanthus roseus* and *Nicotiana tabacum* plants. Acta Physiol Plant 35:867–880. doi:10.1007/s11738-012-1130-8

Antunes PM, de Varennes A, Rajcan I, Goss MJ (2006) Accumulation of specific flavonoids in soybean (*Glycine max* (L.) Merr.) as a function of the early tripartite symbiosis with arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* (Kirchner) Jordan. Soil Biol Biochem 38:1234–1242. doi:10.1016/j.soilbio.2005.09.016

Araim G, Saleem A, Arnason JT, Charest C (2009) Root colonization by an arbuscular mycorrhizal (AM) fungus Increases growth and secondary metabolism of purple Coneflower, Echinacea purpurea (L) Moench. J Agric Food Chem 57:2255–2258

Awasthi A, Bharti N, Nair P, Singh R, Shukla AK, Gupta MM, Darokar MP, Kalra A (2011) Synergistic effect of *Glomus mosseae* and nitrogen fixing *Bacillus subtilis* strain Daz26 on artemisinin content in *Artemisia annua* L. Appl Soil Ecol 49:125–130. doi:http://dx.doi.org/10.1016/j.apsoil.2011.06.005

Babikova Z, Gilbert LBruce TJA, Birkett M, Caulfield JC, Woodcock C, Pickett JA, Johnson D (2013) Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. Ecol Lett 16:835–843. doi:10.1111/ele.12115

Babikova Z, Gilbert L, Bruce T, Dewhirst SY, Pickett JA, Johnson D (2014a) Arbuscular mycorrhizal fungi and aphids interact by changing host plant quality and volatile emission. Funct Ecol 28:375–385

Babikova Z, Johnson D, Bruce T, Pickett J, Gilbert L (2014b) Underground allies: how and why do mycelial networks help plants defend themselves? Bioessays 36:21–26. doi:10.1002/bies.201300092

Babst BA et al (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. New Phytol 167:63–72. doi:10.1111/j.1469-8137.2005.01388.x

Babst BA, Ferrieri RA, Thorpe MR, Orians CM (2008) *Lymantria dispar* herbivory induces rapid changes in carbon transport and partitioning in *Populus nigra*. Entomol Exp Appl 128:117–125

Bacht M (2015) Multitrophic interactions in oak. PhD doctoral thesis, University of Marburg, Marburg

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant-insect interactions. Curr Opin Plant Biol 4:351–358

Ballare CL (2011) Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. Trends Plant Sci 16:249–257. doi:10.1016/j.tplants.2010.12.001

Ballhorn DJ, Heil M, Pietrowski A, Lieberei R (2007) Quantitative effects of cyanogenesis on an adapted herbivore. J Chem Ecol 33:2195–2208. doi:10.1007/s10886-007-9380-4

Ballhorn DJ, Kautz S, Lion U, Heil M (2008) Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). J Ecol 96:971–980

Ballhorn DJ, Kautz S, Rakotoarivelo FP (2009) Quantitative variability of cyanogenesis in *Cathariostachys madagascariensis* – the main food plant of Bamboo Lemurs in Southeastern Madagascar. Am J Primatol 71:305–315

Ballhorn DJ, Kautz S, Schädler M (2013) Induced plant defense via volatile production is dependent on rhizobial symbiosis. Oecologia 172:833–846. doi:10.1007/s00442-012-2539-x

Ballhorn DJ, Godschalx AL, Smart SM, Kautz S, Schadler M (2014a) Chemical defense lowers plant competitiveness. Oecologia 176:811–824. doi:10.1007/s00442-014-3036-1

Ballhorn DJ, Kay J, Kautz S (2014b) Quantitative effects of leaf area removal on indirect defense of lima Bean (*Phaseolus lunatus*) in nature. J Chem Ecol 40:294–296. doi:10.1007/s10886-014-0392-6

Ballhorn DJ, Schädler M, Elias JD, Millar JA, Kautz S (2016) Friend or foe-light availability determines the relationship between mycorrhizal fungi, rhizobia and lima bean (*Phaseolus lunatus*). Plos One 11(5), e0154116. doi:10.1371/journal.pone.0154116

Barber NA (2013) Arbuscular mycorrhizal fungi are necessary for the induced response to herbivores by *Cucumis sativus*. J Plant Ecol 6:171–176. doi:10.1093/jpe/rts026

Barrett LG, Broadhurst LM, Thrall PH (2012) Geographic adaptation in plant-soil mutualisms: tests using *Acacia* spp. and rhizobial bacteria. Funct Ecol 26:457–468. doi:10.1111/j.1365-2435.2011.01940.x

Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. J Ecol 98:745–753. doi:10.1111/j.1365-2745.2010.01658.x

Battaglia D et al (2013) Tomato below ground-above ground interactions: *Trichoderma longibrachiatum* affects the performance of *Macrosiphum euphorbiae* and its natural antagonists. Mol Plant Microbe Interact 26:1249–1256. doi:10.1094/mpmi-02-13-0059-r

Baum C, Toljander YK, Eckhardt KU, Weih M (2009) The significance of host-fungus combinations in ectomycorrhizal symbioses for the chemical quality of willow foliage. Plant and Soil 323:213–224. doi:10.1007/s11104-009-9928-x

Beckers GJM, Spoel SH (2006) Fine-tuning plant defence signalling: salicylate versus jasmonate. Plant Biol 8:1–10. doi:10.1055/s-2005-872705

Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218

Bennett AE, Alers-Garcia J, Bever JD (2006) Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypotheses and synthesis. Am Nat 167:141–152

Bennett AE, Bever JD, Bowers MD (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia 160:771–779. doi:10.1007/s00442-009-1338-5

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. doi:http://dx.doi.org/10.1016/j.tplants.2012.04.001

Bezemer TM, Fountain MT, Barea JM, Christensen S, Dekker SC, Duyts H, van Hal R, Harvey JA, Hedlund K, Maraun M, Mikola J, Mladenov MG, Robin C, de Ruiter PC, Scheu S, Setälä H, Smilauer P, van der Putten WH (2010) Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. Ecology 91:3027–3036

Bi HH, Song YY, Zeng RS (2007) Biochemical and molecular responses of host plants to mycorrhizal infection and their roles in plant defence. Allelopathy J 20:15–27

Biere A, Tack AJM (2013) Evolutionary adaptation in three-way interactions between plants, microbes and arthropods. Funct Ecol 27:646–660. doi:10.1111/1365-2435.12096

Bonfante P, Anca IA (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol 63:363–383. doi:10.1146/annurev.micro.091208.073504

Borowicz VA (1997) A fungal root symbiont modifies plant resistance to an insect herbivore. Oecologia 112:534–542

Borowicz VA (2013) The impact of arbuscular mycorrhizal fungi on plant growth following herbivory: a search for pattern. Acta Oecol 52:1–9. doi:10.1016/j.actao.2013.06.004

Bronstein JL (2001) The exploitation of mutualisms. Ecol Lett 4:277–287. doi:10.1046/j.1461-0248.2001.00218.x

Brunner SM, Goos RJ, Swenson SJ, Foster SP, Schatz BG, Lawley YE, Prischmann-Voldseth DA (2015) Impact of nitrogen fixing and plant growth-promoting bacteria on a phloem-feeding soybean herbivore. Appl Soil Ecol 86:71–81. doi:10.1016/j.apsoil.2014.10.007

Cameron DD, Neal AL, van Wees SCM, Ton J (2013) Mycorrhiza-induced resistance: more than the sum of its parts? Trends Plant Sci 18:539–545. doi:10.1016/j.tplants.2013.06.004

Carreon-Abud Y, Torres-Martinez R, Farfan-Soto B, Hernandez-Garcia A, Rios-Chavez P, Bello-Gonzalez MA, Martinez-Trujillo M, Salgado-Garciglia R (2015) Arbuscular mycorrhizal symbiosis increases the content of volatile terpenes and plant performance in *Satureja macrostema* (Benth.) Briq. Boletin Latinoamericano Y Del Caribe De Plantas Medicinales Y Aromaticas 14:273–279

Cartieaux F, Contesto C, Gallou A, Desbrosses G, Kopka J, Taconnat L, Renou JP, Touraine B (2008) Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp strain ORS278 and *Pseudomonas syringae* pv. *tomato* DC3000 leads to complex transcriptome changes. Mol Plant Microbe Interact 21:244–259. doi:10.1094/mpmi-21-2-0244

Castell W, Fleischmann F, Heger T, Matyssek R (2015) Shaping theoretic foundations of holobiont-like systems. In: Canovas F, Luettge U, Matyssek R (eds) Progress in botany, vol 77. Springer, Heidelberg, pp 219–244. doi:10.1007/978-3-319-25689.7_7

Castillo G, Cruz LL, Tapia-Lopez R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gomez G, Valverde PL, Nunez-Faran J (2014) Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. PLoS One 9. doi:10.1371/journal.pone.0102478

Ceccarelli N, Curadi M, Martelloni L, Sbrana C, Picciarelli P, Giovannetti M (2010) Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. Plant and Soil 335:311–323. doi:10.1007/s11104-010-0417-z

Chandanie WA, Kubota M, Hyakumachi M (2009) Interactions between the arbuscular mycorrhizal fungus Glomus mosseae and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl Soil Ecol 41:336–341

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2016) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stress. J Plant Growth Regul 35:276–300. doi:10.1007/s00344-015-9521-x

Colebatch G, Trevaskis B, Udvardi M (2002a) Functional genomics: tools of the trade. New Phytol 153:27–36

Colebatch G, Trevaskis B, Udvardi M (2002b) Symbiotic nitrogen fixation research in the postgenomics era. New Phytol 153:37–42

Conrath U (2009) Priming of induced plant defense responses. Plant Innate Immun 51:361–395

Corby HDL (1981) The systematic value of leguminous root nodules. In: Polhill RM, Raven PH (eds) Advances in legume systematics, Parts 1 and 2. Vol. 2 of the Proceedings of the International Legume Conference, Kew, Surrey, England, 24–29 July 1978. Xvi+425p. (Part 1); V+622p. (Part 2). Royal Botanic Gardens: Surrey, England. Illus. Paper:P657-670

Cosme M, Stout MJ, Wurst S (2011) Effect of arbuscular mycorrhizal fungi (*Glomus intraradices*) on the oviposition of rice water weevil (*Lissorhoptrus oryzophilus*). Mycorrhiza 21:651–658. doi:10.1007/s00572-011-0399-6

Crawley MJ (1997) Plant-herbivore dynamics. In: Crawley MJ (ed) Plant ecology. Blackwell Science, Oxford, pp 401–474

Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153–166

Crotti E, Balloi A, Hamdi C, Sansonno L, Marzorati M, Gonella E, Favia G, Cherif A, Alma A, Daffonchio D (2012) Microbial symbionts: a resource for the management of insect-related problems. J Microbial Biotechnol 5:307–317. doi:10.1111/j.1751-7915.2011.00312.x

De Lange ES, Balmer D, Mauch-Mani B, Turlings TCJ (2014) Insect and pathogen attack and resistance in maize and its wild ancestors, the teosintes. New Phytol 204:329–341. doi:10.1111/nph.13005

Dean JM, Mescher MC, De Moraes CM (2009) Plant-rhizobia mutualism influences aphid abundance on soybean. Plant and Soil 323:187–196. doi:10.1007/s11104-009-9924-1

Dean JM, Mescher MC, De Moraes CM (2014) Plant dependence on rhizobia for nitrogen influences induced plant defenses and herbivore performance. Int J Mol Sci 15:1466–1480. doi:10.3390/ijms15011466

Demirözer O, Tyler-Julian K, Funderburk J (2014) Association of pepper with arbuscular mycorrhizal fungi influences populations of the herbivore *Frankliniella occidentalis* (Thysanoptera: Thripidae). J Entomol Sci 49:156–165

Demirözer O, Özkaya HÖ, Aldemir T, Karapire M (2015) Does the association of arbuscular mycorrhizal fungi and two-spotted spider mite increase gossypol synthesis in two cotton cultivars? Fresenius Environ Bull 24:4199–4204

Douglas AE (2008) Conflict, cheats and the persistence of symbioses. New Phytol 177:849–858. doi:10.1111/j.1469-8137.2007.02326.x

Dresler-Nurmi A, Fewer DP, Räsänen LA, Lindström K (2007) The diversity and evolution of rhizobia. In: Pawlowski K (ed) Prokaryotic symbionts in plants. Springer, Heidelberg, pp 4–41. doi:10.1007/7171_2007_099

Eisenhauer N, Konig S, Sabais ACW, Renker C, Buscot F, Scheu S (2009) Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. Soil Biol Biochem 41:561–567

Eom AH, Wilson GWT, Hartnett DC (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia 93:233–242

Erb M, Veyrat N, Robert CAM, Xu H, Frey M, Ton J, Turlings TCJ (2015) Indole is an essential herbivore-induced volatile priming signal in maize. Nat Commun 6. doi:10.1038/ncomms7273

Fontana A, Reichelt M, Hempel S, Gershenzon J, Unsicker SB (2009) The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of *Plantago lanceolata* L. J Chem Ecol 35:833–843. doi:10.1007/s10886-009-9654-0

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Fritz M, Jakobsen I, Lyngkjaer MF, Thordal-Christensen H, Pons-Kuhnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16:413–419. doi:10.1007/s00572-006-0051-z

Frost CJ, Hunter MD (2008) Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. New Phytol 178:835–845

Gange AC, Ayres RL (1999) On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. Oikos 87:615–621. doi:10.2307/3546829

Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. New Phytol 128:79–87

Gange AC, Bower E, Brown VK (2002a) Differential effects of insect herbivory on arbuscular mycorrhizal colonization. Oecologia 131:103–112

Gange AC, Stagg PG, Ward LK (2002b) Arbuscular mycorrhizal fungi affect phytophagous insect specialism. Ecol Lett 5:11–15

Gange AC, Brown VK, Aplin DM (2003) Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. Ecol Lett 6:1051–1055

Gange AC, Gane DRJ, Chen Y, Gong M (2005) Dual colonization of *Eucalyptus urophylla* S.T. Blake by arbuscular and ectomycorrhizal fungi affects levels of insect herbivore attack. Agric For Entomol 7:253–263. doi:10.1111/j.1461-9555.2005.00268.x

Garcia I, Mendoza R (2012) Impact of defoliation intensities on plant biomass, nutrient uptake and arbuscular mycorrhizal symbiosis in Lotus tenuis growing in a saline-sodic soil. Plant Biol 14:964–971. doi:10.1111/j.1438-8677.2012.00581.x

Garrido E, Bennett AE, Fornoni J, Strauss SY (2010) Variation in arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to above-ground defoliation. J Ecol 98:43–49. doi:10.1111/j.1365-2745.2009.01586.x

Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. Trends Ecol Evol 9:251–255

Gehring CA, Whitham TG (2002) Mycorrhizae-herbivore interactions: population and community consequences. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, Berlin, pp 295–320

Genre A, Russo G (2016) Does a common pathway transduce symbiotic signals in plant-microbe interactions? Front Plant Sci 7:96. doi:10.3389/fpls.2016.00096

Gerlach N, Schmitz J, Polatajko A, Schlüter U, Fahnenstich H, Witt S, Fernie AR, Uroic K, Scholz U, Sonnewald U, Bucher M (2015) An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. Plant Cell Environ 38:1591–1612. doi:10.1111/pce.12508

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227. doi:10.1146/annurev.phyto.43.040204.135923

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117. doi:10.1139/m95-015

Godschalx AL, Schadler M, Trisel JA, Balkan MA, Ballhorn DJ (2015) Ants are less attracted to the extrafloral nectar of plants with symbiotic, nitrogen-fixing rhizobia. Ecology 96:348–354. doi:10.1890/14-1178.1

Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2015) Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech 5:355–377. doi:10.1007/s13205-014-0241-x

Groten K, Nawaz A, Nguyen NHT, Santhanam R, Baldwin IT (2015) Silencing a key gene of the common symbiosis pathway in *Nicotiana attenuata* specifically impairs arbuscular mycorrhizal infection without influencing the root-associated microbiome or plant growth. Plant Cell Environ 38:2398–2416. doi:10.1111/pce.12561

Guerrieri E, Lingua G, Digilio MC, Massa N, Berta G (2004) Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? Ecol Entomol 29:753–756

Halldorsson G, Sverrisson H, Eyjolfsdottir GG, Oddsdottir ES (2000) Ectomycorrhizae reduce damage to Russian larch by *Otiorhynchus larvae*. Scand J For Res 15:354–358

Hart M et al (2015) Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. Mycorrhiza 25:359–376. doi:10.1007/s00572-014-0617-0

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342. doi:10.1146/annurev.ento.54.110807.090614

Hause B, Schaarschmidt S (2009) The role of jasmonates in mutualistic symbioses between plants and soil-born microorganisms. Phytochemistry 70:1589–1599. doi:10.1016/j.phytochem.2009.07.003

Heath KD, Lau JA (2011) Herbivores alter the fitness benefits of a plant-rhizobium mutualism. Acta Oecol 37:87–92. doi:10.1016/j.actao.2010.12.002

Heath KD, McGhee KE (2012) Coevolutionary constraints? The environment alters tripartite interaction traits in a legume. PLoS One 7. doi:10.1371/journal.pone.0041567

Heath KD, Stock AJ, Stinchcombe JR (2010) Mutualism variation in the nodulation response to nitrate. J Evol Biol 23:2494–2500. doi:10.1111/j.1420-9101.2010.02092.x

Hector A et al (1999) Plant diversity and productivity experiments in European grasslands. Science 286:1123–1127

Heil M (2004) Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by Wild Lima Bean (*Phaseolus lunatus*). J Chem Ecol 30:1289–1295. doi:10.1023/B:JOEC.0000030299.59863.69

Heil M (2011) Nectar: generation, regulation and ecological functions. Trends Plant Sci 16:191–200. doi:http://dx.doi.org/10.1016/j.tplants.2011.01.003

Heil M, Bueno JCS (2007) Herbivore-induced volatiles as rapid signals in systemic plant responses: how to quickly move the information? Plant Signal Behav 2:191–193

Hempel S et al (2009) Specific bottom-up effects of arbuscular mycorrhizal fungi across a plant-herbivore-parasitoid system. Oecologia 160:267–277. doi:10.1007/s00442-009-1294-0

Hendrickson OQ, Burgess D, Perinet P, Tremblay F, Chatatpaul L (1993) Effects of *Frankia* on field performance of *Alnus* clones and seedlings. Plant and Soil 150:295–302. doi:10.1007/BF00013027

Henke C et al (2015) Analysis of volatiles from *Picea abies* triggered by below-ground interactions. Environ Exp Bot 110:56–61. doi:10.1016/j.envexpbot.2014.09.009

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335. doi:10.1086/417659

Hermsmeier D, Schittko U, Baldwin IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. Plant Physiol 125:683–700

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil 311:1–18

Herrmann S, Buscot F (2007) Cross talks at the morphogenetic, physiological and gene regulation levels between the mycobiont *Piloderma croceum* and oak microcuttings (*Quercus robur*) during formation of ectomycorrhizas. Phytochemistry 68:52–67. doi:10.1016/j.phytochem.2006.09.028

Hobbie JE, Hobbie EA (2006) N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. Ecology 87:816–822

Hoeksema JD, Piculell BJ, Thompson JN (2009) Within-population genetic variability in mycorrhizal interactions. Commun Integr Biol 2:110–112

Hoffmann D, Vierheilig H, Riegler P, Schausberger P (2009) Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite Tetranychus urticae. Oecologia 158:663–671

Hoffmann D, Vierheilig H, Peneder S, Schausberger P (2011a) Mycorrhiza modulates aboveground tri-trophic interactions to the fitness benefit of its host plant. Ecol Entomol 36:574–581. doi:10.1111/j.1365-2311.2011.01298.x

Hoffmann D, Vierheilig H, Riegler P, Schausberger P (2011b) Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite *Tetranychus urticae* (vol 158, pg 663, 2009). Oecologia 165:545. doi:10.1007/s00442-010-1852-5

Hoffmann D, Vierheilig H, Schausberger P (2011c) Arbuscular mycorrhiza enhances preference of ovipositing predatory mites for direct prey-related cues. Physiol Entomol 36:90–95. doi:10.1111/j.1365-3032.2010.00751.x

Hoffmann D, Vierheilig H, Schausberger P (2011d) Mycorrhiza-induced trophic cascade enhances fitness and population growth of an acarine predator. Oecologia 166:141–149. doi:10.1007/s00442-010-1821-z

Hol WHG et al (2010) Reduction of rare soil microbes modifies plant-herbivore interactions. Ecol Lett 13:292–301. doi:10.1111/j.1461-0248.2009.01424.x

Hui D, Iqbal J, Lehmann K, Gase K, Saluz HP, Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host

Nicotiana attenuata: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. Plant Physiol 131:1877–1893. doi:10.1104/pp.102.018176

Irmer S et al (2015) New aspect of plant-rhizobia interaction: alkaloid biosynthesis in *Crotalaria* depends on nodulation. Proc Natl Acad Sci U S A 112:4164–4169. doi:10.1073/pnas.1423457112

Jogaiah S, Abdelrahman M, Tran L-SP, Shin-ichi I (2013) Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. J Exp Bot 64:3829–3842. doi:10.1093/jxb/ert212

Johnson SN, Rasmann S (2015) Root-feeding insects and their interactions with organisms in the rhizosphere. In: Berenbaum MR (ed) Annual review of entomology, vol 60. Annual Reviews, Palo Alto, pp 517–535

Johnson ND, Liu B, Bentley BL (1987) The effects of nitrogen-fixation, soil nitrate, and defoliation on the growth, alkaloids, and nitrogen levels of *Lupinus succulentus* (Fabaceae). Oecologia 74:425–431

Johnson ND, Bentley, BL, Barbosa A, Krischik VA, Jones CG (1991) Symbiotic N 2 -fixation and the elements of plant resistance to herbivores: lupine alkaloids and tolerance to defoliation. In: Barbosa P, Krischik VA, Jones CG (eds) Microbial mediation of plant-herbivore interactions. Wiley Interscience, pp 45–63

Johnson SN, Birch ANE, Gregory PJ, Murray PJ (2006) The 'mother knows best' principle: should soil insects be included in the preference–performance debate? Ecol Entomol 31:395–401. doi:10.1111/j.1365-2311.2006.00776.x

Jones CG, Last FT, Barbosa A, Krischik VA (1991) Ectomycorrhizae and tress: implications for aboveground herbivory. Microbial mediation of plant-herbivore interactions. Wiley Interscience, pp 65–103

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. J Chem Ecol 38:651–664. doi:10.1007/s10886-012-0134-6

Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. Mycorrhiza 17:581–587. doi:10.1007/s00572-007-0135-4

Karst J et al (2015) Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. New Phytol 208:904–914. doi:10.1111/nph.13492

Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biol Biochem 41:1233–1244. doi:10.1016/j.soilbio.2009.03.005

Katayama N, Nishida T, Zhang ZQ, Ohgushi T (2010) Belowground microbial symbiont enhances plant susceptibility to a spider mite through change in soybean leaf quality. Popul Ecol 52:499–506. doi:10.1007/s10144-010-0207-8

Katayama N, Zhang ZQ, Ohgushi T (2011a) Belowground rhizobia positively affect abundances of aboveground sap feeding and leaf chewing herbivores. J Plant Interact 6:173–174. doi:10.1080/17429145.2010.536264

Katayama N, Zhang ZQ, Ohgushi T (2011b) Community-wide effects of below-ground rhizobia on above-ground arthropods. Ecol Entomol 36:43–51. doi:10.1111/j.1365-2311.2010.01242.x

Katayama N, Silva AO, Kishida O, Ushio M, Kita S, Ohgushi T (2014) Herbivorous insect decreases plant nutrient uptake: the role of soil nutrient availability and association of below-ground symbionts. Ecol Entomol 39:511–518. doi:10.1111/een.12125

Kempel A, Brandl R, Schädler M (2009) Symbiotic soil microorganisms as players in aboveground plant-herbivore interactions – the role of rhizobia. Oikos 118:634–640

Kempel A, Schmidt AK, Brandl R, Schädler M (2010) Support from the underground: induced plant resistance depends on arbuscular mycorrhizal fungi. Funct Ecol 24:293–300. doi:10.1111/j.1365-2435.2009.01647.x

Kempel A, Schädler M, Chrobock T, Fischer M, van Kleunen M (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proc Natl Acad Sci U S A 108:5685–5689. doi:10.1073/pnas.1016508108

Kempel A, Nater P, Fischer M, van Kleunen M (2013) Plant-microbe-herbivore interactions in invasive and non-invasive alien plant species. Funct Ecol 27:498–508. doi:10.1111/1365-2435.12056

Khaitov B, Patiño-Ruiz JD, Pina T, Schausberger P (2015) Interrelated effects of mycorrhiza and free-living nitrogen fixers cascade up to aboveground herbivores. Ecol Evol 5:3756–3768. doi:10.1002/ece3.1654

Kiers ET, Denison RF (2008) Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Annu Rev Ecol Evol Syst 39:215–236

Kiers ET, Adler LS, Grman EL, van der Heijden MGA (2010) Manipulating the jasmonate response: how do methyl jasmonate additions mediate characteristics of aboveground and belowground mutualisms? Funct Ecol 24:434–443. doi:10.1111/j.1365-2435.2009.01625.x

Koller R, Rodriguez A, Robin C, Scheu S, Bonkowski M (2013a) Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytol 199:203–211. doi:10.1111/nph.12249

Koller R, Scheu S, Bonkowski M, Robin C (2013b) Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. Soil Biol Biochem 65:204–210. doi:10.1016/j.soilbio.2013.05.020

Koorneef A, Pieterse CMJ (2008) Cross-talk in defense signalling. Plant Physiol 146:839–844

Koricheva J, Gange AC, Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. Ecology 90:2088–2097

Koschier EH, Khaosaad T, Vierheilig H (2007) Root colonization by the arbuscular mycorrhizal fungus *Glomus mosseae* and enhanced phosphorous levels in cucumber do not affect host acceptance and development of *Frankliniella occidentalis*. J Plant Interact 2:11–15. doi:10.1080/17429140701231459

Kost C, Heil M (2008) The defensive role of volatile emission and extrafloral nectar secretion for Lima Bean in nature. J Chem Ecol 34:2–13. doi:10.1007/s10886-007-9404-0

Krupa S, Fries N (1971) Studies on ectomycorrhizae of pine. I Production of volatile organic compounds. Can J Bot 49:1425–1431. doi:10.1139/b71-200

Krupa S, Andersson J, Marx DH (1973) Studies on ectomycorrhizae of pine III. Volatile organic compounds in mycorrhizal and nonmycorrhizal root systems of Pinus echinata Mill. Eur J For Pathol 3:194–200. doi:10.1111/j.1439-0329.1973.tb00394.x

Kula AAR, Hartnett DC (2015) Effects of mycorrhizal symbiosis on aboveground arthropod herbivory in tallgrass prairie: an in situ experiment. Plant Ecol 216:589–597. doi:10.1007/s11258-015-0461-0

Kula AAR, Hartnett DC, Wilson GWT (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. Ecol Lett 8:61–69

Laird R, Addicott J (2007) Arbuscular mycorrhizal fungi reduce the construction of extrafloral nectaries in *Vicia faba*. Oecologia 152:541–551. doi:10.1007/s00442-007-0676-4

Landgraf R, Schaarschmidt S, Hause B (2012) Repeated leaf wounding alters the colonization of *Medicago truncatula* roots by beneficial and pathogenic microorganisms. Plant Cell Environ 35:1344–1357. doi:10.1111/j.1365-3040.2012.02495.x

Larimer AL, Bever JC, Clay K (2010) The interactive effects of plant microbial symbionts: a review and meta-analysis. Symbiosis 51:139–148. doi:10.1007/s13199-010-0083-1

Lehr NA, Schrey SD, Bauer R, Hampp R, Tarkka MT (2007) Suppression of plant defence response by a mycorrhiza helper bacterium. New Phytol 174:892–903. doi:10.1111/j.1469-8137.2007.02021.x

Leimu R, Koricheva J (2006) A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112:1–9. doi:10.1111/j.0030-1299.2006.41023.x

Leitner M, Kaiser R, Hause B, Boland W, Mithofer A (2010) Does mycorrhization influence herbivore-induced volatile emission in *Medicago truncatula*? Mycorrhiza 20:89–101. doi:10.1007/s00572-009-0264-z

Li H et al (2013) Impact of the earthworm *Aporrectodea trapezoides* and the arbuscular mycorrhizal fungus *Glomus intraradices* on N-15 uptake by maize from wheat straw. Biol Fertil Soils 49:263–271. doi:10.1007/s00374-012-0716-z

Liu JY et al (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. Plant Cell 15:2106–2123. doi:10.1105/tpc.014183

Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529–544. doi:10.1111/j.1365-313X.2007.03069.x

Manninen AM (1999) Susceptibility of Scots pine seedlings to specialist and generalist insect herbivores – importance of plant defense and mycorrhizal status. Natural and Environmental Sciences 100. PhD, University Kuopio, Kuopio University Publications C

Manninen AM, Holopainen T, Holopainen JK (1998) Susceptibility of ectomycorrhizal and nonmycorrhizal Scots pine (*Pinus sylvestris*) seedlings to a generalist insect herbivore, *Lygus rugulipennis*, at two nitrogen availability levels. New Phytol 140:55–63

Manninen AM, Holopainen T, Holopainen JK (1999) Performance of grey pine aphid, *Schizolachnus pineti*, on ectomycorrhizal and non-mycorrhizal Scots pine seedlings at different levels of nitrogen availability. Entomol Exp Appl 93:117–120

Manninen AM, Holopainen T, Lyytikaeinen-Saarenmaa P, Holopainen JK (2000) The role of low-level ozone exposure and mycorrhizas in chemical quality and insect herbivore performance on Scots pine seedlings. Glob Chem Biol 5:1–11

Markkola A, Kuikka K, Rautio P, Harma E, Roitto M, Tuomi J (2004) Defoliation increases carbon limitations in ectomycorrhizal symbiosis of *Betula pubescens*. Oecologia 140:234–240. doi:10.1007/s00442-004-1587-2

Martinuz A, Schouten A, Menjivar RD, Sikora RA (2012) Effectiveness of systemic resistance toward *Aphis gossypii* (Hom., Aphididae) as induced by combined applications of the endophytes *Fusarium oxysporum* Fo162 and Rhizobium etli G12. Biol Control 62. doi:10.1016/j.biocontrol.2012.05.006

Marx J (2004) The roots of plant-microbe collaborations. Science 304:234–236

Matyssek R, Koricheva J, Schnyder H, Ernst D, Munch JC, Oßwald W, Pretzsch H (2012) The balance between resource sequestration and retention: a challenge in plant science. In: Matyssek R, Schnyder R, Oßwald W, Ernst D, Munch JC, Pretzsch H (eds) Growth and defence in plants. Springer, Heidelberg, pp 3–23. doi:10.1007/978-3-642-30645-7_1

Mechri B, Tekaya M, Cheheb H, Attia F, Hammami M (2015) Accumulation of flavonoids and phenolic compounds in olive tree roots in response to mycorrhizal colonization: a possible mechanism for regulation of defense molecules. J Plant Physiol 185:40–43. doi:10.1016/j.jplph.2015.06.015

Medina A, Probanza A, Gutierrez Mañero FJ, Azcón R (2003) Interactions of arbuscular-mycorrhizal fungi and *Bacillus* strains and their effects on plant growth, microbial rhizosphere activity (thymidine and leucine incorporation) and fungal biomass (ergosterol and chitin). Appl Soil Ecol 22:15–28. doi:http://dx.doi.org/10.1016/S0929-1393(02)00112-9

Michel K, Abderhalden O, Bruggmann R, Dudler R (2006) Transcriptional changes in powdery mildew infected wheat and *Arabidopsis* leaves undergoing syringolin-triggered hypersensitive cell death at infection sites. Plant Mol Biol 62:561–578. doi:10.1007/s11103-006-9045-7

Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, Henning JA, Schultz PA, Bever JD (2015) Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6:276. doi:10.1890/ES15-00152.1

Mikola J, Nieminen M, Ilmarinen K, Vestberg M (2005) Belowground responses by AM fungi and animal trophic groups to repeated defoliation in an experimental grassland community. Soil Biol Biochem 37:1630–1639

Milcu A, Bonkowski M, Collins CM, Crawley MJ (2015) Aphid honeydew-induced changes in soil biota can cascade up to tree crown architecture. Pedobiologia 58:119–127. doi:10.1016/j.pedobi.2015.07.002

Miller RE, Gleadow RM, Cavagnaro TR (2014) Age versus stage: does ontogeny modify the effect of phosphorus and arbuscular mycorrhizas on above- and below-ground defence in forage sorghum? Plant Cell Environ 37:929–942. doi:10.1111/pce.12209

Moon DC, Barnouti J, Younginger B (2013) Context-dependent effects of mycorrhizae on herbivore density and parasitism in a tritrophic coastal study system. Ecol Entomol 38:31–39. doi:10.1111/j.1365-2311.2012.01399.x

Morandi D (1996) Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions, and their potential role in biological control. Plant and Soil 185:241–251. doi:10.1007/BF02257529

Mueller RC, Sthultz CM, Martinez T, Gehring CA, Whitham TG (2005) The relationship between stem-galling wasps and mycorrhizal colonization of *Quercus turbinella*. Can J Bot 83:1349–1353

Murrell EG, Hanson CR, Cullen EM (2015) European corn borer oviposition response to soil fertilization practices and arbuscular mycorrhizal colonization of corn. Ecosphere 6. doi:10.1890/es14-00501.1

Ness JH (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. Oikos 113:506–514. doi:10.1111/j.2006.0030-1299.14143.x

Neuhauser C, Fargione JE (2004) A mutualism-parasitism continuum model and its application to plant-mycorrhizae interactions. Ecol Model 177:337–352. doi:10.1016/j.ecolmodel.2004.02.010

Nishida T, Izumi N, Katayama N, Ohgushi T (2009) Short-term response of arbuscular mycorrhizal association to spider mite herbivory. Popul Ecol 51:329–334

Okada K, Abe H, Arimura G (2015) Jasmonates induce both defense responses and communication in monocotyledonous and dicotyledonous plants. Plant Cell Physiol 56:16–27. doi:10.1093/pcp/pcu158

Orrelland P, Bennett AE (2013) How can we exploit above-belowground interactions to assist in addressing the challenges of food security? Front Plant Sci 4. doi:10.3389/fpls.2013.00432

Ossler JN, Zielinski CA, Heath KD (2015) Tripartite mutualism: facilitation or trade-offs between rhizobial and mycorrhizal symbionts of legume hosts. Am J Bot 102:1332–1341. doi:10.3732/ajb.1500007

Pangesti N, Pineda A, Pieterse CMJ, Dicke M, van Loon JJA (2013) Two-way plant-mediated interactions between root-associated microbes and insects: from ecology to mechanisms. Front Plant Sci 4. doi:10.3389/fpls.2013.00414

Pangesti N, Pineda A, Dicke M, van Loon JJA (2015a) Variation in plant-mediated interactions between rhizobacteria and caterpillars: potential role of soil composition. Plant Biol 17:474–483. doi:10.1111/plb.12265

Pangesti N, Weldegergis BT, Langendorf B, van Loon JJA, Dicke M, Pineda A (2015b) Rhizobacterial colonization of roots modulates plant volatile emission and enhances the attraction of a parasitoid wasp to host-infested plants. Oecologia 178:1169–1180. doi:10.1007/s00442-015-3277-7

Pankoke H, Höpfner I, Matuszak A, Beyschlag W, Müller C (2015) The effects of mineral nitrogen limitation, competition, arbuscular mycorrhiza, and their respective interactions, on morphological and chemical plant traits of *Plantago lanceolata*. Phytochemistry 118:149–161. doi:http://dx.doi.org/10.1016/j.phytochem.2015.07.014

Pastore AI, Russell FL (2012) Insect herbivore effects on resource allocation to shoots and roots in *Lespedeza capitata*. Plant Ecol 213:843–851. doi:10.1007/s11258-012-0046-0

Piculell BJ, Hoeksema JD, Thompson JN (2008) Interactions of biotic and abiotic environmental factors in an ectomycorrhizal symbiosis, and the potential for selection mosaics. BMC Biol 6. doi:10.1186/1741-7007-6-23

Pieterse CMJ et al (2008) Cross-talk between signaling pathways leading to defense against pathogens and insects. In: Lorito M, Woo SL, Dicke M (eds) Biology of plant-microbe interactions. International Society for Molecular Plant-Microbe Interactions, St. Paul, pp 1–9

Pieterse CMJ, Poelman EH, Van Wees SCM, Dicke M (2013) Induced plant responses to microbes and insects. Front Plant Sci 4. doi:10.3389/fpls.2013.00475

Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51:403–415. doi:10.1007/s00374-015-0996-1

Pineda A, Zheng SJ, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci 15:507–514. doi:10.1016/j.tplants.2010.05.007

Pineda A, Zheng SJ, van Loon JJA, Dicke M (2012) Rhizobacteria modify plant-aphid interactions: a case of induced systemic susceptibility. Plant Biol 14:83–90. doi:10.1111/j.1438-8677.2011.00549.x

Pineda A, Dicke M, Pieterse CMJ, Pozo MJ (2013a) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? Funct Ecol 27:574–586. doi:10.1111/1365-2435.12050

Pineda A, Soler R, Weldegergis BT, Shimwela MM, Van Loon JJA, Dicke M (2013b) Non-pathogenic rhizobacteria interfere with the attraction of parasitoids to aphid-induced plant volatiles via jasmonic acid signalling. Plant Cell Environ 36:393–404. doi:10.1111/j.1365-3040.2012.02581.x

Planchamp C, Glauser G, Mauch-Mani B (2015) Root inoculation with *Pseudomonas putida* KT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. Front Plant Sci 5. doi:10.3389/fpls.2014.00719

Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398. doi:10.1016/j.pbi.2007.05.004

Pozo MJ, Van Loon LC, Pieterse CMJ (2004) Jasmonates – signals in plant-microbe interactions. J Plant Growth Regul 23:211–222. doi:10.1007/s00344-004-003105

Rapparini F, Llusià J, Peñuelas J (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua* L. Plant Biol 10:108–122. doi:10.1055/s-2007-964963

Remy W, Taylor T, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular mycorrhizae. Proc Natl Acad Sci 91:11841–11843

Rieske LK (2001) Influence of symbiotic fungal colonization on oak seedling growth and suitability for insect herbivory. Environ Entomol 30:849–854

Roda AL, Baldwin IT (2003) Molecular technology reveals how the induced direct defenses of plants work. Basic Appl Ecol 4:15–26

Rodriguez-Echeverria S, Traveset A (2015) Putative linkages between below- and aboveground mutualisms during alien plant invasions. Aob Plants 7. doi:10.1093/aobpla/plv062

Roger A, Getaz M, Rasmann S, Sanders IR (2013) Identity and combinations of arbuscular mycorrhizal fungal isolates influence plant resistance and insect preference. Ecol Entomol 38:330–338. doi:10.1111/een.12022

Rostás M, Turlings TCJ (2008) Induction of systemic acquired resistance in *Zea mays* also enhances the plant's attractiveness to parasitoids. Biol Control 46:178–186. doi:http://dx.doi.org/10.1016/j.biocontrol.2008.04.012

Sampedro L, Moreira X, Zas R (2011) Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. J Ecol 99:818–827. doi:10.1111/j.1365-2745.2011.01814.x

Santhanam R, Luu VT, Weinhold A, Goldberg J, Oh Y, Baldwin IT (2015) Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. Proc Natl Acad Sci 112:E5013–E5020. doi:10.1073/pnas.1505765112

Saravesi K, Ruotsalainen AL, Cahill JF (2014) Contrasting impacts of defoliation on root colonization by arbuscular mycorrhizal and dark septate endophytic fungi of *Medicago sativa*. Mycorrhiza 24:239–245. doi:10.1007/s00572-013-0536-5

Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. Soil Biol Biochem 87:25–33. doi:http://dx.doi.org/10.1016/j.soilbio.2015.04.001

Schädler M, Jung G, Brandl R, Auge H (2004) Secondary succession is influenced by belowground insect herbivory on a productive site. Oecologia 138:242–252

Schädler M, Brandl R, Kempel A (2010) Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. Entomol Exp Appl 135:162–169. doi:10.1111/j.1570-7458.2010.00976.x

Schausberger P, Peneder S, Jurschik S, Hoffmann D (2012) Mycorrhiza changes plant volatiles to attract spider mite enemies. Funct Ecol 26:441–449. doi:10.1111/j.1365-2435.2011.01947.x

Schenk PM, McGrath KC, Lorito M, Pieterse CMJ (2008) Plant-microbe and plant-insect interactions meet common grounds. New Phytol 179:251–255

Schmidt DD, Voelckel C, Hartl M, Schmidt S, Baldwin IT (2005) Specificity in ecological interactions. Attack from the same lepidopteran herbivore results in species-specific transcriptional responses in two solanaceous host plants. Plant Physiol 138:1763–1773. doi:10.1104/pp.105.061192

Schreck TK, David SJ, Mooney KA (2013) Effects of *Brassica nigra* and plant-fungi interactions on the arthropod community of *Deinandra fasciculata*. Biol Invasions 15:2443–2454. doi:10.1007/s10530-013-0464-5

Selosse MA, Strullu-Derrien C, Martin FM, Kamoun S, Kenrick P (2015) Plants, fungi and oomycetes: a 400-million year affair that shapes the biosphere. New Phytol 206:501–506. doi:10.1111/nph.13371

Shrivastava G et al (2015) Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. Symbiosis 65:65–74. doi:10.1007/s13199-015-0319-1

Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. Can J Bot 82:1140–1165

Simard SW, Jones MD, Durall DM (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. Mycorrhizal Ecol 157:33–74

Simonsen AK, Stinchcombe JR (2014) Herbivory eliminates fitness costs of mutualism exploiters. New Phytol 202:651–661. doi:10.1111/nph.12668

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, New York

Spehn EM et al (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98:205–218

Sprent JI (2001) Nodulation in legumes. Kew Royal Botanical Gardens, Kew

Stadler B, Solinger S, Michalzik B (2001) Insect herbivores and the nutrient flow from the canopy to the soil in coniferous and deciduous forests. Oecologia 126:104–113

Summers MC, Mondor EB (2011) *Rhizobium* alters inducible defenses in broad bean, *Vicia faba*. Open J Ecol 1:57–62

Sun XG, Tang M (2013) Effect of arbuscular mycorrhizal fungi inoculation on root traits and root volatile organic compound emissions of *Sorghum bicolor*. S Afr J Bot 88:373–379. doi:10.1016/j.sajb.2013.09.007

Tao L, Gowler CD, Ahmad A, Hunter MD, de Roode JC (2015) Disease ecology across soil boundaries: effects of below-ground fungi on above-ground host–parasite interactions. Proc R Soc Lond B Biol Sci 282. doi:10.1098/rspb.2015.1993

Tao L, Ahmad A, de Roode JC, Hunter MD (2016) Arbuscular mycorrhizal fungi affect plant tolerance and chemical defences to herbivory through different mechanisms. J Ecol 104:561–571. doi:10.1111/1365-2745.12535

Tarkka M, Hampp R (2008) Secondary metabolites of soil streptomycetes in biotic interactions. In: Karlovsky P (ed) Secondary metabolites in soil ecology. Springer, Heidelberg, pp 107–128

Tarkka MT et al (2013) OakContigDF159.1, a reference library for studying differential gene expression in Quercus robur during controlled biotic interactions: use for quantitative transcriptomic profiling of oak roots in ectomycorrhizal symbiosis. New Phytol 199:529–540. doi:10.1111/nph.12317

Tawaraya K et al (2012) Leaf herbivory by *Spodoptera litura* increases arbuscular mycorrhizal colonization in roots of soybean. Soil Sci Plant Nutr 58:445–449. doi:10.1080/00380768.2012.704520

Techau MEC, Bjornlund L, Christensen S (2004) Simulated herbivory effects on rhizosphere organisms in pea (*Pisum sativum*) depended on phosphate. Plant and Soil 264:185–194

Thamer S, Schädler M, Bonte D, Ballhorn DJ (2011) Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant. Plant and Soil 341:209–219. doi:10.1007/s11104-010-0635-4

Thompson JN (2005) The geographic mosaic of coevolution. University Chicago Press, Chicago

Tkacz A, Poole P (2015) Role of root microbiota in plant productivity. J Exp Bot 66:2167–2175. doi:10.1093/jxb/erv157

Toussaint JP, Smith FA, Smith SE (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza 17:291–297. doi:10.1007/s00572-006-0104-3

Trocha LK, Weiser E, Robakowski P (2016) Interactive effects of juvenile defoliation, light conditions, and interspecific competition on growth and ectomycorrhizal colonization of *Fagus sylvatica* und *Pinus sylvestris* seedlings. Mycorrhiza 26:47–56. doi:10.1007/s00572-015-0645-4

Ueda K et al (2013) Effects of arbuscular mycorrhizal fungi on the abundance of foliar-feeding insects and their natural enemy. Appl Entomol Zool 48:79–85. doi:10.1007/s13355-012-0155-1

Valdez Barillas JR, Paschke MW, Ralphs MH, Child RD (2007) White locoweed toxicity is facilitated by a fungal endophyte and nitrogen-fixing bacteria. Ecology 88:1850–1856

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091

van der Heijden MGA et al (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

van der Heijden MGA et al (2006) Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. FEMS Microbiol Ecol 56:178–187

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483. doi:10.1146/annurev.phyto.36.1.453

Vannette RL, Hunter MD (2009) Mycorrhizal fungi as mediators of defence against insect pests in agricultural systems. Agric For Entomol 11:351–358. doi:10.1111/j.1461-9563.2009.00445.x

Vannette RL, Hunter MD (2011) Plant defence theory re-examined: nonlinear expectations based on the costs and benefits of resource mutualisms. J Ecol 99:66–76. doi:10.1111/j.1365-2745.2010.01755.x

Vannette RL, Hunter MD (2013) Mycorrhizal abundance affects the expression of plant resistance traits and herbivore performance. J Ecol 101:1019–1029. doi:10.1111/1365-2745.12111

Varga S, Kytöviita M-M, Siikamäki P (2009) Sexual differences in response to simulated herbivory in the gynodioecious herb *Geranium sylvaticum*. Plant Ecol 202:325–336. doi:10.1007/s11258-008-9492-0

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255:571–586. doi:10.1023/A:1026037216893
Veyrath N, Robert CAM, Turlings TCJ, Erb M (2016) Herbivore intoxication as a potential primary function of an inducible volatile plant signal. J Ecol 104:591–600. doi:10.1111/1365-2745.12526
Wagner D (1997) The influence of ant nests on *Acacia* seed production, herbivory and soil nutrients. J Ecol 85:83–93. doi:10.2307/2960629
Wall L (2000) The actinorhizal symbiosis. J Plant Growth Regul 19:167–182
Wang M, Bezemer TM, van der Putten WH, Biere A (2015) Effects of the timing of herbivory on plant defense induction and insect performance in ribwort plantain (*Plantago lanceolata L.*) depend on plant mycorrhizal status. J Chem Ecol 41:1006–1017. doi:10.1007/s10886-015-0644-0
Wardle DA (2002) Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton
Waughman GJ, French JRJ, Jones K (1981) Nitrogen fixation in some terrestrial environments. In: Broughton WJ (ed) Nitrogen fixation, vol 1. Clarendon, Oxford, pp 135–192
Wearn JA, Gange AC (2007) Above-ground herbivory causes rapid and sustained changes in mycorrhizal colonization of grasses. Oecologia 153:959–971
Weber MG, Keeler KH (2012) The phylogenetic distribution of extrafloral nectaries in plants. Ann Bot. doi:10.1093/aob/mcs225
Weese DJ, Heath KD, Dentinger BTM, Lau JA (2015) Long-term nitrogen addition causes the evolution of less-cooperative mutualists. Evolution 69:631–642. doi:10.1111/evo.12594
Whitaker MRL, Katayama N, Ohgushi T (2014) Plant-rhizobia interactions alter aphid honeydew composition. Arthropod Plant Interact 8:213–220. doi:10.1007/s11829-014-9304-5
Wiemken V, Boller T (2002) Ectomycorrhiza: gene expression, metabolism and the wood-wide web. Curr Opin Plant Biol 5:355–361
Wilson K, Stinner RE (1984) A potential influence of rhizobium activity on the availability of nitrogen to legume herbivores. Oecologia 61:337–341. doi:10.1007/BF00379631
Wink M (1988) Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor Appl Genet 75:225–233
Wooley SC, Paine TD (2007) Can intra-specific genetic variation in arbuscular mycorrhizal fungi (*Glomus etunicatum*) affect a mesophyll-feeding herbivore (*Tupiocoris notatus* Distant)? Ecol Entomol 32:428–434
Wooley SC, Paine TD (2011) Infection by mycorrhizal fungi increases natural enemy abundance on tobacco (*Nicotiana rustica*). Environ Entomol 40:36–41. doi:10.1603/en10145
Wullschleger SD, Leakey ADB, St Clair SB (2007) Functional genomics and ecology – a tale of two scales. New Phytol 176:735–739
Yang HS, Dai YJ, Wang XH, Zhang Q, Zhu LQ, Bian XM (2014) Meta-analysis of interactions between arbuscular mycorrhizal fungi and biotic stressors of plants. Scientific World Journal. doi:10.1155/2014/746506
Yao Q, Zhu HH, Zeng RS (2007) Role of phenolic compounds in plant defence: induced by arbuscular mycorrhizal fungi. Allelopathy J 20:1–13
Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microbe Interact 25:139–150. doi:10.1094/mpmi-06-11-0179
Zubek S, Rola K, Szewczyk A, Majewska ML, Turnau K (2015) Enhanced concentrations of elements and secondary metabolites in *Viola tricolor* L. induced by arbuscular mycorrhizal fungi. Plant and Soil 390:129–142. doi:10.1007/s11104-015-2388-6

Unraveling the Importance of Inter- and Intraspecific Competition for the Adaptation of Forests to Climate Change

Christian Ammer

Contents

Abstract Climate change implies a new and challenging source of uncertainty for forestry and requires adaptation measures. In the context of silviculture two main approaches have recently been discussed: adapting target tree species composition, and adapting stand density. This review shows that creating mixed stands and controlling stand density through thinning are effective adaptation principles, which both may reduce resource competition among trees. Mixed stands composed of species with different functional traits and foraging strategies increase the likelihood of complementary effects because of reduced (intraspecific) competition pressure and/or facilitation effects. Thinning stands leads to lowered interception and increases in throughfall and soil water availability, improving tree recovery and resilience after drought events. For an adequate interpretation of tree growth responses to drought it is important to distinguish between the term *sensitivity*, which describes the magnitude of the individual's response to stress, and the term *vulnerability*, which describes whether or not the actual stress is crucial for both long-term performance and tree or stand survival, respectively.

C. Ammer (✉)
Silviculture and Forest Ecology of the Temperate Zones and Center for Biodiversity and Sustainable Land Use, Göttingen University, Büsgenweg 1, 37077 Göttingen, Germany
e-mail: christian.ammer@forst.uni-goettingen.de

Progress in Botany (2017) 78: 345–368, DOI 10.1007/124_2016_14,
Published online: 15 June 2016

1 Introduction

Due to the long time span between tree establishment and harvest, forestry always has had to deal with uncertainties. The probability of a stand to be affected by abiotic factors, like storms or fires or biotic factors like insect outbreaks and/ or social upheavals, increases with the length of its rotation period (i.e., time span between stand establishment and final harvest). In the past, these uncertainties have repeatedly required adaptation measures by forest management (Bolte et al. 2009). However, climate change implies a new and far more challenging source of uncertainty (Seidl and Lexer 2013). In contrast to annual plant species, long-living tree species are and increasingly will be facing the impacts of climate change within their life-span. It is not yet clear which of the expected changes will impact the different tree species most: warming, summer drought, or episodic weather extremes of severe drought in combination with heat waves (IPCC 2013; Lindner et al. 2014; Teskey et al. 2015). Tree growth is significantly reduced and the mortality risk increases under extreme or repeated severe drought (Lévesque et al. 2014; Meir et al. 2015). A lot of research has been conducted to predict the response of tree species to the climatic changes, i.e., if species are able to adapt or to migrate to other environments. However, the suitability of species distribution models as a result of such research has recently been questioned (see Keenan 2015). For example, Dobrowski et al. (2013) pointed out that complex interactions between temperature and water availability exist, which cannot be modeled precisely on a regional scale, but are required because mean values of temperature and precipitation changes are of limited value.

Even though Europe is characterized by a comparably low number of tree species (Schulze et al. 2016) the variety of woody species seems sufficiently high to assure forest persistence independent of the underlying climate change scenario (Thuiller et al. 2006). However, from the perspective of forest management the future of economically important species and the prospect of presently existing stands are of special interest and concern. Ecologists, nature conservationists, and foresters may further be concerned about preserving forest functions and the fate of rare species and genetic diversity. In a comprehensive overview, Lindner et al. (2010) summarized the existing knowledge about observed and projected impacts of climate change along bioclimatic regions. For each region they assessed exposure and sensitivity of the respective forests and the potential impacts of the expected changes in forest goods and services. Even though some regions may benefit from climate change, the authors conclude that severe and wide-ranging negative impacts have to be expected in most European regions towards the end of the twenty-first century (Lindner et al. 2010).

The question is, and will be for the up-coming decades, if forest ecosystems composed of desired tree species will be adaptive enough to respond to the expected changes, without drastically losing their ability to provide ecosystem services important to society, such as timber production, carbon sequestration, and soil protection. In the context of climate change, Smit et al. (2000) defined the term

"adaptation" as "adjustment in ecological-socio-economic systems in response to actual or expected climatic stimuli, their effects or impacts" (see also Sect. 2). Following this definition there are basically two ways for forests to adapt to a changing climate. First, adaptation through species responses, and second, adaptation through forest management actions. With respect to the ability of tree species to respond to climate change, there is some evidence from genetic studies that many genes of tree species underlie locally adaptive traits (Aitken et al. 2008). For example, European beech seedlings from the southern distribution limit showed a higher intrinsic water-use efficiency when exposed to drought than other provenances (Dounavi et al. 2016). In another recent study Schuldt et al. (2016) examined vessel diameter and density of European beech trees along a precipitation gradient in Germany. These traits are associated with xylem efficiency, safety, and growth (Schuldt et al. 2016). The study showed that vessel diameter and hydraulic efficiency decreased with decreasing precipitation while embolism resistance increased. Additionally, epigenetics seem to play an important role in the ability of a species to adapt to environmental changes (Aitken et al. 2008; Keenan 2015). Aitken et al. (2008) suggest that populations which are able to maintain high fecundity and genetic variability should be able to adapt to most of the expected climate changes although lags in genetic adaptation may occur. In contrast, adaptation of species which are characterized by small, fragmented populations, or trees species with low fecundity or late sexual maturity may be impeded (Aitken et al. 2008). However, many economically important tree species are characterized by the latter traits which raise the question if and to what extent management measures may enhance the resistance and resilience of forest ecosystems and thus their ability to adapt to the continuing changes (Millar et al. 2007). The uncertainties associated with these changes require a portfolio of measures suitable to address the wide range of potential future climate conditions (Seppälä 2009; Keenan 2015). By combining vulnerability assessment, ecosystem modeling, and multi-criteria decision analysis Seidl et al. (2011) showed that measures conducted to adapt forests to climatic changes and related processes, such as insect outbreaks, significantly reduced climate-related detrimental effects on productivity and also stand sensitivity to climate-related disturbances.

In this chapter I will look at different adaptation measures which may be carried out to reduce the vulnerability of existing and future forests to climate change. More specifically I will focus on measures to adapt forests to drought. Such measures take place at different scales, ranging from stands to landscapes and larger entities. Recent contributions have mainly addressed the latter and were focused on concepts rather than distinct descriptions of measures operating at the stand level (Keskitalo 2011; Brang et al. 2014). This review presents an overview of the most important adaptation measures on stand level. In this context I will examine the ecophysiological and/or ecological response of trees and stands to such measures. The better we understand how trees and forest stands respond to measures of forest management, the better these measures can be adjusted. This general rule of ecologically based silviculture is of particular importance for measures aiming at adapting forests to climate change. Due to the "time lag

between action and consequence in forest management" (Pinkard et al. 2015) silvicultural changes have too often been initiated by best guesses rather than decisions based on a profound mechanistic understanding of tree and stand responses to management practices.

2 Adaptive Forest Management

Generally speaking, adaptations are risk prevention measures resulting from environmental hazards that affect the vulnerability of systems important for human needs and wellbeing (Smit and Wandel 2006). Adaptive *forest* management aims at preserving and developing the functionality of forests as a prerequisite for ensuring the full range of potential future forest ecosystem services (Wagner 2004). Adaptation of forests to *climate change* implies strategies and measures which either improve the resistance or the resilience of existing or future forests even though the actual degree of climate change and its effects remain uncertain (Joyce et al. 2009; Keskitalo 2011; Keenan 2015). However, as management objectives, site conditions, adaptive potential of the species and populations, and the actual degree of change vary strongly, there is no unique strategy or measure, but a variety of suitable adaptation approaches (Spittlehouse and Stewart 2003; Bolte et al. 2010). The measures potentially available focus on modifying forest composition, forest structures, and rotation length (Bolte et al. 2009). A large number of studies have already presented different concepts and practices of adaptive forest management (see, for example, Millar et al. 2007; Bolte et al. 2009; Joyce et al. 2009; Keskitalo 2011; Seidl et al. 2011; Rist and Moen 2013). They cover different sectors, management levels, timeframes, and spatial levels (Schoene and Bernier 2012) but most of them are more or less conceptual descriptions of possible approaches rather than elaborated guidelines (Brang et al. 2014). Further, the approaches differ in their degree of effectiveness. There may be strategies that result in benefits even in the absence of climate change ("no-regret" option according to Hallegatte 2009) whereas others might overshoot the target and result in detrimental situations if the actual climate change is less severe than predicted or will lead to unexpected outcomes. An example for overshooting the target under moderate climate changes may be the replacement of productive but less drought tolerant species by other species which may be more tolerant to drought but also much less productive. Hence, if drought events stay behind the expected intensity, the species replacement might not have been necessary in the first place.

According to the three most recent reviews on adaptive forest management (Brang et al. 2014; Keenan 2015; Pinkard et al. 2015) the different approaches address different stakeholders and can be grouped into strategic and operational considerations. Strategic elements ("principles" according to Brang et al. 2014) are the following:

- Increase tree species richness; create and promote mixed forests

- Increase structural diversity
- Increase and maintain genetic variation within tree species
- Increase resistance and resilience of individual trees and stands against biotic and abiotic stressors
- Replace high-risk stands
- Keep growing stock low (i.e., short rotation period, early final harvest, stands consisting of younger and less tall trees, or stands of low density)

At the operational level these principles may be transferred to silvicultural measures ("practices" according to Brang et al. 2014). Most of these measures not only address a single but also several of the principles. For example, maintaining seed trees and promoting natural regeneration over longer time spans does not only support the genetic variability of tree species but also increases structural stand diversity. Further measures at the operational level are regeneration cuts, shorter rotation periods to keep the growing stock low, tending, thinning, adding new species by artificial regeneration, introducing other provenances of a species which are already present at a given site, control ungulate density to prevent browsing, etc. (Brang et al. 2014).

Most of the "principles" mentioned above focus on two basic silvicultural measures: (1) adapting target species composition and (2) adapting management (felling) intensity (Seidl et al. 2011). More specifically, forest management may adjust the present tree species composition by replacing highly vulnerable stands with stands composed of less sensitive species, mostly while creating mixed stands, and apply thinnings and other felling operations. Actually, the two options are partly based on a single rationale: reducing the competition for resources within a given stand (Lebourgeois et al. 2013). In mixtures, however, facilitation effects may also play a key role in explaining the better performance of mixed stands (see Sect. 3.1). In the following we will examine the two silvicultural measures in more detail.

3 Management Options

3.1 Choice of Species and Mixture Type

In recent years, research on growth dynamics of mixed stands has led to a fundamental knowledge increase on species interactions. For example, different studies showed that mixed stands can be more productive than suggested by the weighted average of the component species in monocultures (Amoroso and Turnblom 2006; Pretzsch and Schütze 2009; Pretzsch et al. 2010, 2013a, 2015). However, since factors such as species composition, site quality, and stand density come into play, no monocausal patterns of overyielding were observed.

Species composition has been shown to be a major driver of mixed stand performance compared to monocultures because complementarity effects require

mixtures of species with different functional traits, resulting in different resource demands and different abilities of resource uptake and use (Lebourgeois et al. 2013). If, for example, tree species growing in mixture do not differ much in shade tolerance, complementarity in canopy space exploration and thus higher light acquisition by the mixed stand is less likely to be observed compared to a mixture of species differing in this trait. Along a gradient across Europe it was, for example, shown that a mixture of a light demanding (*Pinus sylvestris*) and a shade tolerant species (*Fagus sylvatica*) significantly increased many aspects of structural heterogeneity related to canopy exploration (vertical structure, density, size distribution patterns, etc.) compared to the respective monocultures (Pretzsch et al. 2016). Positive effects of mixtures on tree growth (but also on other responses such as water consumption) may be caused by at least one of the two processes: reduction of competition, i.e., lower interspecific competition compared to intraspecific competition, and facilitation, i.e., at least one species has a positive influence on growth or survival of the other species (Forrester 2014).

The importance of *site quality* on the performance of a mixed stand is reflected by the stress-gradient hypothesis (SGH, Maestre et al. 2009). It suggests that facilitation will increase, and competition decrease, with increasing ecological mostly edaphic constraints and hence harsher environmental conditions (Bertness and Callaway 1994). Accordingly, Jucker et al. (2016) found that tree diversity influenced forest productivity more strongly in environmental conditions that limit productivity than in more favorable conditions. This finding is of special importance for forest adaptation as climate change is changing the environmental conditions. A nice example for the complex interactions of the response of tree species growing in mixtures and site quality was reported by Pretzsch et al. (2010). They found that the growth of Norway spruce (*Picea abies*) was fostered by admixed European beech (*Fagus sylvatica*) on poor but not on fertile soils. On poor sites beech tapped nutrients in deeper soil layers and its litterfall improved the nutrient status of spruce, a shallow rooting species. However, beech benefitted more from admixed spruce on fertile sites than on poor sites. The authors suggested that admixed spruce caused a reduction of the strong intraspecific competition of beech on fertile sites. On these sites, beech exhibits rapid canopy and root expansion (Pretzsch et al. 2010). In contrast, on poor sites intraspecific competition of beech may have been less severe which means that an admixture of spruce had a less pronounced effect (Pretzsch et al. 2010). Because of the usually superior height growth of spruce on such sites, beech may even suffer from competition for light by spruce (Pretzsch and Schütze 2009). A comparable example was reported by Condés and del Río (2015) for *Fagus sylvatica* and *Pinus sylvestris*. While European beech in mixture benefited under various site conditions, Scots pine benefited on low precipitation sites only. Interestingly, the effect of mixtures not only varies spatially but also temporally. Del Río et al. (2014) showed that annual variations in abiotic factors most likely lead to changes in the importance of competition resulting in interspecific interactions on tree growth with positive effects in low-growth years and negative effects in high-growth years.

Finally, stand *density* has also to be taken into account because it may alter the complementary effects mentioned above (Forrester and Pretzsch 2015). At low density levels, facilitative effects may be too weak, but at high density and low resource availability, interspecific competition may not sufficiently be reduced to allow for complementary effects (Forrester 2014). Accordingly, increasing stand density has been shown to both increase and reduce complementary effects in mixed-species forests (Amoroso and Turnblom 2006; Condés et al. 2013; Forrester 2014). In a recent study, Forrester (2015) could show that in a Tasmanian blue gum (*Eucalyptus globulus*) monoculture growth and evapotranspiration declined as density increased whereas no such pattern was observed in mixture with Australian acacia (*Acacia mearnsii*). The author suggested that this finding was due to intensified interactions with increasing density, some of which were complementary (Forrester 2015). He concluded that the interactions between density and complementarity and the respective growth responses are likely to differ between forests, which impedes generalizations. Similarly to density, complementary effects may also change during stand ageing, i.e., species which may weakly compete during early stages increase competition in later stages or vice versa (Filipescu and Comeau 2007; Forrester et al. 2011).

From the perspective of forest adaptation it is of special importance whether or not tree species respond differently to droughts if growing in monocultures or mixtures. One may argue that mixed stands producing more biomass than monocultures of comparable age and density consume more water than monospecific stands because transpiration is increased (Matyssek et al. 2009; Schwendenmann et al. 2015; Forrester 2015). One may further conclude that mixed stands are more susceptible to droughts than monocultures because complementary effects may be lost due to excessive water competition (Forrester 2015). However, other factors aside of transpiration have to be taken into account which may also differ between a mixed stand and the weighted average of monoculture components (Forrester 2015)[1]: (1) higher canopy throughfall and changed throughfall distribution, for example, through a higher amount of stemflow (Schume et al. 2004), (2) temporal dissimilarities of the species' water use (Gebauer et al. 2012; Schwendenmann et al. 2015), (3) different strategies (e.g., rooting pattern) of water acquisition (Rothe 1997; Schmid 2002; Schume et al. 2004; Meinzer et al. 2007), (4) different stomatal response to water shortage (Bréda et al. 2006), (5) hydraulic lift by one species which may improve the water availability of another species (Dawson 1996), and (6) different rooting plasticity and belowground competitiveness of the species involved (Grossiord et al. 2014a). As a result, different findings on

[1] An attribute A (e.g. productivity) of a mixed stand composed of species 1 (with mixing proportion x) and species 2 (with mixing proportion y) ($A_{1,2}$) may be compared to the expected attribute A' calculated from the monoculture components ($A'_{1,2}$ = attribute of species 1 in monoculture · mixing proportion x + attribute of species 2 in monoculture · mixing proportion y). $A_{1,2}$ may be equal (no interaction between the two species), higher (positive interactions) or lower (negative interaction) than $A'_{1,2}$.

water consumption and susceptibility of mixed stands to droughts were reported. In the following, I will depict some of the most illustrative studies.

In a pan-European study Grossiord et al. (2014b) tested the influence of drought on the relationship between tree species diversity and the increase in stand-level carbon isotope composition between a wet and dry year. While a reduced susceptibility of a mixed stand to drought would be indicated by a negative relationship between stand-level increase in $\delta^{13}C$ and tree species diversity, a positive relationship would indicate that forests rich in species were more affected by drought stress. Interestingly either no relationship (hemiboreal, mountainous beech, and Mediterranean forests) or negative relationships (temperate beech, thermophilous deciduous forests) between stand-level increase in $\delta^{13}C$ values and tree species diversity were detected (Grossiord et al. 2014c). The authors concluded "that higher tree species diversity offers a greater resistance to drought events in some forest types but that this pattern cannot be generalized to all forest ecosystems." It is worth mentioning that competition intensity was a confounding factor for two of the forest types which showed no relationship between species diversity and the increase in stand-level carbon isotope composition between the wet and dry year (hemiboreal and mountainous beech forests) (Grossiord et al. 2014c). This finding indicates that higher drought resistance of mixed stands is likely to be observed if interspecific competition is lower than the corresponding intraspecific competition in a monoculture at the same site (see Sect. 4). Moreover, if interspecific competition is higher than intraspecific interactions and no facilitation effects occur, even detrimental effects of interspecific competition for water resources were found. Grossiord et al. (2014b) investigated the transpiration of two oak species (*Quercus cerris* and *Quercus petraea*) in a Mediterranean forest. While the transpiration response of sessile oak (*Quercus petraea*) to soil drought did not differ between monocultures and mixed plots, transpiration of Turkey oak (*Quercus cerris*) was more reduced in mixed plots than in monoculture. The authors hypothesized that sessile oak roots had outcompeted Turkey oak roots in the deeper and wetter soil horizons (Grossiord et al. 2014b).

The degree of competitive interactions in mixtures compared to the respective monocultures may explain why species exhibiting strong intraspecific competition seem to increase drought resistance more when growing in mixtures than species exhibiting less severe intraspecific interference. The latter species may (if facilitation comes into play) or may not benefit from mixtures (see the *Quercus* example above). It has been repeatedly reported that European beech, for example, clearly benefits from the presence of other, less competitive species (Schume et al. 2004; Pretzsch et al. 2013b; Grossiord et al. 2014a; Mölder and Leuschner 2014; Metz et al. 2016). As shade tolerant species, European beech is characterized by strong intraspecific competition which results in a steeper self-thinning slope than those of co-occurring species (Pretzsch and Biber 2005; Bosela et al. 2015). Regardless of tree age, European beech shows high plasticity above- and belowground (Grams et al. 2002; Schall et al. 2012). As a result, it is more effective in exploring and occupying above- and belowground space than other species. It tends to shift its roots to deeper soil horizons when growing in mixtures (Schmid 2002;

Bolte and Villanueva 2006). By using deuterium labeling in a 10-year-old plantation, Grossiord et al. (2014a) showed that European beech extracted water from deeper soil layers when growing in mixture with conifers. Schume et al. (2004) compared soil water depletion and soil water recharge of Norway spruce and European beech monocultures with a mixed stand composed of the two species. Soil water depletion and recharge turned out to be non-additive, i.e., the values of the mixed stand did not represent the mean of the (lower) spruce evapotranspiration and the (higher) beech evapotranspiration but showed an overproportionate evapotranspiration which could, at the expense of spruce, exclusively be attributed to the beech component (Schume et al. 2004).

Pretzsch et al. (2013b) examined the basal area increment at breast height of Norway spruce and European beech in monospecific and mixed stands, and sessile oak (*Quercus petraea*) and beech in monocultures and mixed stands, along an ecological gradient through Southern Germany. Ratios of basal area increment data of dry years (1976 and 2003) and corresponding pre- or post-drought periods were computed according to Lloret et al. (2011).[2] *Resistance* (Rt) towards drought was calculated as ratio between mean basal area increment during drought (low-growth period) and mean basal area increment during respective pre-drought periods (3 years in the case of Pretzsch et al. 2013b), whereas a smaller value indicates lower resistance. *Resilience* (Rs), which describes the capacity to reach pre-drought performance levels, was calculated as the ratio between post-drought (3 years) and pre-drought increment. Values < 1 indicate a low-growth level after the episodic stress. *Recovery* (Rc) corresponds to the ratio between the post-drought mean basal area increment and the respective value during the dry period. Rc values < 1 indicate a low ability to recover relative to the dry episode (Lloret et al. 2011). In the study only beech, but not the two other species, responded positively in Rt, Rs, and Rc when growing in mixed stands compared to its Rt, Rs, and Rc values in monoculture. The positive effect of admixed species was most pronounced if beech grew together with oak (Pretzsch et al. 2013b). A similar finding was reported by Manso et al. (2015) who found that especially suppressed and intermediate beech trees benefited from growing in mixture with oak. Jonard et al. (2011) investigated the effect of oak on beech in dry years. They showed that the sensitivity of beech in stomatal conductance to extractable water was lower in a mixed than in a monospecific stand. However, it remains unknown which processes cause this effect. Zapater et al. (2011) who analyzed a mixed beech-oak stand, found no evidence of a hydraulic-uplift through oak, from with beech is assumed to benefit.

Metz et al. (2016) combined a comparable dendrochronological approach with measurements of carbon isotope composition of tree rings. Aside from also calculating Rt, Rs, and Rc values from cores of European beech trees growing in different neighborhoods, the wood $\delta^{13}C$ – signature of the beech year rings including the low precipitation pointer years 1976 and 2003 were analyzed. The target

[2] $\mathrm{Rt} = \frac{\mathrm{BAI_{DryYear}}}{\mathrm{MeanBAI_{PreDryYears}}}$ $\mathrm{Rc} = \frac{\mathrm{MeanBAI_{PostDryYears}}}{\mathrm{BAI_{DryYear}}}$ $\mathrm{Rs} = \frac{\mathrm{MeanBAI_{PostDryYears}}}{\mathrm{MeanBAI_{PreDryYears}}}$.

tree beeches used in the study grew on forest plots of the German Biodiversity Exploratories, which are spread over three major geographic regions across Germany (see Fischer et al. 2010). In all three Exploratories, beech occurs in monospecific and mixed stands. However, as the site conditions differ between the study regions the main admixed tree species (Scots Pine (*Pinus sylvestris*), valuable hardwoods (mainly *Fraxinus excelsior*, *Acer pseudoplatanus*), and Norway spruce (*Picea abies*) varies depending on the study area. For further details of the experimental setup, see Metz et al. (2013). For both dry pointer years (1976 and 2003) Rc and Rs of beech radial increment in the local mixture was higher than in the respective monospecific stands (except for Rs at the Swabian Alb). In contrast Rt was higher in intraspecific stands. However, this finding was mainly caused by the predominantly narrower mean pre-drought ring widths of target tree beeches in monocultures. As the narrow tree rings of the beech trees in the intraspecific neighborhood were reduced to approximately the same level than the wide pre-drought tree rings of the beeches growing in mixture, resistance in monocultures was apparently higher than in the respective mixtures (Metz et al. 2016). Beech trees growing under interspecific competition showed lower $\delta^{13}C$ – values. This indicates that these trees were less stressed throughout both dry periods compared to the trees growing in intraspecific competition. Interestingly the $\delta^{13}C$ – values in 2003 were closely correlated to competition intensity when calculated on a single tree basis by a competition index (Metz et al. 2013). The higher the competition, the higher the $\delta^{13}C$ – values, and, thus, the higher the degree of drought stress. This suggests that competition rather than neighborhood identity may have determined the degree of stress and it may explain why Scots pine had the most significant effect on the performance of beech in dry years. Scots pine is known to exert low competition on beech compared to other tree species (Dieler and Pretzsch 2013; Metz et al. 2013). Consequently the response of beech to the neighborhood of valuable hardwoods, such as ash or sycamore maple, both species which are more similar to beech in terms of basic plant traits, was weaker (but still apparent). Accordingly, Mölder and Leuschner (2014) who also examined the response of beech in the neighborhood of other hardwood species found that beech growth was less sensitive to environmental fluctuations in the presence of allospecific neighbors compared to a conspecific neighborhood. Apparently, the effect of mixtures is higher if the neighboring species differ in functional traits (Grossiord et al. 2014a; Metz et al. 2016). This view is supported by Lebourgeois et al. (2013). In the latter study silver fir (*Abies alba*) monocultures were compared to those of three different mixture types along a climatic gradient: fir with beech, fir with spruce, and fir with beech and spruce. It was found that mixture type changed the response of fir to summer drought but only under the most limiting conditions. This result, which again confirms the SGH, suggests that positive effects of other species increase when resources are limited. On the poor site it was further shown that fir was fostered more by beech than by spruce. This may be explained by the fact that the resource foraging strategies and functional traits of the two conifers are more similar to each other than that of fir relative to beech (Lebourgeois et al. 2013). The interception of spruce and fir, for example, is much higher than

that of beech (Schume et al. 2004) and fir may have additionally benefitted from the high beech stemflow.

It seems as if positive complementary effects for a given species, for example, through improved resource availability, are only rarely associated with a higher stress level of the admixed species. However, complementarity and lower stress levels of the species in mixed stands were not always observed (Jucker et al. 2014; Toïgo et al. 2015; Merlin et al. 2015). The contrasting findings may be explained by refining the SGH, as suggested by Maestre et al. (2009). It seems as is the SGH holds true more frequently if "the interacting species (relative tolerance to stress versus competitive ability) and the characteristics of the stress factor (resource versus non-resource)" are taken into account (Maestre et al. 2009). Therefore the degree of facilitation firstly depends on whether the abiotic stress may come from non-resource-related conditions such as heat, cold, wind, salinity, or soil structure, or from resource-related conditions such as water, light, and nutrients (Maestre et al. 2009). Secondly, the degree of facilitation may further depend on the type of species: when both the "benefactor" and the "beneficiary species" are competitive species facilitation may become important at other stress levels compared to mixtures where both species are stress tolerant but less competitive, or where one of the two species is stress tolerant and the other is competitive (Maestre et al. 2009). This important specification is illustrated in Fig. 1. Assume a species such as European beech, which effectively competes for light, growing in mixture with a less competitive species such as Scots pine which is more stress tolerant, e.g., towards drought (see, for example, Condés and del Río 2015). Due to weak competition, and direct and indirect facilitation, e.g., by hydraulic lift and/or by higher throughfall, beech increasingly benefits from the mixture with Scots pine,

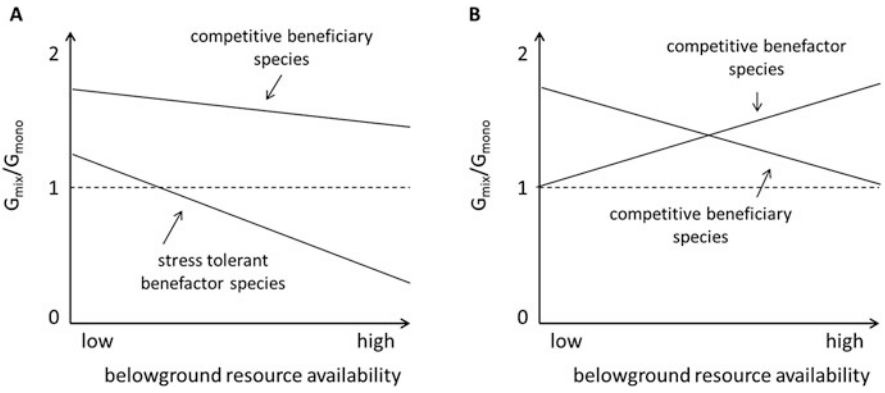

Fig. 1 Theoretical scheme showing the different potential outcomes of facilitation (and release from competition) in tree mixtures. (**a**) Depicts a mixture of a competitive beneficiary species which benefits in growth (G_{mix}, relative to its growth on an adjacent monoculture G_{mono}) across the entire gradient of site quality and a stress-tolerant benefactor species. The latter species benefits on poor sites only. (**b**) Shows a mixture of two competitive species where both species benefit from the mixture but show an opposing trend in the dependency of their growth response to site quality

while Scots pine, the benefactor species, is positively affected only on harsh sites (e.g., by improved nutrient availability). On good sites where beech displays its full competitive power, the growth of pine may respond negatively (Fig. 1a). Another outcome may be expected if two competitive species like Norway spruce and European beech interact (see, for example, Pretzsch et al. 2010). Norway spruce benefits from admixed European beech on nutrient poor sites, due to the Ca-pump effect of beech (Berger et al. 2004). On goods sites, however, where Norway spruce may not be limited by base cation availability this positive response does not occur. The admixture of beech may even result in negative effects on spruce growth, as beech shows rapid canopy and root expansion on these sites and benefits from reduced intraspecific competition when growing in mixture (Pretzsch et al. 2010). On poor sites intraspecific competition of beech is less intensive and thus admixed spruce does not imply better growing conditions (Pretzsch et al. 2010).

Summarizing the findings presented in this section shows that mixed stands composed of species with different functional traits and different nourishing strategies increase the likelihood of complementary effects because of reduced (intraspecific) competition pressure. Facilitation may play an additional role, aside of competition release (Holmgren et al. 1997). Both effects have the potential to reduce drought stress at least for some of the involved species and/or some individuals (Forrester 2015). However, it is not clear yet, to what extent "true" facilitation plays a role in addition to merely competition release. In any case, adaption strategies fostering mixed stands may help reduce the degree of drought stress in forest stands (Neuner et al. 2015). Because the functional traits of the species involved are of crucial importance, species identity seems to be more important than species diversity (Lübbe et al. 2016).

3.2 Reduction of Tree Density

In the previous section the preeminent character of competition for tree performance, resistance, and recovery from drought stress has already been highlighted. Thinnings controlling stand density are an apparent adaptation strategy that take advantage of releasing target trees from competition and have been advocated for enhancing resistance and resilience to drought. In the recent past, dendrochronological studies, isotope analyses and measurements of tree and stand transpiration, soil water availability, and tree growth have been carried out to evaluate this strategy. In the following, I will summarize main findings from some meaningful investigations. The selected studies cover a wide range of geographic regions, tree species, and time spans since the initial thinnings occurred. However, they provide a rather consistent picture on the options and limits of adapting forest stands by stand density reductions.

Measurements of tree and stand water consumption are the most laborious but most exact ways of determining the effect of thinnings on tree response to drought. Pioneer work in this field has already been carried out in the 1980s (Morikawa

et al. 1986; Aussenac and Granier 1988). Basically, the effect of a thinning event on the water status of a stand is twofold. Firstly, it decreases the interception losses and thus increases the amount of precipitation reaching the forest floor (Stogsdill et al. 1992; Simonin et al. 2007). Secondly, it reduces the transpiring surface to a certain degree, i.e., the leaf area is reduced (Bréda et al. 1995). As a consequence the extractable soil water content is increased (Cotillas et al. 2009) while stand transpiration, and hence stand water consumption is decreased (Morikawa et al. 1986; Aussenac and Granier 1988; del Campo et al. 2014; Gebhardt et al. 2014). A contradictory example was reported by Lagergren et al. (2008). In their study the transpiration level of the thinned plot was already higher in the second season after the thinning event, than that of the unthinned control. This unexpected finding could not be attributed to environmental variables, but was most likely caused by other factors, such as fertilization (Lagergren et al. 2008). Note that while stand transpiration of the stand decreases after thinning, transpiration of the remaining individual trees is enhanced due to increased light availability of formerly shaded crown parts and crown expansion increases in the years following thinning (Morikawa et al. 1986; Simonin et al. 2007; del Campo et al. 2014; Gebhardt et al. 2014). Fernandes et al. (2016), for example, reported that heavily thinned Aleppo pines (*Pinus halepensis*) transpired three times the amount of water than trees from the control plot. Due to the improved water status, predawn water potential of thinned trees was found to be higher than that of unthinned control trees (Bréda et al. 1995; White et al. 2009). The immediate response of the remaining trees to higher water availability and higher exposure to radiation, which triggers transpiration, explains why the reduction in basal area and leaf area index (LAI) was found to be higher than the reduction in stand transpiration (Morikawa et al. 1986; Bréda et al. 1995; Gebhardt et al. 2014). Neither interception nor stand transpiration is proportional to stand density reduction (Bréda et al. 1995). Therefore the effect of thinning on stand water status is partly determined by thinning intensity. On the one hand, it is apparent that a high amount of removed trees increases throughfall and soil water availability, on the other hand, very heavy reductions of stand density may also have certain constraints. Firstly, total stand productivity may fall under a desired threshold, if the remaining trees cannot fully compensate the increment loss through tree removal. Secondly, understorey vegetation may be vitalized and, through competition with trees, reduce the gain in extractable soil water (Simonin et al. 2007). Gebhardt et al. (2014), for example, found that the surprisingly fast establishment of a vital ground vegetation of a heavily thinned plot (removal of 65% of basal area) diminished the differences in stand transpiration between thinned plots and control plots by 26%.

According to the balanced-growth-hypothesis (Shipley and Meziane 2002), increasing water availability after thinning fosters carbon allocation towards the transpiring surface, i.e., leaf area (A_l) at the expense of the rooting area (Giuggiola et al. 2013; Fernández-de-Uña et al. 2016). As a consequence, the ratio between leaf area and water supplying sapwood area (A_s) is expected to increase under high water availability and to decrease under dry conditions (Giuggiola et al. 2013). Actually the A_l:A_s ratio was found to decrease with decreasing stand density due to

thinning (McDowell et al. 2006; Giuggiola et al. 2013). The increase in leaf area, and, as a result, stem growth, can most efficiently be achieved if thinnings were applied early enough, i.e., at ages when the trees are still able to substantially expand their crowns after competition release (Pretzsch 2003). In these cases diameter growth tends to be higher than the increase in tree transpiration resulting in a higher biomass production per unit water (WUE) of thinned trees compared to unthinned control trees (Gebhardt et al. 2014; Fernandes et al. 2016). If thinnings are applied at later stages, i.e., when a tree's ability to respond to increased resourced availability is limited, an improved WUE is less likely to occur. Interestingly, no effect of thinning on intrinsic water-use efficiency (WUE_i, the ratio between photosynthetic assimilation (A) and stomatal conductance (g_s)) was found (Martín-Benito et al. 2010; Fernandes et al. 2016). Surprisingly, this was not even the case in the dry years (Fernández-de-Uña et al. 2016). On the one hand, thinning increases g_s due to improved soil water availability and increased exposure to radiation which should result in reduced WUE_i following thinning (McDowell et al. 2006). On the other hand, thinning reduces competition for light and, depending on the site conditions, for nutrients. This should increase A, and hence WUE_i (Fernández-de-Uña et al. 2016). It seems as if "the effect of reduced competition in intrinsic water-use efficiency depends on the strength that the different factors exert on A and g_s" which may explain contrasting results (Fernández-de-Uña et al. 2016). Even in cases where WUE_i was lower for thinned trees in dry years it was found to be balanced via changes in above-ground architecture (A_l:A_s) (McDowell et al. 2006). One may conclude that trees respond to changes in resource availability mainly through structural changes rather than changing WUE_i (Fernández-de-Uña et al. 2016). This view is supported by recent findings that showed (1) a large increase in the size and productivity of the fine root system of trees responding to low precipitation (Hertel et al. 2013), and (2) a decrease in height growth relative to radial growth with increasing soil water deficits (Trouvé et al. 2015).

In addition to real time measurements of stand transpiration *dendrochronological approaches*, often combined with *isotope analyses*, provide a useful tool to examine the response of thinned stands to drought (Martín-Benito et al. 2010). Dendrochronological approaches examine tree-ring series and try to differentiate the climate signal from thinning, both of which influence growth variations (Misson et al. 2003). Analyses of carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) of tree rings are another tool to disentangle climate and management practices (McDowell et al. 2006; Giuggiola et al. 2016). For methodical details, see Grams et al. (2007). Giuggiola et al. (2016) examined heavily thinned Scots pine (70% removal of basal area) on a xeric site based on a dual isotope approach. They found that Scots pine growth increased strongly after thinning. Moreover, during the 30 post-thinning years, $\delta^{13}C_{tree\text{-}ring}$ of the heavy-thinning plot remained significantly lower, indicating lower drought stress. Compared with $\delta^{13}C_{tree\text{-}ring}$ and basal area increment, $\delta^{18}O_{tree\text{-}ring}$ showed a shorter response to thinning most likely because of canopy closure. Evaporative enrichment in source water was the prominent factor explaining $\delta^{18}O_{tree\text{-}ring}$. As explanation, the authors suggest higher water

availability at the thinned plots where trees were able to use "more accessible, shallower water sources that are relatively more ^{18}O enriched" (Giuggiola et al. 2016). Many dendrochronological studies focus on exceptionally dry years (Sohn et al. 2013; Metz et al. 2016). For example, Kohler et al. (2010) analyzed Norway spruce (*Picea abies*) trees from a thinning experiment and examined reductions in radial growth during drought years and their subsequent recovery by calculating indices for resistance, recovery, and resilience (see Sect. 3.1). They found a higher *resilience* of trees in heavily thinned stands towards extreme drought, even if the drought event had occurred more than 10 years after the last thinning intervention (Kohler et al. 2010). However, the study shows that the degree of tree responses may depend on (1) the time during the year when the drought occurs (spring or late summer), and (2) the time span between the last thinning intervention and the drought event. Working on the same plots Sohn et al. (2013) found that in recently thinned stands increased water availability allowed trees to better maintain growth rates during droughts compared to control stands. Interestingly, growth *recovery* of trees after the drought was improved through thinning irrespective of the time span between thinning and drought (Sohn et al. 2013). A comparable result was reported by Misson et al. (2003) who analyzed tree ring-width series from increment cores sampled in control plots along with three different thinning intensity treatments. The authors concluded that heavy-thinning intensity has made trees more resistant towards drought stress, at least 6 years after treatment. This effect seemed to be more apparent for the humid stand compared to the dry stand (Misson et al. 2003).

As mentioned above, a crucial question for forest adaption is how long the positive effects of thinnings will persist. While D'Amato et al. (2013) reported increased sensitivity of thinned stands to drought at later stand development stages due to long-term acclimation of the hydraulic architecture, others found a persisting effect of thinnings (Giuggiola et al. 2016). However, possibly D'Amato et al.'s (2013) finding is not related to tree age per se but rather to the fact that dominant trees are more abundant during later stages. Dominant trees, promoted by thinnings, are known to be more susceptible to drought in relative terms (Mérian and Lebourgeois 2011; Sánchez-Salguero et al. 2015) but it seems as if these trees absolutely still grow more than suppressed trees in dry episodes (Martínez-Vilalta et al. 2012) and recover much faster after severe droughts. The privileged condition of a tree released from competition leads to an apparent oxymoron when it is exposed to drought: while trees already suffering from competition are believed to be predisposed to decline given an additional short-term stress, such as a severe drought (see, e.g., Linares et al. 2010), dominant and large trees are described to be more sensitive to drought than smaller trees (see, e.g., Sánchez-Salguero et al. 2015). However, one should distinguish between *sensitivity*, which describes the magnitude of response of an individual to stress and *vulnerability*, which describes whether or not the actual stress is crucial for both long-term performance and survival of the tree. In other words: while a tree released from competition may become more sensitive towards droughts, it is, according to the results presented by the studies summarized in this review, less vulnerable to this kind of stress.

From a practical point of view the question remains how intense and how often a stand should be thinned. Summarizing the studies compiled in Sect. 3.2 moderate to strong and repeated thinnings appear to be the most effective way of improving resistance and resilience of forests stands. This view is confirmed by Cescatti and Piutti (1998), working on European beech, and Magruder et al. (2013), studying red pine (*Pinus resinosa*). They found that an intermediate thinning intensity resulted in a higher drought resiliency while maintaining a valuable yield. In conclusion thinnings are, when conducted early enough and applied repeatedly, without doubt a powerful tool to promote climatic resilient forest stands through competition regulation.

4 Concluding Remarks

In the previous section the prominent roles of competition and facilitation for the performance of a tree in dry periods have been highlighted. One can even go further and state that climate only modulates the growth response of competition intensity (Trouvé et al. 2014; Sánchez-Salguero et al. 2015; Condés and del Río 2015). Growing in a mixture of trees with differing demands supplemented by facilitation, and/or growing under conditions of low intraspecific competition both seem to improve the access of a tree to limiting resources, here, specifically water. Thus, the status of competition driving important aspects of the adaptive capacity, potential facilitation effects, and the genetically controlled stress tolerance determine the vulnerability of a tree and/or stand (Fig. 2).

On the one hand, the vulnerability of a forest stands depends on endogenous factors, which are based on the species autecology and stand structural attributes. On the other hand, it is partly determined by exogenous factors such as climate variables. However, only endogenous factors can be addressed by forest management, which operates at strategic and operational levels. While the choice of tree species composition at a given site is the main management decision on the strategic level, management decisions on the operational level mainly focus on controlling stand density (Schall and Ammer 2013). Both levels need to be addressed for forest adaptation, aiming at composing and managing forests which should be as invulnerable as possible. First, species (and accordingly provenances) should be preferred for regeneration which tolerate present and future abiotic and biotic stresses. Second, stand management should take all measures presented in Sect. 3 into account to make forest stands as resistant and resilient as possible to climate extremes (Fig. 2).

Section 3 showed that silvicultural practices controlling stand density and/or creating mixed stands of tree species with different functional traits are promising management options to improve the adaptability of forest ecosystems towards drought. However, the success of adaptation measures will also always be restricted by the extent of endogenous changes. Adaptation measures alone will therefore not prevent forests from detrimental impacts of climate change. There is an urgent need

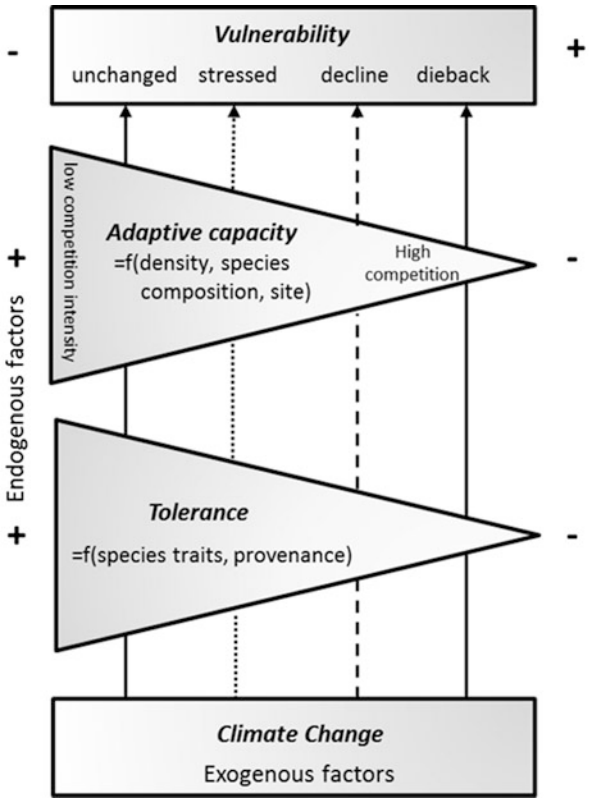

Fig. 2 Conceptual framework for the vulnerability assessment of trees and/or forest stands (adopted from Linares et al. 2010 and adjusted). Adaptation measures aiming at making forests as invulnerable as possible are most promising if they focus on species known to be tolerant against present and future abiotic and biotic stressors, and if they use all management options related to controlling species composition and stand density (for further explanation, see text)

for adaptation measures to be complemented by substantial efforts combating the anthropogenic causes of climate change, i.e., the high CO_2 emissions on the global scale. From a methodical point of view, this review underlines the value of joint research in the fields of ecophysiology, forest ecology, and silviculture for augmenting our functional understanding of neighbor tree effects. This knowledge is essential to evaluate mixed-species management options (Burkhart and Tham 1992) and to optimize stand density regulation for consolidated forest management under exacerbating water limitation.

Acknowledgements I am grateful to Rainer Matyssek who initiated this work and who gave valuable comments to an earlier version of the manuscript. I also thank Peter Annighöfer for commenting on the manuscript and linguistic corrections. Finally, I would like to thank two anonymous reviewers for critical remarks and helpful suggestions.

References

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol Appl 1:95–111

Amoroso MM, Turnblom EC (2006) Comparing productivity of pure and mixed Douglas-fir and western hemlock plantations in the Pacific Northwest. Can J For Res 36:1484–1496

Aussenac G, Granier A (1988) Effects of thinning on water stress and growth in Douglas-fir. Can J For Res 18:100–105

Berger TW, Köllensperger G, Wimmer R (2004) Plant-soil feedback in spruce (*Picea abies*) and mixed spruce-beech (*Fagus sylvatica*) stands as indicated by dendrochemistry. Plant and Soil 264:69–83

Bertness M, Callaway RM (1994) Positive interactions in communities. Trends Ecol Evol 9:191–193

Bolte A, Villanueva I (2006) Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.). Eur J For Res 125:15–26

Bolte A, Ammer C, Löf M, Madsen P, Nabuurs GJ, Schall P, Spathelf P, Rock J (2009) Adaptive forest management in central Europe: climate change impacts, strategies and integrative concept. Scand J For Res 24:473–482

Bolte A, Ammer C, Löf M, Nabuurs G-J, Schall P, Spathelf P (2010) Adaptive forest management: a prerequisite for sustainable forestry in the face of climate change. In: Spathelf P (ed) Sustainable forest management in a changing world: a European perspective, vol 19, Managing forest ecosystems. Springer, Heidelberg, pp 115–139

Bosela M, Tobin B, Šebeň V, Petráš R, Larocque GR (2015) Different mixtures of Norway spruce, silver fir, and European beech modify competitive interactions in central European mature mixed forests. Can J For Res 45:1577–1586

Brang P, Spathelf P, Larsen JB, Bauhus J, Bončìna A, Chauvin C, Drössler L, García-Güemes C, Heiri C, Kerr G, Lexer MJ, Mason B, Mohren F, Mühlethaler U, Nocentini S, Svoboda M (2014) Suitability of close-to-nature silviculture for adapting temperate European forests to climate change. Forestry 87:492–503

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann For Sci 63:625–644

Bréda N, Granier A, Aussenac G (1995) Effects of thinning on soil and tree water relations, transpiration and growth in an oak forest (*Quercus petraea (Matt.) Liebl.*). Tree Physiol 15:295–306

Burkhart HE, Tham Å (1992) Predictions from growth and yield models of the performance of mixed-species stands. In: Cannell MGR, Malcolm DC, Robertson PA (eds) The ecology of mixed stands of trees. Blackwell Scientific Publications, Oxford, pp 21–34

Cescatti A, Piutti E (1998) Silvicultural alternatives, competition regime and sensitivity to climate in a European beech forest. For Ecol Manage 102:213–223

Condés S, del Río M (2015) Climate modifies tree interactions in terms of basal area growth and mortality in monospecific and mixed *Fagus sylvatica* and *Pinus sylvestris* forests. Eur J For Res 134:1095–1108

Condés S, del Río M, Sterba H (2013) Mixing effect on volume growth of *Fagus sylvatica* and *Pinus sylvestris* is modulated by stand density. For Ecol Manage 292:86–95

Cotillas M, Sabate S, Gracia C, Espelta JM (2009) Growth response of mixed Mediterranean oak coppices to rainfall reduction: could selective thinning have any influence on it? For Ecol Manage 258:1677–1683

D'Amato AW, Bradford JB, Fraver S, Palik BJ (2013) Effects of thinning on drought vulnerability and climate response in north temperate forest ecosystems. Ecol Appl 23:1735–1742

Dawson TE (1996) Determining water use by trees and forests from isotopic, energy balance and transpiration analyses: the roles of tree size and hydraulic lift. Tree Physiol 16:263–272

del Campo AD, Fernandes TJ, Molina AJ (2014) Hydrology-oriented (adaptive) silviculture in a semiarid pine plantation: how much can be modified the water cycle through forest management? Eur J For Res 133:879–894

del Río M, Schütze G, Pretzsch H (2014) Temporal variation of competition and facilitation in mixed species forests in Central Europe. Plant Biol 16:166–176

Dieler J, Pretzsch H (2013) Morphological plasticity of European beech (*Fagus sylvatica* L.) in pure and mixed-species stands. For Ecol Manage 295:97–108

Dobrowski SZ, Abatzoglou J, Swanson AK, Greenberg JA, Mynsberge AR, Holden ZA, Schwartz MK (2013) The climate velocity of the contiguous United States during the 20th century. Glob Chang Biol 19:241–251

Dounavi A, Netzer F, Celepirovic N, Ivanković M, Burger J, Figueroa AG, Schön S, Simon J, Cremer E, Fussi B, Konnert M, Rennenberg H (2016) Genetic and physiological differences of European beech provenances (*F. sylvatica* L.) exposed to drought stress. For Ecol Manage 361:226–236

Fernandes TJG, del Campo AD, Herrera R, Molina AJ (2016) Simultaneous assessment, through sap flow and stable isotopes, of water use efficiency (WUE) in thinned pines shows improvement in growth, tree-climate sensitivity and WUE, but not in WUEi. For Ecol Manage 361:298–308

Fernández-de-Uña L, McDowell NG, Cañellas I, Gea-Izquierdo G (2016) Disentangling the effect of competition, CO_2 and climate on intrinsic water-use efficiency and tree growth. J Ecol 104:678–690

Filipescu CN, Comeau PG (2007) Competitive interactions between aspen and white spruce vary with stand age in boreal mixedwoods. For Ecol Manage 247:175–184

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S (2010) Implementing large-scale and long-term functional biodiversity research: the Biodiversity Exploratories. Basic Appl Ecol 11:473–485

Forrester DI (2014) The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. For Ecol Manage 312:282–292

Forrester DI (2015) Transpiration and water-use efficiency in mixed-species forests versus monocultures. Effects of tree size, stand density and season. Tree Physiol 35:289–304

Forrester DI, Pretzsch H (2015) Tamm review: on the strength of evidence when comparing ecosystem functions of mixtures with monocultures. For Ecol Manage 356:41–53

Forrester DI, Vanclay JK, Forrester RI (2011) The balance between facilitation and competition in mixtures of Eucalyptus and Acacia changes as stands develop. Oecologia 166:265–272

Gebauer T, Horna V, Leuschner C (2012) Canopy transpiration of pure and mixed forest stands with variable abundance of European beech. J Hydrol 442:2–14

Gebhardt T, Häberle K-H, Matyssek R, Schulz C, Ammer C (2014) The more, the better? Water relations of Norway spruce stands after progressive thinning intensities. Agric For Meteorol 197:235–243

Giuggiola A, Bugmann H, Zingg A, Dobbertin M, Rigling A (2013) Reduction of stand density increases drought resistance in xeric Scots pine forests. For Ecol Manage 310:827–835

Giuggiola A, Ogée J, Rigling A, Gessler A, Bugmann H, Treydte K (2016) Improvement of water and light availability after thinning at a xeric site: which matters more? A dual isotope approach. New Phytol 210:108–121. doi:10.1111/nph.13748

Grams TEE, Kozovits AR, Reiter IM, Barbro Winkler J, Sommerkorn M, Blaschke H, Häberle K-H, Matyssek R (2002) Quantifying competitiveness in woody plants. Plant Biol 4:153–158

Grams TEE, Kozovits AR, Häberle K-H, Matyssek R, Dawson TE (2007) Combining $\delta^{13}C$ and $\delta^{18}O$ analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. Plant Cell Environ 30:1023–1034

Grossiord C, Gessler A, Granier A, Berger S, Bréchet C, Hentschel R, Hommel R, Scherer-Lorenzen M, Bonal D (2014a) Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation. J Hydrol 519:3511–3519

Grossiord C, Gessler A, Granier A, Pollastrini M, Bussotti F, Bonal D (2014b) Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. For Ecol Manage 318:54–61

Grossiord C, Granier A, Ratcliffe S, Bouriaud O, Bruelheide H, Chećko E, Forrester DI, Dawud SM, Finér L, Pollastrini M, Scherer-Lorenzen M, Valladares F, Bonal D, Gessler A (2014c) Tree diversity does not always improve resistance of forest ecosystems to drought. Proc Natl Acad Sci 111:14812–14815

Hallegatte S (2009) Strategies to adapt to an uncertain climate change. Glob Environ Chang 19:240–247

Hertel D, Strecker T, Müller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient – is optimal resource partitioning theory applicable to water-limited mature trees? J Ecol 101:1183–1200

Holmgren M, Scheffer M, Huston MA (1997) The interplay of facilitation and competition in plant communities. Ecology 78:1966–1975

IPCC (2013) Climate Change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Cambridge University Press, United Kingdom and New York, NY, USA, 1535 pp, doi:10.1017/CBO9781107415324

Jonard F, André F, Ponette Q, Vincke C, Jonard M (2011) Sap flux density and stomatal conductance of European beech and common oak trees in pure and mixed stands during the summer drought of 2003. J Hydrol 409:371–381

Joyce LA, Blate GM, McNulty SG, Millar CI, Moser S, Neilson RP, Peterson DL (2009) Managing for multiple resources under climate change: national forests. Environ Manag 44:1022–1032

Jucker T, Bouriaud O, Avacaritei D, Dănilă I, Duduman G, Valladares F, Coomes DA (2014) Competition for light and water play contrasting roles in driving diversity–productivity relationships in Iberian forests. J Ecol 102:1202–1213

Jucker T, Avăcăriței D, Bărnoaiea I, Duduman G, Bouriaud O, Coomes DA (2016) Climate modulates the effects of tree diversity on forest productivity. J Ecol 104:388–398

Keenan RJ (2015) Climate change impacts and adaptation in forest management: a review. Ann For Sci 72:145–167

Keskitalo ECH (2011) How can forest management adapt to climate change? Possibilities in different forestry systems. Forests 2:415–430

Kohler M, Sohn J, Nägele G, Bauhus J (2010) Can drought tolerance of Norway spruce (Picea abies (L.) Karst.) be increased through thinning? Eur J For Res 129:1109–1118

Lagergren F, Lankreijer H, Kučera J, Cienciala E, Mölder M, Lindroth A (2008) Thinning effects on pine-spruce forest transpiration in central Sweden. For Ecol Manage 255:2312–2323

Lebourgeois F, Gomez N, Pinto P, Mérian P (2013) Mixed stands reduce *Abies alba* tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. For Ecol Manage 303:61–71

Lévesque M, Rigling A, Bugmann H, Weber P, Brang P (2014) Growth response of five co-occurring conifers to drought across a wide climatic gradient in Central Europe. Agric For Meteorol 197:1–12

Linares JC, Camarero JJ, Carreira JA (2010) Competition modulates the adaptation capacity of forests to climatic stress: insights from recent growth decline and death in relict stands of the Mediterranean fir *Abies pinsapo*. J Ecol 98:592–603

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709

Lindner M, Fitzgerald JB, Zimmermann NE, Reyer C, Delzon S, van der Maaten E, Schelhaas M-J, Lasch P, Eggers J, van der Maaten-Theunissen M, Suckow F, Psomas A, Poulter B, Hanewinkel M (2014) Climate change and European forests: what do we know, what are the uncertainties, and what are the implications for forest management? J Environ Manage 146:69–83

Lloret F, Keeling EG, Sala A (2011) Components of tree resilience: effects of successive low-growth episodes in old ponderosa pine forests. Oikos 120:1909–1920

Lübbe T, Schuldt B, Coners H, Leuschner C (2016) Species diversity and identity effects on the water consumption of tree sapling assemblages under ample and limited water supply. Oikos 125:86–97

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J Ecol 97:199–205

Magruder M, Chhin S, Palik B, Bradford JB (2013) Thinning increases climatic resilience of red pine. Can J For Res 43:878–889

Manso R, Morneau F, Ningre F, Fortin M (2015) Effect of climate and intra-and inter-specific competition on diameter increment in beech and oak stands. Forestry 88:540–551

Martín-Benito D, del Río M, Heinrich I, Helle G, Cañellas I (2010) Response of climate-growth relationships and water use efficiency to thinning in a *Pinus nigra* afforestation. For Ecol Manage 259:967–975

Martínez-Vilalta J, López B, Loepfe L, Lloret F (2012) Stand-and tree-level determinants of the drought response of Scots pine radial growth. Oecologia 168:877–888

Matyssek R, Wieser G, Patzner K, Blaschke H, Häberle KH (2009) Transpiration of forest trees and stands at different altitude: consistencies rather than contrasts? Eur J For Res 128:579–596

McDowell NG, Adams HD, Bailey JD, Hess M, Kolb TE (2006) Homeostatic maintenance of ponderosa pine gas exchange in response to stand density changes. Ecol Appl 16:1164–1182

Meinzer FC, Warren JM, Brooks JR (2007) Species-specific partitioning of soil water resources in an old-growth Douglas-fir–western hemlock forest. Tree Physiol 27:871–880

Meir P, Mencuccini M, Dewar RC (2015) Drought-related tree mortality: addressing the gaps in understanding and prediction. New Phytol 207:28–33

Mérian P, Lebourgeois F (2011) Size-mediated climate–growth relationships in temperate forests: a multi-species analysis. For Ecol Manage 261:1382–1391

Merlin M, Perot T, Perret S, Korboulewsky N, Vallet P (2015) Effects of stand composition and tree size on resistance and resilience to drought in sessile oak and Scots pine. For Ecol Manage 339:22–33

Metz J, Seidel D, Schall P, Scheffer D, Schulze E-D, Ammer C (2013) Crown modeling by terrestrial laser scanning as an approach to assess the effect of aboveground intra- and interspecific competition on tree growth. For Ecol Manage 310:275–288

Metz J, Annighöfer P, Schall P, Zimmermann J, Kahl T, Schulze E-D, Ammer C (2016) Site-adapted admixed tree species reduce drought susceptibility of mature European beech. Glob Chang Biol 22:903–920. doi:10.1111/gcb.13113

Millar CI, Stephenson NL, Stephens SL (2007) Climate change and forests of the future: managing in the face of uncertainty. Ecol Appl 17:2145–2151

Misson L, Nicault A, Guiot J (2003) Effects of different thinning intensities on drought response in Norway spruce (*Picea abies* (L.) Karst.). For Ecol Manage 183:47–60

Mölder I, Leuschner C (2014) European beech grows better and is less drought sensitive in mixed than in pure stands: tree neighbourhood effects on radial increment. Trees 28:777–792

Morikawa Y, Hattori S, Kiyono Y (1986) Transpiration of a 31-year-old *Chamaecyparis obtusa* Endl. stand before and after thinning. Tree Physiol 2:105–114

Neuner S, Albrecht A, Cullmann D, Engels F, Griess VC, Hahn WA, Hanewinkel M, Härtl F, Kölling C, Staupendahl K, Knoke T (2015) Survival of Norway spruce remains higher in mixed stands under a dryer and warmer climate. Glob Chang Biol 21:935–946

Pinkard E, Battaglia M, Bruce J, Matthews S, Callister AN, Hetherington S, Last I, Mathieson S, Mitchell C, Mohammed C, Musk R, Ravenwood I, Rombouts J, Stone C, Wardlaw T (2015) A

history of forestry management responses to climatic variability and their current relevance for developing climate change adaptation strategies. Forestry 88:155–171
Pretzsch H (2003) Diversität und Produktivität von Wäldern. Allg Forst Jagdztg 174:88–98
Pretzsch H, Biber P (2005) A re-evaluation of Reineke's rule and stand density index. For Sci 51:304–320
Pretzsch H, Schütze G (2009) Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: evidence on stand level and explanation on individual tree level. Eur J For Res 128:183–204
Pretzsch H, Block J, Dieler J, Dong PH, Kohnle U, Nagel J, Spellmann H, Zingg A (2010) Comparison between the productivity of pure and mixed stands of Norway spruce and European beech along an ecological gradient. Ann For Sci 67:712
Pretzsch H, Bielak K, Block J, Bruchwald A, Dieler J, Ehrhart HP, Kohnle U, Nagel J, Spellmann H, Zasada M, Zingg A (2013a) Productivity of mixed versus pure stands of oak (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) and European beech (*Fagus sylvatica* L.) along an ecological gradient. Eur J For Res 132:263–280
Pretzsch H, Schütze G, Uhl E (2013b) Resistance of European tree species to drought stress in mixed versus pure forests: evidence of stress release by inter-specific facilitation. Plant Biol 15:483–495
Pretzsch H, del Río M, Ammer C, Avdagic A, Barbeito I, Bielak K, Brazaitis G, Coll L, Dirnberger G, Drössler L, Fabrika M, Forrester DI, Godvod K, Heym M, Hurt V, Kurylyak V, Löf M, Lombardi F, Mohren F, Motta R, den Ouden J, Pach M, Ponette Q, Schütze G, Schweig J, Skrzyszewski J, Sramek V, Sterba H, Stojanovic D, Svoboda M, Vanhellemont M, Verheyen K, Wellhausen K, Zlatanov T, Bravo-Oviedo A (2015) Growth and yield of mixed versus pure stands of Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) analyzed along a productivity gradient through Europe. Eur J For Res 134:927–947
Pretzsch H, del Río M, Schütze G, Ammer C, Annighöfer P, Avdagic A, Barbeito I, Bielak K, Brazaitis G, Coll L, Drössler L, Fabrika M, Forrester DI, Kurylyak V, Löf M, Lombardi F, Mohren F, Motta R, Pach M, Ponette Q, Sramek V, Sterba H, Stojanović D, Svoboda M, Verheyen K, Zlatanov T, Bravo-Oviedo A (2016) Mixing of Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) enhances structural heterogeneity, and the effect increases with humidity. For Ecol Manag 373:149–166
Rist L, Moen J (2013) Sustainability in forest management and a new role for resilience thinking. For Ecol Manage 310:416–427
Rothe A (1997) Einfluss des Baumartenanteils auf Durchwurzelung, Wasserhaushalt, Stoffhaushalt und Zuwachsleistung eines Fichten-Buchen-Mischbestandes am Standort Höglwald. Forstliche Forschungsberichte München 163:1–174
Sánchez-Salguero R, Linares JC, Camarero JJ, Madrigal-González J, Hevia A, Sánchez-Miranda Á, Ballesteros-Cánovas JA, Alfaro-Sánchez R, García-Cervigón AI, Bigler C, Rigling A (2015) Disentangling the effects of competition and climate on individual tree growth: a retrospective and dynamic approach in Scots pine. For Ecol Manage 358:12–25
Schall P, Ammer C (2013) How to quantify forest management intensity in Central European Forests. Eur J For Res 132:379–396
Schall P, Lödige C, Beck M, Ammer C (2012) Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. For Ecol Manage 266:246–253
Schmid I (2002) The influence of soil type and interspecific competition on the fine root system of Norway spruce and European beech. Basic Appl Ecol 3:339–346
Schoene DH, Bernier PY (2012) Adapting forestry and forests to climate change: a challenge to change the paradigm. Forest Policy Econ 24:12–19
Schuldt B, Knutzen F, Delzon S, Jansen S, Müller-Haubold H, Burlett R, Clough Y, Leuschner C (2016) How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? New Phytol 210:443–458

Schulze ED, Aas G, Grimm GW, Gossner MM, Walentowski H, Ammer C, Kühn I, Bouriaud O, von Gadow K (2016) A review on plant-diversity and forest management of European beech forests. Eur J For Res 135:51–67

Schume H, Jost G, Hager H (2004) Soil water depletion and recharge patterns in mixed and pure forest stands of European beech and Norway spruce. J Hydrol 289:258–274

Schwendenmann L, Pendall E, Sanchez-Bragado R, Kunert N, Hölscher D (2015) Tree water uptake in a tropical plantation varying in tree diversity: interspecific differences, seasonal shifts and complementarity. Ecohydrology 8:1–12

Seidl R, Lexer MJ (2013) Forest management under climatic and social uncertainty: trade-offs between reducing climate change impacts and fostering adaptive capacity. J Environ Manage 114:461–469

Seidl R, Rammer W, Lexer MJ (2011) Adaptation options to reduce climate change vulnerability of sustainable forest management in the Austrian Alps. Can J For Res 41:694–706

Seppälä R (2009) A global assessment on adaptation of forests to climate change. Scand J For Res 24:469–472

Shipley B, Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and roots biomass allocation. Funct Ecol 16:326–331

Simonin K, Kolb TE, Montes-Helu M, Koch GW (2007) The influence of thinning on components of stand water balance in a ponderosa pine forest stand during and after extreme drought. Agric For Meteorol 143:266–276

Smit B, Wandel J (2006) Adaptation, adaptive capacity and vulnerability. Glob Environ Chang 16:282–292

Smit B, Burton I, Klein R, Wandel J (2000) An anatomy of adaptation to climate change and variability. Clim Change 45:223–251

Sohn J, Gebhardt T, Ammer C, Bauhus J, Häberle K-H, Matyssek M, Grams TEE (2013) Mitigation of drought by thinning: short-term and long-term effects on growth and physiological performance of Norway spruce (*Picea abies*). For Ecol Manage 308:188–197

Spittlehouse DL, Stewart RB (2003) Adaptation to climate change in forest management. BC J Ecosyst Manag 4:1–11

Stogsdill WR, Wittwer RF, Hennessey TC, Dougherty PM (1992) Water use in thinned loblolly pine plantations. For Ecol Manage 50:233–245

Teskey R, Wertin T, Bauweraerts I, Ameye M, McGuire MA, Steppe K (2015) Responses of tree species to heat waves and extreme heat events. Plant Cell Environ 38:1699–1712

Thuiller W, Lavorel S, Sykes MT, Araújo MB (2006) Using niche-based modelling to assess the impact of climate change on tree functional diversity in Europe. Divers Distribution 12:49–60

Toïgo M, Vallet P, Tuilleras V, Lebourgeois F, Rozenberg P, Perret S, Courbaud B, Perot T (2015) Species mixture increases the effect of drought on tree ring density, but not on ring width, in *Quercus petraea–Pinus sylvestris* stands. For Ecol Manage 345:73–82

Trouvé R, Bontemps J-D, Collet C, Seynave I, Lebourgeois F (2014) Growth partitioning in forest stands is affected by stand density and summer drought in sessile oak and Douglas-fir. For Ecol Manage 334:358–368

Trouvé R, Bontemps J-D, Seynave I, Collet C, Lebourgeois F (2015) Stand density, tree social status and water stress influence allocation in height and diameter growth of *Quercus petraea* (Liebl.). Tree Physiol 35:1035–1046

Wagner S (2004) Möglichkeiten und Beschränkungen eines funktionsorientierten Waldbaus. Forst und Holz 59:105–111

White DA, Crombie DS, Kinal J, Battaglia M, McGrath JF, Mendham DS, Walker SN (2009) Managing productivity and drought risk in *Eucalyptus globulus* plantations in south-western Australia. For Ecol Manage 259:33–44

Zapater M, Hossann C, Bréda N, Bréchet C, Bonal D, Granier A (2011) Evidence of hydraulic lift in a young beech and oak mixed forest using ^{18}O soil water labelling. Trees 25:885–894

Plants and Atmospheric Aerosols

Jürgen Burkhardt and David A. Grantz

Contents

Abstract Atmospheric aerosols are liquid, solid, or mixed suspensions of heterogeneous chemical composition, ranging from a few nanometers to almost 100 μm in diameter. Plants are sources and sinks of these diverse aerosols. Vegetation is

J. Burkhardt (✉)
Institute of Crop Science and Resource Conservation, University of Bonn,
Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany
e-mail: j.burkhardt@uni-bonn.de

D.A. Grantz
Institute of Crop Science and Resource Conservation, University of Bonn,
Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany

University of California at Riverside, Kearney Agricultural Center, 9240 S. Riverbend Ave.,
Parlier, CA 93648, USA
e-mail: dagrantz@ucanr.edu

Progress in Botany (2017) 78: 369–406, DOI 10.1007/124_2016_12,
Published online: 28 June 2016

influenced by aerosols through the water cycle, radiation balance, and nutrient transport, on global and regional scales, but direct interactions of aerosols with plant ecophysiology have not been considered in sufficient detail. Plant surface characteristics and aerodynamic factors control deposition. These factors may be manipulated in efforts to mitigate aerosol concentrations using urban vegetation as efficient aerosol collectors. Hygroscopic aerosols deposited on leaves generate concentrated solutions that reduce surface tension and generate thin liquid films. These films are shown to enter the stomatal pores, facilitating foliar nutrient uptake and enhancing liquid water loss that is poorly controlled by stomata. Aerosol pollution can reduce plant drought tolerance and alter nutrient balance. Anthropogenic aerosols now exceed natural aerosols, particularly in urban areas. The effects of these aerosols on plants require a focused research effort.

1 Introduction

Atmospheric aerosols cover a size range of less than 1 nm in aerodynamic diameter (d_p) to more than 100 μm, about five orders of magnitude. Aerosols $\leq$100 nm are termed ultrafine, 0.1–1 μm fine, and >1 μm coarse. Accumulation mode aerosols are 0.1–1 μm and are important carriers of plant nutrients. Typical number concentrations range from ~100 cm^{-3} in pristine marine environments to ~100,000 cm^{-3} in polluted urban regions, corresponding to mass concentrations of 1–100 μg m^{-3} (Pöschl 2005). Atmospheric lifetimes in the troposphere range from seconds to months, allowing intercontinental transport of fine aerosols (Yu et al. 2012). In this way, aerosols transport large amounts of pollutants and nutrients (Field et al. 2010; Lequy et al. 2012). Some ecosystems, e.g., those in the Amazon and in the Hawaiian Islands, are dependent upon mineral input by deposition of such aerosols from remote sources (Chadwick et al. 1999; Newman 1995; Swap et al. 1992).

Atmospheric aerosols can be classified in several ways, in addition to size. Natural and anthropogenic sources may differ in composition and source characteristics, but may overlap, as in the cases of dust from disturbed soil and nitrate from fertilized agriculture. Primary aerosols are directly emitted, from abrasion or combustion processes, while secondary aerosols are formed in the atmosphere by the transformation of directly emitted precursor gases (Fuzzi et al. 2015). The major chemical constituents of aerosols are inorganic ions (mainly nitrate, sulfate, and ammonium), mineral dust from geologic sources, abrasion products such as automobile brake lining fragments, sea salt, and carbonaceous materials including secondary organic compounds and elemental or black carbon (Fuzzi et al. 2015; Zhang et al. 2007). Aerosols may be of biological origin, including viable bacteria, viruses, fungal spores, pollen, and nonviable plant debris (Despres et al. 2012). Plants emit biogenic precursor gases that are transformed to secondary aerosols, including dimethyl sulfide (DMS) from phytoplankton (Charlson et al. 1987) and

monoterpenes and isoprene from forest vegetation (Claeys et al. 2004; Ehn et al. 2014; Tunved et al. 2006). Plants thus contribute to atmospheric aerosol and are, in turn, affected by aerosol-induced changes in radiation; thus, aerosols participate in the biological feedback regulation of climate (Boucher 2015).

Human disturbance of the global aerosol system is an indicator of the Anthropocene (Mahowald et al. 2011; Pöschl and Shiraiwa 2015). Climatic and health effects suggest a poorly characterized planetary tipping point, whereby crossing an unknown boundary could generate abrupt or irreversible environmental changes (Steffen et al. 2015). Combustion, construction, industry, agriculture, and desertification have more than doubled the global mean aerosol number concentration since preindustrial times (Hamilton 2015). This has serious public health implications, particularly in highly polluted urban and industrial environments (USEPA 2004, 2009; WHO 2013). Aerosols are defined for regulatory purposes by size classes that differ in human respirability. Aerosol with $d_p < 10$ μm enters into deep lung airways, while aerosol with $d_p < 2.5$ μm, and particularly with $d_p < 1$ μm, may enter the blood stream, reaching extrapulmonary tissues such as the heart, reproductive and digestive systems, brain, and liver. Chronic and acute exposure studies indicate that increases in aerosol concentration are causally associated with cardiovascular and respiratory morbidity and mortality (Anderson et al. 2012; Brook et al. 2010; Fuzzi et al. 2015; Pope and Dockery 2006). Model projections indicate that by 2050 outdoor air pollution may be the largest global source of premature death (OECD 2012). Emission controls and phytoremediation through urban vegetation appear the most effective approaches to mitigating aerosols in the human environment.

Indirect, mass-related biogeochemical effects of aerosols on ecosystems have been more thoroughly investigated than possible direct effects of aerosols on individual plants (Grantz et al. 2003). Direct effects from dust may come from abrasion, radiative heating, reduced light reaching the photosynthetic tissues (Grantz et al. 2003; Sharifi et al. 1997), physical blockage of stomata (Ricks and Williams 1974; Flückiger et al. 1977; Farmer 1993), or stomatal entry of sub-micrometer aerosols (Gmur et al. 1983). Deposition of hygroscopic aerosols on leaves can also have direct effects on plants. In the atmosphere, aerosols govern cloud condensation behavior, and they act similarly after deposition, attracting water from humid air, including that generated at the leaf surface by transpiration. The chaotropicity of hygroscopic aerosols determines their effects on water surface tension (Dutcher et al. 2010) which facilitates the penetration of liquid into stomatal pores (Burkhardt et al. 2012), contrary to a long-standing paradigm based on the properties of pure water.

Once deposited to plant surfaces, aerosols nominally cease being aerosols, i.e., surrounded by air molecules. Their physicochemical properties likely persist on the new hydrophobic substrate (Burkhardt 2010; Burkhardt and Eiden 1994), and the term "aerosol" is used throughout this review to reflect this essential character. Thus, "aerosol" is synonymous with "particles," "particulate matter," and "PM," as used elsewhere. In the following discussion, we consider the composition and deposition of aerosols to vegetation and the resulting direct and indirect impacts

of deposited aerosol on plants and ecosystems. Recent developments suggest the importance of aerosols as an environmental factor and highlight the need for further characterization of complex aerosol-plant interactions.

2 Atmospheric Aerosols

The combination of varied source types, different emission strengths, and short atmospheric lifetimes compared to many trace gases causes substantial temporal and spatial heterogeneity in aerosol distribution, with the largest concentrations occurring close to natural or anthropogenic emission sources (Hamilton 2015). Nevertheless, the global atmospheric burden of aerosols influences planetary climate and radiation available for plant growth.

Aerosols scatter, absorb, and reflect solar radiation and serve as cloud condensation nuclei (CCN, Andreae and Crutzen 1997; Charlson et al. 1987; Lohmann and Feichter 2005). A hygroscopicity parameter κ (kappa) has been developed to describe the CCN activity of aerosols (Petters and Kreidenweis 2007). While size is the most important factor, the chemistry of aerosols plays an important secondary role in CCN activity (Dusek et al. 2006). Fine aerosols are strongly affected by a continuous process of atmospheric aging. Repeated condensation, evaporation, and oxidation processes lead to homogeneous "internally mixed" aerosol populations (Fuzzi et al. 2015). Timescales of less than 1 day are required in photochemically active environments for the conversion of complex aerosol mixtures to internally mixed populations (Wang et al. 2010). Nevertheless, many primary aerosols maintain a nonvolatile core that may represent a small fraction of the mass but may be useful in source attribution (Fuzzi et al. 2015).

The net effect of atmospheric aerosols on radiation is to increase planetary albedo, reflecting sunlight back into space. Albedo has increased due to CCN activity in the postindustrial era of the last 150 years. This has masked the full extent of climate warming caused by greenhouse gas emissions in the same period (Boucher 2015), although at intermediate elevations aerosols absorb infrared radiation and may, themselves, increase atmospheric warming. These physical effects have been recently reviewed by Fuzzi et al. (2015). Effects on vegetation are a net result of numerous and sometimes conflicting aerosol impacts. Reduced direct beam radiation reduced photosynthesis in a model treatment of crop loss due to severe aerosol pollution in China (Chameides et al. 1999), suggesting a 5–30 % reduction in yield of most crops. On the other hand, diffuse radiation penetrates more uniformly into plant canopies which may increase productivity by better balancing radiation interception by upper and lower leaves (Hoyt 1978; Mercado et al. 2009). A model treatment of global plant productivity from 1950 to 1980 suggested increased productivity due to light scattering, despite decreased total radiation interception due to regional haze.

2.1 Natural Aerosols

Natural primary aerosols have both inorganic and organic components. Inorganic aerosols include 1,000–10,000 Tg year^{-1} of global marine sea salt, formed by wind shear and the action of breaking waves, plus 1,000–3,000 Tg year^{-1} of geologic material largely transported from deserts (Boucher 2015; de Leeuw et al. 2011; Fuzzi et al. 2015; Hamilton et al. 2014). Annual emissions of organic aerosol from wildfires are about 20–35 Tg year^{-1} (Carslaw et al. 2010) and of primary biological aerosols up to 1,000 Tg year^{-1} (Despres et al. 2012; Jaenicke 2005). Due to the relatively large size of many natural primary aerosols, particularly sea salt and dust, they dominate aerosol mass in the atmosphere.

Secondary aerosols are usually smaller than primary aerosols and contribute less to mass, but dominate aerosol number and surface area. This latter property is important for atmospheric chemistry. Natural secondary aerosols are formed in the atmosphere from gaseous precursors such as SO_2 from volcanic activity (6–-20 Tg S year^{-1}), dimethyl sulfide (DMS) emitted by phytoplankton in marine environments (20–40 Tg year^{-1}), and terpenoid hydrocarbons emitted by plants (40–400 Tg year^{-1}) (Fuzzi et al. 2015). Natural aerosol sources vary substantially on seasonal, interannual, and decadal timescales.

There appear to be no places on Earth, particularly in the Northern Hemisphere, with truly pristine conditions. Aerosols approaching pristine background may be approached over the oceans in the Southern Hemisphere. In these areas, aerosols consist of sea salt, organics, sulfates from the oxidation of biogenic DMS, mineral dust, and possibly smoke from wildfires. In biologically productive ocean regions, typical concentrations of CCN are in the low hundreds per cm^3, but over the midlatitude oceans in winter, when biological and photochemical activity are low, only a few tens of CCN per cm^3 are observed (Andreae 2007; Hamilton et al. 2014).

2.2 Anthropogenic Aerosols

Human perturbation has considerably changed the planetary aerosol system. Anthropogenic primary aerosols are from industrial dust (40–130 Tg year^{-1}), biomass burning (50–90 Tg year^{-1}), soot (25–40 Tg year^{-1}), and soil abrasion from agricultural and construction activities (Boucher 2015; Mulitza et al. 2010). Anthropogenic secondary aerosols are mostly sulfate derived from precursor SO_2 (70–90 Tg S year^{-1}), volatile organic compounds (VOCs, 100–560 Tg C year^{-1}), NH_3 (20–50 Tg N year^{-1}), and nitrate derived from precursor NO_x (30–-40 Tg N year^{-1}) (Boucher 2015).

Human impacts on aerosol production started with land surface manipulation by fire and plow but increased strongly over the last 250 years through industrialization and intensified land use change (Andreae 2007; Mulitza et al. 2010). Anthropogenic

emissions have caused large enhancement of aerosol mass loadings, including transport to remote parts of the continents, with typical enhancements of 50–300 % over remote regions of Asia, North America, and South America (Andreae 2007). Such transport to remote regions has regulatory implications. For example, the mass of aerosol arriving in North America is comparable to domestic emissions (Yu et al. 2012).

Continental aerosol number concentrations are typically higher than marine levels, though these may have been similar prior to human disturbance (Andreae 2007). However, the dominance of secondary organic aerosols over water-soluble sulfates and inorganic nitrates has increased (Andreae 2007; Pajunoja et al. 2015). Anthropogenic aerosols are characterized by high concentrations of fine aerosols (Kaufman et al. 2002), reducing the number fraction of natural aerosols with minor effects on their mass fraction. Number concentrations of CCN have increased by over twofold globally and up to tenfold in highly polluted continental regions (Hamilton 2015), with a corresponding reduction in the relative importance of natural aerosols (Hamilton 2015).

Agriculture is the largest source of $PM_{2.5}$ in Europe, Russia, Turkey, Korea, Japan, and the Eastern USA, up to 40 % of the total in many European countries. Agricultural releases of ammonia (NH_3) from fertilizer use and domesticated animals affect air quality through several multiphase chemical pathways, forming aerosol ammonium sulfate and nitrate. Mitigation strategies are complex and region specific. Combined strategies reducing both secondary and primary aerosol are needed (Fuzzi et al. 2015; Paulot and Jacob 2014). During 2001–2010, a decrease in aerosol number concentration was observed over Europe and the Northern Hemisphere, associated with decreasing emissions of primary aerosols and SO_2 (Asmi et al. 2013). NH_3 often limits atmospheric production of $PM_{2.5}$, so that reduction of NH_3 emissions may also effectively reduce aerosol formation (Lelieveld et al. 2015). However, reductions of precursor gases are less effective in low latitudes, because the oxidation capacity of the atmosphere is higher and gas-to-particle conversion is more rapid (Manktelow et al. 2007).

The anthropogenic-dominated aerosol environment represents a novel evolutionary driver for plants.

3 Deposition of Aerosols

3.1 Wet and Occult Deposition

Wet deposition of aerosols is carried to the earth's surface by interaction with hydrometeors (i.e., raindrops, snowflakes, hail; Lovett 1994). Such interactions may be by washout below the cloud level. In this case, both impaction of hydrometeors with coarse aerosols and diffusion of ultrafine and CCN aerosols to the falling droplets scrub aerosol from the atmosphere and carry it to the land surface.

Alternatively, rainout within forming clouds occurs as hygroscopic fine aerosols are incorporated by nucleation into cloud droplets, eventually growing sufficiently massive that they become hydrometeors themselves. Rainout is more effective in removing accumulation mode aerosols than washout.

Most wet-deposited aerosol reaches the soil, although in extremely polluted air, wet deposition may increase aerosol loading on leaves (Wang et al. 2015). In most cases, rain decreases the element accumulation on leaves, although rainfall does not effectively clean leaf surfaces. Simulated heavy rainfall, by aqueous washing of leaves with agitation, only removed about half of the aerosol deposited to a range of plant species (Dzierzanowski et al. 2011). Two days of continuous natural rainfall did not substantially reduce the mass of coarse or fine aerosols on the foliage of five urban tree species (Freer-Smith et al. 2005). While total aerosol is greater on upper leaf surfaces (Wang et al. 2015), fine aerosol is also deposited on lower surfaces where it is less accessible to cleaning by rainfall. In the case of cement and limestone quarry dust (Brandt and Rhoades 1972, 1973; Lerman and Darley 1975), the formation of crusts impedes washing by rain.

Resistance of deposited aerosol to removal by rainfall also reflects the embedding of aerosol in waxes. Studies with two-stage extraction of leaf surfaces with water followed by chloroform (Mo et al. 2015; Popek et al. 2013; Saebo et al. 2012; Sgrigna et al. 2015), and analysis of magnetic properties of leaves in high-aerosol urban environments (Hofman et al. 2014), have shown that fine aerosols, including those with magnetic properties, become embedded in the surface waxes. This confirms earlier results with other techniques (Kozlov et al. 2000; Lin and Schuepp 1996; Oliva and Raitio 2003; Oliva and Valdes 2004; Simmleit et al. 1989).

A distinct mode of particulate deposition is known, for historical rather than physical reasons, as occult deposition. In this case, deposition is driven by advective wind and impaction of aerosol-laden fog droplets on vegetation elements. Occult deposition was hidden (thus "occult") from early measurement efforts that captured dry and wet deposition. Nevertheless, occult deposition may be of substantial significance in some ecosystems. The existence of cloud forests and the quantifiable inputs of fog to crop irrigation requirements (Snyder 2015) suggest the importance of occult deposition of water. Forested hillsides intercept four- to sixfold greater inputs of wet and occult deposition than short vegetation at lower elevations due to topographic factors and greater micrometeorological coupling to the atmosphere (Unsworth and Wilshaw 1989).

3.2 Dry Deposition

3.2.1 Deposition Processes

While wet and occult deposition dominate aerosol accumulation by vegetated landscapes in humid regions, in more arid environments dry deposition dominates. Dry deposition occurs by sedimentation of coarse aerosol and, for fine aerosol, by

impaction through turbulent transport. Resuspension (i.e., removal of deposited aerosols by wind) decreases with time and increases with aerosol size and wind speed (Nicholson 1993; Ould-Dada and Baghini 2001). Resuspension rates of fine aerosol are extremely small (Gillette et al. 2004; Nicholson 1993; Peters and Eiden 1992; Pryor et al. 2008; Reynolds 2000).

The distinction between fine and coarse aerosols is somewhat arbitrary, subject to differing regulatory definitions in different jurisdictions. However, for fine aerosol deposition, the interaction of sedimentation, governed by Stokes' law, and impaction, governed by eddy dynamics and near-surface diffusion, is well understood (see Grantz et al. 2003). Stokes' law predicts increasing deposition velocities (V_d, ratio of flux to concentration) due to sedimentation with increasing aerodynamic diameter. In contrast, the relationships describing Brownian diffusion predict decreasing V_d with increasing diameter. These theoretical relationships intersect at a minimum V_d near 1 μm, providing a relatively robust cutoff between fine and coarse aerosols. Experimental data (Little and Wiffen 1977; Peters and Eiden 1992), influenced by turbulence and receptor morphology that are not included in these theoretical relationships, follow the predictions relatively closely. Measured minimum V_d, however, was somewhat lower d_p than the theoretical prediction of 1 μm. The low V_d of the largely anthropogenic fraction between 0.1 and 1 μm increases its atmospheric residence time and potential for aging as described above. As this fraction becomes more oxidized, it develops greater hygroscopicity and in humid air increases more in size than other fractions. This affects both its deposition and potential activity on the leaf surface.

The deposition of fine aerosols is affected both by environmental factors, such as wind speed, temperature, humidity, aerosol concentrations, and urban structure (Mori et al. 2015b), and by the biophysical characteristics of the plant stand, including plant density, leaf surface area index, phenology, and height. As noted above for wet and occult deposition, tall vegetation such as forests generates more frictional drag and higher aerodynamic roughness than short canopies, leading to higher rates of fine aerosol deposition (Erisman and Draaijers 2003; Fowler et al. 1999).

The overall V_d depends on aerosol transport through the leaf boundary layer (LBL). The LBL is directly adjacent to the surface and thus represents the lowest part of the atmosphere, where wind speed is reduced by surface friction and where temperature and humidity are significantly influenced by the leaf (Schuepp 1993). The thickness of the LBL is in the range of micrometers to millimeters, increasing with leaf size and decreasing with wind speed. The boundary layer is thinnest at leading edges (Grace and Wilson 1976), increasing deposition near leaf margins (Huang et al. 2015; Lin and Schuepp 1996; Wang et al. 2015; Wild et al. 2006). Within the viscous (quasi-laminar, turbulence-free) sublayer of the LBL, transport of gases and ultrafine aerosols by Brownian motion leads to rapid diffusion to the leaf surface. For fine aerosols $0.1 < d_p < 1$ μm, neither inertial impaction nor diffusion is very effective, resulting in the minimum V_d of accumulation mode aerosol described above (Slinn 1982).

The extent of aerosol deposition to a plant canopy may be defined by aerosol capture efficiency (C_p), the ratio of captured aerosols to aerosols available for capture (Beckett et al. 2000; Belot et al. 1976; Räsänen et al. 2013). Plants differ substantially in C_p (Freer-Smith et al. 2005; Mori et al. 2015a; Popek et al. 2013; Saebo et al. 2012) reflecting differences in morphology, foliage density, and position but also individual leaf properties, including hairiness and microroughness of the leaves, characteristic dimension, thickness, and wax surface microroughness (Burkhardt et al. 1995; Chamberlain 1967; Howsam et al. 2000; Martell 1974; Meyers et al. 2006; Rauret et al. 1994; Slinn 1982). Leaves with roughness elements such as trichomes accumulate more aerosol than glabrous leaves (Kuki et al. 2008; Yang et al. 2015) and waxy conifer needles more than broadleaf leaves (Räsänen et al. 2013; Reznik and Schmidt 2008). A correlation between the amount of waxes and aerosol collection (d_p 2.5–10 μm) has been observed (Popek et al. 2013).

In *Quercus robur*, $C_p = 6 \times 10^{-4}$ for 1.2 μm aerosols at a wind speed of 2 m s^{-1} but increased twofold at 10 ms^{-1} (Reinap et al. 2009). In *Pinus nigra* for 0.8 μm aerosols, $C_p = 13 \times 10^{-4}$ at 1 m s^{-1} but increased 22-fold at 10 m s^{-1} (Beckett et al. 2000). While this effect of wind speed is well known, the mechanism remains uncertain, particularly for fine aerosol. Turbulent bursts entering the viscous sublayer may accelerate aerosol transport (Peters and Eiden 1992), in part by aerosols migrating against the turbulence gradient (turbophoresis) and accumulating within the viscous sublayer (Guha 2008). Deposition may be enhanced by residual turbulent fluctuations very near the deposition surface ("near-wall" effects, Botto et al. 2005; Guha 2008; Marchioli et al. 2006; Parker et al. 2008). These effects are poorly characterized and require further consideration to reconcile theory with the results of wind tunnel experiments and environmental loading of fine aerosol to leaves (Burkhardt et al. 1995; Freer-Smith et al. 2004; Huang et al. 2015; Wiman 1986).

3.2.2 Quantification of Deposited Aerosols

Approaches to describe aerosol deposition fluxes include big-leaf schemes in climate models, rough boundary layers used in air quality models, and multilayered models used in ecosystem studies (Feng 2008; Huang et al. 2014, 2015; Katul et al. 2010; Peters and Eiden 1992; Petroff et al. 2008; Wesely and Hicks 2000). Deposition models require a general understanding of the processes involved, and credible results can be achieved when relevant environmental factors are sufficiently known. Micrometeorological approaches such as eddy covariance or gradient techniques combined with aerosol concentration measurements can address deposition fluxes of number, mass, or chemical constituents of aerosol over large areas (Deventer et al. 2013; Nemitz et al. 2009; Peters and Eiden 1992; Pryor et al. 2008; Wyers and Duyzer 1997).

Differences (up to an order of magnitude) that are frequently observed between micrometeorological measurements and model predictions (Feng 2008; Gallagher et al. 1997; Guha 1997; Meyers et al. 2006; Pryor et al. 2008; Reynolds 2000;

Wesely and Hicks 2000; Wyers and Duyzer 1997) might relate to leaf microroughness effects or to difficulties in quantifying volatile aerosols such as NH_4NO_3. Such aerosols may evaporate below the instrument height and then deposit as ammonia and nitric acid gases, with greater V_d than aerosol (Fuzzi et al. 2015). The opposite effect, i.e., apparent emission of aerosol due to growth to the size range detectable by a particle counter, has been observed over fertilized grassland, where NH_3 concentration is high near the ground and adsorbs to existing fine aerosol (Nemitz et al. 2009).

A direct approach to determining deposition of aerosols on plant leaves uses the accumulation method (Petroff et al. 2008), determined after washing the leaf. Leaves may be washed with water and/or organic solvents such as chloroform, followed by filtration and weighing or chemical analysis of the fractions (Freer-Smith et al. 2005; Mo et al. 2015; Oliva and Raitio 2003; Saebo et al. 2012; Wyttenbach and Tobler 1998). The advantage of this approach is that it includes the actual microroughness and other local parameters. The concentration of deposited aerosols on a specified leaf surface area is measured, so that average fluxes can be determined over specified periods. These are net fluxes because aerosols are both removed by resuspension, rainfall, and migration into the leaf by stomatal or cuticular uptake, and added to the surface by leaching from the leaf interior. Leaves or filters may be scanned by electron microscopy, allowing number concentrations to be quantified (Neinhuis and Barthlott 1998; Ottele et al. 2010; Sternberg et al. 2010; Wang et al. 2015).

Technical advances have extended the resolution of single deposited aerosols on leaves down to 90 nm (Wang et al. 2015). In polluted air, the percentage of leaf area covered by particles can be more informative than particle number, as it reflects the high degree of aggregation between single aerosol particles (Wang et al. 2015). In the extremely polluted air of Beijing, 10–50 % of adaxial and 3–35 % of abaxial surfaces of *Ulmus pumila*, *Salix babylonica*, and *Gingko biloba* were covered by aerosol.

Quantification of total leaf loading of aerosol-borne compounds may be achieved by proton-induced X-ray emission (PIXE) (Lin and Schuepp 1996; Simmleit et al. 1989) or by inductively coupled plasma (ICP), either of material on the leaf surface or of material washed from the surface (Bertolotti et al. 2014; Kalinovic et al. 2016; Mori et al. 2015a; Quayle et al. 2010; Santos et al. 2015; Schleppi et al. 2000). Measurement of magnetic susceptibility or saturation isothermal remanent magnetization (SIRM) is a relatively new method to determine foliar-deposited aerosols in urban vegetation (Hofman et al. 2014; Kardel et al. 2011; Matzka and Maher 1999; Sant'Ovaia et al. 2012; Urbat et al. 2004). This technique has demonstrated that metallic $PM_{2.5}$ from road and rail traffic, including fuel combustion, brake abrasion, and corrosion, is deposited to leaves and may become embedded in them (Hofman et al. 2014; Muxworthy et al. 2003; Urbat et al. 2004). SIRM of urban tree leaves has proven to be a good estimator of traffic-derived aerosol (Kardel et al. 2011; Maher et al. 2013; Rai 2013).

Understanding the processes governing aerosol deposition to leaves can guide pollution mitigation efforts. Phytoremediation through planting of urban forests,

roadside shrubs, and green walls to reduce urban aerosol concentrations is under active investigation (Beckett et al. 1998; Escobedo and Nowak 2009; Freer-Smith et al. 1997; Manso and Castro-Gomes 2015; Pugh et al. 2012). Urban forests were found to remove 770 Mg of PM_{10} $year^{-1}$ in central Beijing (Mo et al. 2015) and 64 Mg $year^{-1}$ in Atlanta (Nowak et al. 2013). Although impressive, these adsorption levels represent <10 % of the ambient atmospheric aerosol burden in polluted environments (McDonald et al. 2007; Pugh et al. 2012). Higher effectiveness, up to 50 % reduction for aerosol below 10 μm in diameter, has been calculated for vegetated "green walls" in urban street canyons (Pugh et al. 2012).

3.2.3 The Role of Air Humidity

Humidity and temperature influence the dry deposition and impact of aerosols on vegetation. Hygroscopic aerosol particles absorb water from humid air and increase in size, thereby increasing their V_d. The steep increase in humidity toward the leaf surface within the leaf boundary layer also increases aerosol size (Wesely and Hicks 2000). Aerosol may achieve >95 % equilibration with the increased humidity prior to impact with the surface. These effects may be large, i.e., a sevenfold increase in mass of a dry particle of ammonium sulfate under saturating humidity (Ruijgrok et al. 1997). On the leaf surface, the concentration of constituents contained in deposited aerosol increases in dry air due to solvent water evaporation. This concentration effect increases the reactivity and absorption of the deposited material. However, evaporation to dryness strongly reduces these processes (Swietlik and Faust 1984). Models of aerosol deposition must account for the greater size, mass, and likelihood of deposition by impaction of hydrated particles, relative to particles measured in the free atmosphere (Ruijgrok et al. 1997; Zhang et al. 2001).

4 Direct Aerosol/Plant Interactions

4.1 Plant Adaptation to Foliar Accumulation of Aerosols

Aerosols transferred from the atmosphere to foliar surfaces may reside on the leaf, twig, or bark surface for extended periods, be taken up through the leaf surface, or be removed from the plant to the soil by resuspension, washing by rainfall, or litterfall (Grantz et al. 2003). There is increasing evidence for many plant species that leaves accumulate aerosols throughout their lifetime, reaching high aerosol loads particularly in urban environments. However, net accumulation of aerosols after budbreak in three deciduous tree species reached a plateau after 4–7 days, reflecting inputs and outputs (Wang et al. 2015).

The loading of aerosols on leaf surfaces is generally between 20 and 100 μg cm^{-2}, although lower and higher values have been observed (Burkhardt 2010; Mo et al. 2015; Popek et al. 2013; Przybysz et al. 2014; Sgrigna et al. 2015). This is similar to the range of epicuticular waxes (35–100 μg cm^{-2}). SEM measurements of number concentrations ranged up to 500,000 cm^{-2} aerosol loading on leaves of *Q. robur* (Neinhuis and Barthlott 1998) and up to 2.9 million cm^{-2} on *Hedera helix* leaves (Ottele et al. 2010; Sternberg et al. 2010). Positive correlations between surface wax and deposited aerosol have been reported (Popek et al. 2013).

Aerosols have always been part of the atmospheric environment of plants. Despite occasional emissions of dust from geologic disturbances and ash and precursor gases from volcanic events, emissions were probably relatively constant over geologic time. Therefore physiological and morphological adaptations have been slow. Yet, as noted above, the influence of leaf surface properties on aerosol interception is so significant that evolutionary adaptations seem likely, to increase deposition and aerosol adherence in some environments and to decrease deposition and accelerate removal in others.

Plants in resource-poor environments may benefit from increased aerosol loading through increased leaf roughness, trichome proliferation, and altered surface wax micromorphology (microroughness for fine aerosols, stickiness for coarse aerosols; Beckett et al. 1998; Chamberlain 1975). Accumulation mode aerosols (0.1–1 μm diameter) are often hygroscopic and carry ionic and easily available nutrients which may be taken up by the leaf across the cuticle or through the stomata (Fernandez and Eichert 2009). In arid environments, hygroscopic aerosol facilitates condensation of water on leaf surfaces (Konrad et al. 2015) which may be absorbed by the leaf. Deposition to needles of *Pinus sylvestris*, *Picea abies*, and *Abies alba* was greatest near stomata due to local wax microroughness (Burkhardt et al. 1995), possibly facilitating stomatal uptake.

The increase in nutrient-rich anthropogenic fine aerosol may eventually lead to selective pressure toward traits that reduce aerosol capture. In the natural environment, aerosol loading is unavoidably linked to deposition and colonization of leaf surfaces by microorganisms, including pathogenic fungi and bacteria. As these organisms require free water for germination, hygroscopic particles may be deleterious, as in marsh plants (Neinhuis and Barthlott 1997) and irrigated fields in arid environments (Michailides and Morgan 1992). Plants with long adaptation to these conditions have developed highly hydrophobic leaf surfaces that hinder the growth of microorganisms by reducing deposition of aerosol and pathogens and the amount and duration of leaf wetness. An extreme example is the lotus effect observed in *Nelumbo nucifera* Gaertn, *Brassica oleracea* L., and other species. This effect is achieved through an elaborate leaf microroughness regime that maintains an air layer between the leaf surface and aqueous particles, reducing adherence of water and pathogens and facilitating their removal by rain or splashed water (Barthlott and Neinhuis 1997; Neinhuis and Barthlott 1997; Patankar 2004).

4.2 Hygroscopic Aerosols Induce Microscopic Leaf Wetness

A critical characteristic of aerosols, both in the atmosphere and on leaf surfaces, is the ability to attract water molecules from unsaturated air, i.e., hygroscopicity. The average hygroscopicity of aerosol over the continents has increased with industrialization and desertification, while over marine regions it has decreased (Pringle et al. 2010). Deposited aerosols on leaf surfaces act as "dew condensation nuclei" in analogy to CCN. The interaction of hygroscopic aerosols with plants follows several stages which include foliar wetting, overcoming surface tension of water, expansion into the stomata, hydraulic activation of stomata (HAS), wicking, and effects on plant water relations. These processes depend on aerosol modification of the physical properties of water.

On leaf surfaces, the deliquescence relative humidity (DRH) is a physiologically important aerosol parameter. This is the relative air humidity at which a salt in equilibrium with the water activity of the surrounding air accumulates sufficient liquid water to dissolve. Examples are sea salt (DRH 75 %), ammonium sulfate (DRH 80 %), ammonium bisulfate (DRH 40 %), or calcium chloride (DRH 30 %). Aerosol salt exposed to its DRH becomes a saturated salt solution, approximately doubling its volume (Pilinis et al. 1989) and making both the water and the solute physiologically available for transport and assimilation.

Efflorescence is the reverse of deliquescence. Salts crystallize when the relative humidity of the air (RH) is reduced. Many organic substances, including carboxylic acids, carbohydrates, and proteins, form amorphous rather than crystalline phases upon precipitation, which follows cooling or drying of aqueous solution droplets (Mikhailov et al. 2009). Amorphous behavior of dried $NaNO_3$ at room temperature has been observed (Dette and Koop 2015). Many aerosols arrive at the leaf surface already in liquid phase, remaining amorphous rather than becoming crystalline. This makes them difficult to detect by scanning electron microscopy (Burkhardt et al. 2001). Such behavior has been observed directly in still and video photographs of leaves undergoing cycles of changing RH (Burkhardt and Pariyar 2014).

Deliquescent aerosols on leaf surfaces in humid air generate nearly saturated solutions (up to several Molar), so that ion-specific properties become important. Compounds have been classified along a chaotropic to kosmotropic spectrum, the Hofmeister series, originally on the basis of differential ability to dissolve proteins (Hofmeister 1887; Kunz et al. 2004). More recently, a general description of chaotropicity has been proposed, with a numerically continuous scale (Cray et al. 2013). Concentrated solutions of chaotropic ions reduce the surface tension of water (Pegram and Record 2007; Dutcher et al. 2010; Burkhardt et al. 2012). These ions alter the viscosity and the ability to solubilize nonelectrolytes into aqueous solution (Cray et al. 2013). The solubility is related to the respective distribution of the ions between the surface and the bulk of a water droplet according to the order of the Hofmeister series (dos Santos et al. 2010; Dutcher et al. 2010; Zhang and Cremer 2010; Ball and Hallsworth 2015).

Electrical conductance measurements demonstrate the presence of liquid water on leaf surfaces even during hot and dry summer days (Burkhardt and Eiden 1994; Burkhardt and Hunsche 2013). This microscopic water remains available for the dissolution of atmospheric gases such as ammonia (Burkhardt and Eiden 1994; Burkhardt and Hunsche 2013). This water is the result of absorption from humid air by hygroscopic aerosols after deposition on the leaves. The sources of the condensing water vapor are both ambient humidity and the transpiration stream, depending on ambient RH, prevailing turbulence, and stomatal aperture (Burkhardt and Hunsche 2013; Burkhardt et al. 1999).

4.3 Hydraulic Activation of Stomata

The minute amounts of water involved in such microfilms of saturated solutions are not relevant to the hydrologic or latent heat balance of leaves. However, fluctuations in RH at the leaf surface arise from transient heating and cooling due to sunflecks, leaf movements, or turbulence, and result in migration of deposited aerosol by repeated drying/wetting cycles (Fig. 1). This process is generally hindered by the hydrophobicity of the leaf surface, but this hydrophobic effect is overcome at concentrations above approximately 100 mM (Lo Nostro and Ninham 2012) by chaotropic reduction of viscosity and surface tension caused by the chemical properties of the aerosol (dos Santos et al. 2010; Dutcher et al. 2010; Pegram and Record 2007). It has been demonstrated by SEM videos taken under environmentally relevant conditions (ESEM) that thin (<100 nm) liquid films of solutions of deliquescent, chaotropic salts can and do enter stomatal cavities (Fig. 2). Dynamic capture of this process can be seen in the original videos (Burkhardt and Hunsche 2013, Supplementary Material). This is contrary to long-standing interpretation of liquid behavior on the leaf surface, based on calculations showing that (pure) water could not enter the stomata (Schönherr and Bukovac 1972).

Penetration of a continuous film of aqueous liquid from the atmosphere along the walls of individual stomata into the substomatal cavity leads to "hydraulic activation of stomata" (Burkhardt 2010; Burkhardt et al. 2012). This provides hydraulic linkage between the leaf surface and the apoplast, enabling the bidirectional exchange of liquid water, dissolved and suspended substances, and hydraulic signals through individual stomatal pores (Burkhardt 2010). The presence of a liquid film, traversing a controlling aperture, the stomatal throat, that is assumed to regulate only gas-phase transport, has large and yet underappreciated implications. A liquid-phase link between apoplast and atmospheric boundary layer has the potential to mediate leaf water loss that is not effectively controlled by stomatal closure and that may confound porometric determinations of stomatal conductance and estimates of ecosystem water balance. This transpiration is by wick action in which water is lost from the apoplast through the stomata in liquid form and only passes into the vapor phase at the leaf surface. The water is not proportionally

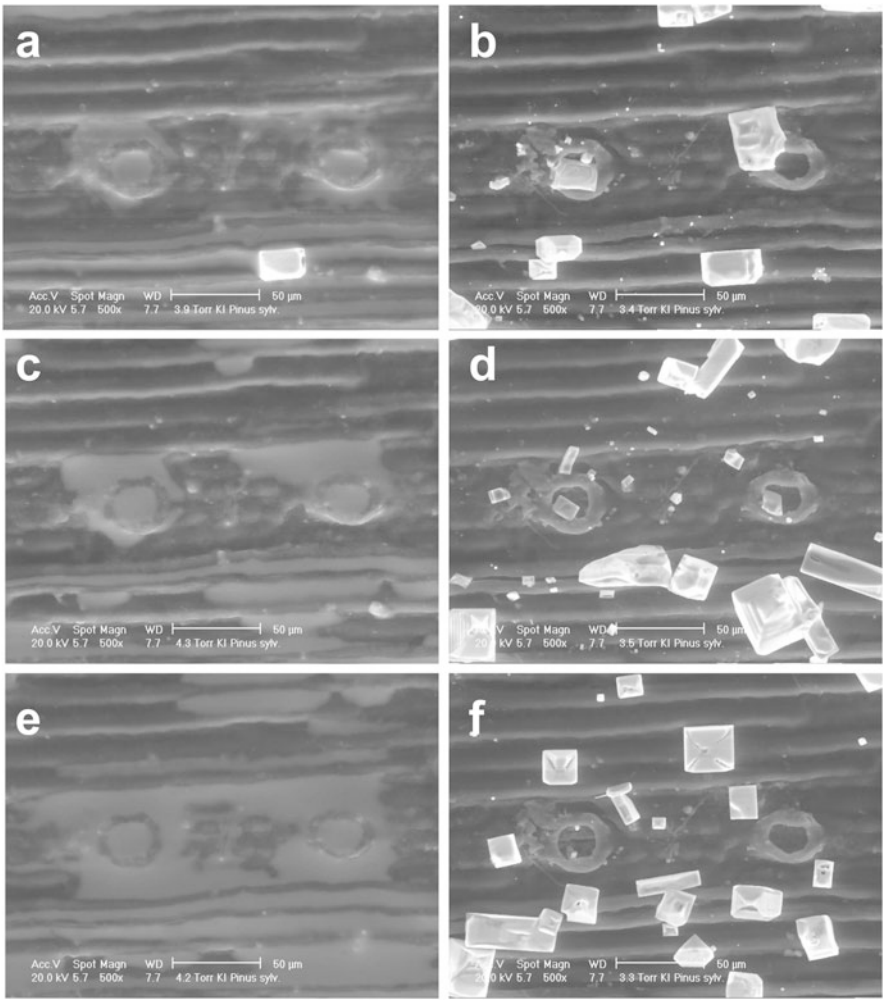

Fig. 1 Three sequential cycles of atmospheric humidity in the environmental scanning electron microscope (ESEM), showing the behavior of potassium iodide (KI) particles on a *Pinus sylvestris* needle. The time difference between subsequent images is approximately 5 min. In (**a**), (**c**), and (**e**), the salt forms a highly concentrated solution. In (**a**), a single crystal remains in the process of deliquescence. In (**b**), (**d**), and (**f**), the salt reforms crystals as the humidity is reduced below the deliquescence humidity of about 70 % (Greenspan 1977). Despite the hydrophobicity of the *Pinus sylvestris* needle surface, the contact angle of the solution is small and individual droplets coalesce. The migration of the salt across the leaf surface with cycles of humidity and repeated deliquescence/efflorescence is reflected in the differences between images (**b**), (**d**), and (**f**). Iodide (I^-) is a naturally occurring, chaotropic anion that reduces the surface tension of water

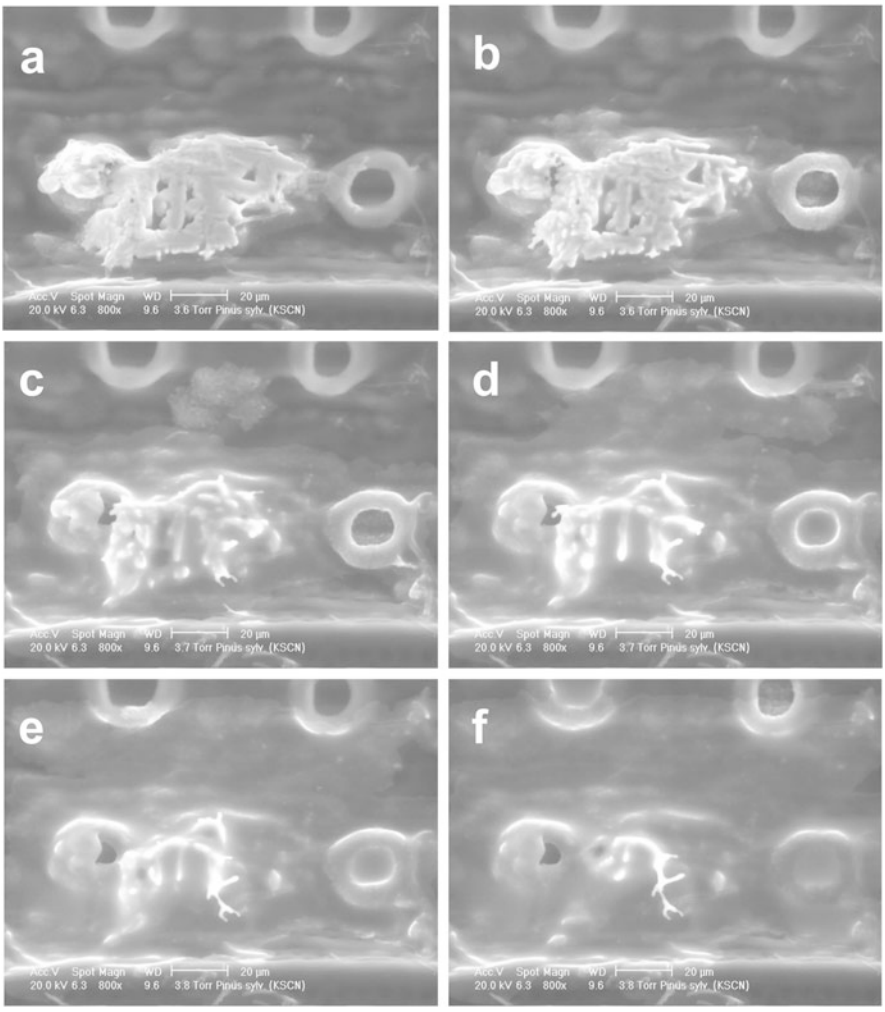

Fig. 2 Deliquescence of potassium thiocyanate (KSCN) on a *Pinus sylvestris* needle. Thiocyanate (SCN^-) is an extremely chaotropic anion. The solution develops a very flat contact angle and while advancing with increasing humidity enters the epistomatal chambers as a thin liquid film [lower right in (**b**), upper right in (**f**)]. This dynamic process is more clearly seen in the original video montage (Burkhardt and Hunsche 2013, supplementary material available at Movie 2). The epistomatal chamber contains the same diverging and converging geometry as an open stoma, previously considered the barrier to entry of pure water (Schönherr and Bukovac 1972)

compensated by uptake of CO_2 and thus is unproductive and decreases water use efficiency. The possible perturbation of such estimates by liquid fluxes remains to be quantified. This connectivity may also facilitate foliar fertilization and the uptake of aerosol-borne materials. Under some conditions, the liquid film may facilitate foliar uptake of hygroscopically derived surface water and also of deposited fog water (Eller et al. 2013).

Accumulation of hygroscopic salts on leaf surfaces may lead to increased total leaf water loss (Burkhardt 2010). This mechanism might play a role in the rapid "chemical desiccation" used in the evaluation of drought resistance in plant breeding, to simulate natural drought (Blum et al. 1983; Nicolas and Turner 1993; Burkhardt 2010), and in the rapid defoliation of crops such as potato and cotton to facilitate harvest. The technique works better with chaotropic than with kosmotropic ions (Wilson et al. 1947; Herrett et al. 1962; Murphy 1968; Renner 1991; Bond and Bollich 2007; Burkhardt et al. 2012).

While salts thus can enhance water loss, the effect may depend on the water relations strategy of the respective plant species. In anisohydric beech seedlings, sap flow increased with increasing vapor pressure deficit (VPD) and responded to simulated aerosol deposition. The slope of the response of sap flow to VPD doubled following application of $(NH_4)_2SO_4$ and tripled following spray application of $NaNO_3$ solution to the foliage of beech seedlings (Burkhardt and Pariyar 2016). In contrast, sap flow of isohydric pine seedlings was not correlated with VPD, and these aerosol applications had no effect on sap flow (Burkhardt and Pariyar 2016). Nevertheless, in both species, and in sunflower, growth in ambient aerosol-containing air, and simulated aerosol deposition with salt solutions, decreased (more negative) the values of $\delta^{13}C$ compared to filtered air. This suggests reduced water use efficiency, but the mechanism in this case, and whether carbon assimilation declined or stomatal conductance increased, remains to be determined (Burkhardt and Pariyar 2016). The minimum epidermal conductance (g_{min}) is an important ecophysiological parameter affecting the drought tolerance of plants. Simulated aerosol application, by salt spray, increased g_{min} of pine seedlings (Burkhardt and Pariyar 2014). This effect and the similar response of sap flow in beech indicate a reduction in the efficacy of stomatal control that could directly impact plant tolerance of soil drought and low air humidity. In this way, recent large-scale tree mortality attributed to "global-change-type drought," with rising temperatures and exponentially increasing VPD (Breshears, et al. 2005; van Mantgem et al. 2009; Allen et al. 2015; Eamus et al. 2013; McDowell et al. 2008; McDowell and Allen 2015; Anderegg et al. 2015), might be enhanced by the wicking mechanism of hygroscopic aerosols. The same mechanism might have contributed to the forest decline observed at the end of the last century in Europe and eastern North America that was attributed to air pollution without consideration of potential direct impacts of aerosol.

Apparent wax degradation has been associated with tree damage symptoms, including winter desiccation (Huttunen et al. 1981), needle loss (Bruck et al. 1989; Mengel et al. 1990; Sauter and Voss 1986; Takamatsu et al. 2001; Trimbacher and Eckmüllner 1997; Turunen and Huttunen 1990), and, notably, increased g_{min} (Anfodillo et al. 2002; Cape and Fowler 1981; Heinsoo and Koppel 1998; Sase et al. 1998; van Gardingen et al. 1991), but a satisfactory mechanism for wax degradation has remained elusive. In some cases, amorphous aerosol deposits following repeated deliquescence and efflorescence (see Figs. 1 and 2) may resemble degraded cuticular waxes (Burkhardt and Pariyar 2014).

4.4 *Effects Caused by Coarse Aerosols*

4.4.1 Contact Effects

Particularly heavy loading of soil-derived dust on foliage is most likely in arid regions due to both the wind erodibility of dry soil and the absence of leaf washing by rain. Heavy loading of atmospheric aerosols may also occur near unpaved roads, open-pit mining and quarrying operations, and construction sites. Soil disturbance by agricultural operations is a dominant source of coarse aerosol, including dust >10 μm. While this material is not generally considered a human health hazard, it may coat leaves with a range of impacts. Volcanic events and regional sandstorms may also lead to substantial foliar loading, as well as impacts on human health. Premature leaf abscission and resultant loss of photosynthetic leaf area and growth may occur.

In special cases, such as near limestone quarries and cement kilns, heavy coatings of dust may form crusts on leaves which resist washing off by rain. Cement dust may exert physical and chemical effects, as it liberates calcium hydroxide on hydration that erodes foliar surface waxes as a consequence of severe alkalinity (>pH 12; Guderian 1986) and leads to lipid hydrolysis, protein denaturation, and plasmolysis. Such effects have been shown to occur in a dose-dependent fashion (Lerman and Darley 1975), requiring sufficient surface moisture to hydrate the dust (Lerman and Darley 1975). Heavy limestone crusts forming near a quarry reduced tree growth and led to ecosystem simplification, with a reduction in biodiversity and dominance shifting from maple and oak to the more resistant *Liriodendron tulipifera* (Brandt and Rhoades 1972, 1973).

Windblown geologic material (dust, >10 μm) erodes cuticles and buries understory vegetation (Armbrust and Retta 2002; Sharifi et al. 1999). Abrasion of leaf and stem surfaces (Hoad et al. 1992; Cleugh et al. 1998) has substantial effects on plant water relations, particularly under dry, windy conditions. For example, plantings of onions and carrots in the generally productive sandy soils of the Mojave Desert of California are frequently scoured and partially buried shortly after emergence by windblown soil, requiring replanting (Grantz, personal observation). Plant surfaces that are not destroyed may receive heavy coatings of dust, which also impact plant growth. Aerosol adhered to the leaf surface has the potential to exert direct effects related to the chemical characteristics of the aerosol, but coarse aerosol that impacts the leaf surface, with or without adherence, may additionally cause abrasion, shading, and potentially occlusion of stomatal pores.

Chemically inert particles are unlikely to form wicks, but can also have an impact on plant water relations. Bean leaves significantly enhanced water loss following application of chemically inert clay and silica of different sizes (Wagner 1939), possibly reflecting altered radiation balance or disruption of stomatal closure. Dust application to leaves of kidney beans and cucumbers reduced stomatal conductance in the light (Hirano et al. 1995), but increased conductance in the dark if stomata were still open during dust application. Similarly, application of

chemically inert aerosol such as silica gel to *Populus tremula* leaves (Flückiger et al. 1977) and of kaolin, silica, talc, copper oxychloride, quartz, and other inert suspensions to *Phaseolus multiflorus*, *Coleus blumei*, *Zebrina pendula*, and *Solanum tuberosum* obstructed stomatal closure and increased water loss and drought injury, with stronger effects at increasing concentration and decreasing aerosol size (Eveling 1969, 1972).

4.4.2 Radiation and Gas Exchange

Coating of leaf surfaces has a number of related effects on leaf radiation balance (Grantz et al. 2003). Localized shading with materials of high albedo may cool the leaf and relieve high radiation stress. However, changes in radiative properties following aerosol deposition often result in a decline in leaf albedo, particularly in the near-infrared region of the spectrum (Hope et al. 1991), where uncoated leaves absorb very little radiation (Nobel 1991). Leaf temperature increases (Eller 1977) despite the accompanying, but rather modest, decline in visible/photosynthetically active radiation. The net impact depends strongly on the composition of the aerosol, as some dust materials absorb efficiently in the infrared (Guderian 1986; Sharifi et al. 1997; Dässler et al. 1972; Spinka 1971), while other materials do not (Eller 1977).

The decline in photosynthetically active radiation (400–700 nm) has impacts on carbon assimilation (Sharifi et al. 1997, 1999), resulting in eventual reduction in chlorophyll content and in uncoupling of the oxygen evolving complex of photosystem II (van Heerden et al. 2007; Naidoo and Chirkoot 2004). Similar declines in photosynthetic pigments were observed with proximity to roadways subject to resuspended dust (Prusty et al. 2005), though roadway dust poses a combined threat of chemical and physical impacts (Daresta et al. 2015). Activity of the antioxidant enzyme, peroxidase, and genetic damage were also observed. Lettuce exposed to road dust <65 μm, collected on a high-volume building air-conditioning filter (Pavlik et al. 2012), inhibited growth and photosynthetic carbon assimilation, as above, when applied directly to leaves, but was also inhibitory when applied to the soil. Effects on leaf amino acid composition were greater in the soil-applied than in the foliar-applied treatment, reflecting the complex interaction of direct and indirect aerosol effects on plants.

Loading of aerosols has been suggested to physically clog stomata, impeding both closing and opening phases (Flückiger et al. 1977; Eamus and Fowler 1990). Fine aerosols <2 μm may preferentially lodge in micro-ridge structures in the cuticle and in stomatal openings (Burkhardt et al. 1995; Song et al. 2015; Wang et al. 2015), but deposition of coarse aerosol may be less specific. Reduced gas exchange (Nanos and Ilias 2007) has been reported, often attributed to stomatal occlusion by aerosol deposition (Krajíková and Mejstĺík 1984; Sharifi et al. 1997; Abdullah and Iqbal 1991), but this has not been fully demonstrated.

5 Indirect Effects of Aerosols on Ecosystems

Indirect effects of aerosols are mediated by changes in radiation penetration through the atmosphere, considered above, and through changes in soil chemistry (Zalud et al. 2012). Aerosol may be deposited directly to soil or may transiently reside on leaf or stem surfaces. Rhizosphere impacts reflect the chemical composition of aerosol and some of these impacts may be exerted at the leaf surface if residence time is sufficient.

5.1 Nutrients

Soil suspension, considered above as a physical threat to vegetation, also results in an atmospheric burden of cations including calcium, potassium, magnesium, iron, aluminum, manganese, silicon, and titanium, i.e., crustal materials typically found in the coarse mode as primary aerosol. As with dust, these are particularly prevalent in arid regions with exposed soil and minimal precipitation. Deposition of the base cations, Ca, Mg, and K, is typically positive for plant growth, providing nutrients, neutralizing pH, and reducing the availability of phytotoxic aluminum.

Direct effects of aerosol N on leaves have not been documented, though N is known to be taken up from the leaf surface. The uptake mechanism remains unclear, but the effectiveness of foliar fertilization of horticultural crops with urea is clear (Weinbaum and Neumann 1977). Throughfall is often depleted in N relative to wet deposition collected above the canopy (Sievering et al. 1996; Lovett and Lindberg 1993), suggesting foliar uptake. Aerosols containing N and/or S have little effect when applied to foliage (Olszyk et al. 1989; Martin et al. 1992) though this may depend on particulate composition. Calcium nitrate is as readily taken up by leaves as urea, but causes foliar injury which urea typically does not (Norton and Childers 1954). Aerosols deposited to leaves eventually reach the soil when leaves abscise or by washing of leaves by rain.

As the developed landscape has increased, suspension, transport, and deposition of base cations have declined. This has theoretical impacts on plant mineral nutrition, but these have not yet been clearly documented. Regulatory efforts have reduced emissions of sulfur gases, thus reducing aerosol sulfate. As a result, projected decreases in soil pH caused by declining deposition of base cations have not materialized, balanced by concomitant declines in acidifying deposition.

Deposition of N-containing aerosols, derived from gas-to-particle transformation of NO_x and NH_x, has increased in the industrial age. Because many ecosystems are N limited (Fenn et al. 1998), aerosol deposition of N increases the growth of some plant species. However, this advantages nitrophiles at the expense of typically slower-growing dominant vegetation, changing competitive relationships. Resulting nutrient imbalances lead to growth disruption, declines in biodiversity, and altered species composition (Aber et al. 1995; Rapport and Whitford 1999).

Other ecosystems have become N saturated, as indicated by the appearance of nitrogen in runoff and potentially in deep drainage waters. This poses a direct threat to surface waters and to groundwater and is an indicator of an extreme case of the effects described above, with deposition exceeding the uptake and sequestration capacity of the vegetation. Nitrogen-containing runoff leads directly to eutrophication and uncontrolled growth (blooms) of algal species in receptor bodies of fresh (Vitousek et al. 1997), brackish, and marine waters (Rabalais 2002). Changes in populations of fish, including extinction events, may result. Excessive deposition of N may also lead to denitrification and emissions of climate forcing N_2O.

5.2 Heavy Metals

Heavy metals such as copper, zinc, cadmium, chromium, manganese, lead, nickel, mercury, and vanadium are generally anthropogenic in origin from industrial and roadside sources. They are mostly deposited by wet deposition of fine aerosols (Reisinger 1990; Smith 1990; Adachi and Tainosho 2004; Gleason et al. 2007). Near sources of heavy metals, concentrations are high on foliage and in litter, eventually contaminating the soil surface layers.

While all heavy metals may biomagnify through trophic levels, mercury from industrial and geologic sources is the most likely to do so. This reflects the bioavailability of methyl mercury and its slow elimination from animals (Croteau et al. 2005; Frescholtz et al. 2003). Sulfur deposition, including aerosol SO_4^-, enhances sulfur reducing bacteria which methylate mercury, increasing its availability. Mercury is absorbed into plants mostly by foliar exchange, which is bidirectional (Millhollen et al. 2006) resulting in steady state levels (Erickson 2003). Other heavy metals reach a dynamic foliar equilibrium within a few days of leaf emergence (Wang et al. 2015) due to the balance of aerosol deposition and removal. Metal-containing aerosols, once hydrated, may penetrate into the stomatal pores and alter mesophyll function (Da Silva et al. 2006; Kuki et al. 2008; Burkhardt et al. 2012).

Plant growth and frost hardiness are inhibited by heavy metal uptake (Audet and Charest 2007) as is the development of microbial ecosystems on foliar surfaces (Taulavuori et al. 2005; Kim et al. 2003). Species competitive relationships are altered by reducing early establishment of some species (e.g., *Sophora tomentosa*; Kuki et al. 2009) more than of others (e.g., *S. terebinthifolius*).

The concentration of phytochelatins across mixed forests in the northeastern USA appears to map the deposition of heavy metals and was related to the spatial distribution of dead and dying trees (Gawel et al. 1996). Downwind of the copper-nickel smelter in Harjavalta, Finland, large deposition levels of Cu, Ni, Zn, and Pb in the decades prior to 1990 changed soil concentrations of metals within 30 km of the smelter, but reductions in plant, bird, and insect growth, and species composition (Kiikkilä 2003) became substantial only within about 2 km of the smelter, suggesting that ecosystem function is relatively tolerant of metal deposition. Within

a 0.5 km radius, vegetation was nearly absent. Accumulation of heavy metals was greatest in bryophytes and lichens (Salemaa et al. 2004), though many species are relatively insensitive (Nash 1975; Tremper et al. 2004; Otnyukova 2007). Due to their high C_p, these species serve as useful receptors in biomonitoring surveys (Wolterbeek 2002).

In forested ecosystems, heavy metals accumulate in litter and upper soil horizons, decreasing with depth (Pueyo et al. 2003), providing some protection for groundwater (Watmough et al. 2004). Soil concentrations of heavy metals are not well correlated with biological availability due to differences in binding and immobilization (Feng et al. 2005; Almas et al. 2004). Low aqueous solubility reduces foliar uptake and enhances metal binding to organic matter, though acidic deposition enhances solubility and bioavailability (Lingua et al. 2008). In general, transport of heavy metals from rhizosphere to shoot is somewhat limited in plants. Materials that are initially concentrated in leaves, mycorrhizae, root hairs, and fine roots are eventually transferred to the soil by abscission (Berthelsen et al. 1995; Soares and Siqueira 2008).

Soil-mediated effects reflect altered function of bacterial and mycorrhizal communities. Microbial enzymes involved in cycling of the macronutrients N, P, and S are particularly sensitive to inhibition by zinc, cadmium, and copper (Kandeler et al. 1996). Metal deposition causes a decline in the availability of base cations essential for plant growth due to displacement from cation exchange sites (Derome and Lindroos 1998). Foliar concentrations of Ca, Mg, and Mn were reduced in Scots pine (*P. sylvestris*; Kiikkilä 2003; Helmisaari et al. 1999). Reduced availability of Ca^{++} led to observed decreases in shell thickness of bird eggs and snails (Eeva and Lehikoinen 2004).

Soil fungi may be more sensitive than bacteria (Vaisvalavicius et al. 2006; Oliveira and Pampulha 2006; Pennanen et al. 1996). In heavily impacted soils, metal-tolerant populations become more prevalent at the expense of decreased biodiversity (Almas et al. 2004; Lakzian et al. 2002: Joynt et al. 2006; Oliveira and Pampulha 2006; Yuangen et al. 2006).

One result of heavy metal impacts on soil microbiota is retardation of litter decomposition (Fritze et al. 1989; Yuangen et al. 2006). This is difficult to document (Johnson and Hale 2008; Wyszkowska et al. 2007) because of different effects on pools of organic carbon differing in lability. An intractable pool may be particularly subject to delayed cycling (Boucher et al. 2005), as reflected in models of soil respiration (Oikawa et al. 2014).

5.3 Organic Materials

Organic matter adsorbed to aerosols may include persistent organic pollutants and semivolatile compounds, as well as pesticides and polyaromatic compounds (Srogi 2007). Some organic compounds are directly phytotoxic, some are likely genotoxic, and others pose a danger when bioaccumulated and passed through trophic levels

into the food chain. Woodburning for domestic heat and land clearing, wildfire, and industrial and vehicle emissions are common sources (Sanderson and Farant 2004). Most of the atmospheric burden is in the fine fraction which dominates surface area for adsorption, but most of the dry deposition occurs in the coarse fraction (>80%; Kaupp and McLachlan 1999), which dominates sedimentation. In contrast, wet deposition is dominated by fine aerosols, due both to effectiveness of washout and to the dominance of organic mass in the fine fraction.

Dry deposition of organic compounds that reversibly partition between gas and particle phases (semivolatile organic compounds; McLachlan 1999) is challenging to predict. The repeated deposition and re-volatilization process has resulted in distribution of these materials globally, including into hitherto pristine polar and alpine environments (Lei and Wania 2004; Hageman et al. 2006; Yogui and Sericano 2008). Reflecting these processes, volatile species exhibit greater deposition in winter than in summer, reflecting the dominance of gaseous over particulate deposition.

Uptake into plants may occur through roots, particularly of low molecular weight organic compounds, as a function of aqueous solubility (Krupa et al. 2008) and mostly at the air-plant interface (Tao et al. 2006). Plant cuticles are composed of waxy materials in which lipophilic organic aerosol may embed or dissolve. While accumulation is easily documented, penetration and assimilation into tissues are less well documented. The organic pesticide, chlordane, was distributed throughout the plant body of zucchini squash (*Cucurbita pepo*; Lee et al. 2006). In contrast, polyaromatic hydrocarbons (PAHs) bind to soil organic matter and are thus sequestered away from plant uptake (Gao and Zhu 2004). PAH in paddy rice was correlated with concentrations in air and water, but not with concentrations in the soil (Jiao et al. 2007). This may be related to the presence of aerial roots. Roadside grass communities and spruce trees in remote Alaskan forests accumulated PAH from the atmosphere, with concentrations increasing with proximity to roadways and urban areas (Crépineau et al. 2003; Howe et al. 2004). Tomato fruit concentrates polyaromatic compounds, representing a potential threat to the human food chain (Camargo and Toledo 2003).

Ambient background particulate matter collected on filters and fed to the rooting medium of tomato plants delayed but did not reduce germination (Daresta et al. 2015), but significantly reduced root elongation and root and shoot biomass. Roots exhibited abnormally enhanced branching and inhibited development of the tap root (Daresta et al. 2015; Kummerova et al. 2013). The particulate matter reduced the chlorophyll a/b ratio and the ratio of total chlorophyll to carotenoids and increased levels of reactive oxygen species (Daresta et al. 2015). Aerosols containing PAH derived from the exhaust of diesel engines were more toxic than those derived from gasoline engines, as determined by effects on chlorophyll levels in *Bacopa monnieri* in India (Durga et al. 2015). Because of stress-induced increases in reactive oxygen species, it may be that photosynthetic inhibition is adaptive in reducing the further synthesis of reactive oxygen during electron transport (Kummerova et al. 2006).

6 Conclusions and Perspectives

Atmospheric aerosols are (and have always been) part of the atmospheric environment of plants. Plants participate in the global "aerosol cycle" as both sources and sinks, exhibiting morphological adaptations to facilitate accumulation or shedding of aerosols in a habitat-dependent manner. Deposited aerosols on leaf surfaces have long been considered inert, though nutrient potential of some ions, and destructive potential of alkaline crusts, has been recognized.

A more recent perspective, noting the essential physicochemical similarity of aerosol in the atmosphere and following deposition to the leaf surface, has focused on hygroscopicity and chaotropicity of aerosol. Hygroscopicity causes condensation of water, as cloud condensation nuclei in the atmosphere and in formation of a liquid film on leaf surfaces. Chaotropicity of surface-deposited aerosol reduces the surface tension of the resulting thin film and enables expansion of the nearly saturated solution on the hydrophobic leaf surface. This expansion has been shown to include penetration into the stomatal cavity. This development of a liquid pathway between the apoplast and the atmosphere must be considered as an important direct effect of aerosols on plant water relations. As it bypasses the normally assumed vapor-phase pathway of transpiration, it may require rethinking of assumptions regarding the efficacy of stomatal control, particularly of minimum stomatal conductance observed at night and during drought.

Aerosol is removed from the atmosphere by wet and dry deposition. Improved understanding of dry deposition processes has suggested mitigation strategies involving establishment of vegetation in highly polluted urban areas for protection of public health. However, capture of aerosol by such plantings unavoidably releases materials contained in the aerosol to the local soil, causing indirect effects mediated through impacts on soil chemistry.

These multiple effects are representative of aerosol impacts on managed and natural ecosystems in general. The positive and negative aspects of nutrient loading by particle deposition have received considerable attention in the ecological literature. Similarly, the effects of regional haze and cloud nucleation on climate and plant radiation balance have been evaluated in depth. However, the direct effects on ecophysiological processes, exerted after deposition and retention of aerosols on leaf surfaces, have not received equivalent attention. The direct effects of toxic aerosols, the influence of hygroscopic aerosols on plant water relations, and the role of chaotropic aerosol on bidirectional fluxes of nutrients through stomatal pores represent poorly characterized risk factors for vegetation. The preliminary data now available suggests, for example, that stomatal regulatory responses to air humidity, long a contentious research topic, may be altered by aerosol deposition in ways not considered by current theory and that may confound current measurement techniques. In most of these cases, the magnitude of potential effects and their relative importance in different ecosystems and environments remain unknown.

References

Abdullah CM, Iqbal MZ (1991) Response of automobiles, stone and cement particulate matters on stomatal clogging of plants. Geobios 18:196–201

Aber JD, Ollinger SV, Federer CA, Reich PB, Goulden ML, Kicklighter DW, Melillo JM, Lathrop RG (1995) Predicting the effects of climate change on water yield and forest production in the northeastern United States. Climate Res 5(3):207–222

Adachi K, Tainosho Y (2004) Characterization of heavy metal particles embedded in tire dust. Environ Int 30:1009–1017

Allen CD, Breshears DD, McDowell NG (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6(8)

Almas AR, Bakken LR, Mulder J (2004) Changes in tolerance of soil microbial communities in Zn and Cd contaminated soils. Soil Biol Biochem 36:805–813

Anderegg WRL, Flint A, Huang CY, Flint L, Berry JA, Davis FW, Sperry JS, Field CB (2015) Tree mortality predicted from drought-induced vascular damage. Nat Geosci 8(5):367–371

Anderson JO, Thundiyil JG, Stolbach A (2012) Clearing the air: a review of the effects of particulate matter air pollution on human health. J Med Toxicol 8(2):166–175

Andreae MO (2007) Aerosols before pollution. Science 315(5808):50–51

Andreae MO, Crutzen PJ (1997) Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. Science 276(5315):1052–1058

Anfodillo T, Di Bisceglie DP, Urso T (2002) Minimum cuticular conductance and cuticle features of Picea abies and Pinus cembra needles along an altitudinal gradient in the Dolomites (NE Italian Alps). Tree Physiol 22(7):479–487

Armbrust DV, Retta A (2002) Wind and sandblast damage to growing vegetation. Ann Arid Zone 39:273–284

Asmi A, Coen MC, Ogren JA, Andrews E, Sheridan P, Jefferson A, Weingartner E, Baltensperger U, Bukowiecki N, Lihavainen H, Kivekas N, Asmi E, Aalto PP, Kulmala M, Wiedensohler A, Birmili W, Hamed A, O'Dowd C, Jennings SG, Weller R, Flentje H, Fjaeraa AM, Fiebig M, Myhre CL, Hallar AG, Swietlicki E, Kristensson A, Laj P (2013) Aerosol decadal trends – Part 2: In-situ aerosol particle number concentrations at GAW and ACTRIS stations. Atmos Chem Phys 13(2):895–916

Audet P, Charest C (2007) Heavy metal phytoremediation from a meta-analytical perspective. Environ Pollut 147:231–237

Ball P, Hallsworth JE (2015) Water structure and chaotropicity: their uses, abuses and biological implications. Phys Chem Chem Phys 17(13):8297–8305

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202(1):1–8

Beckett KP, Freer-Smith PH, Taylor G (1998) Urban woodlands: their role in reducing the effects of particulate pollution. Environ Pollut 99(3):347–360

Beckett KP, Freer-Smith PH, Taylor G (2000) Particulate pollution capture by urban trees: effect of species and windspeed. Glob Chang Biol 6(8):995–1003

Belot Y, Baille A, Delmas JL (1976) Numerical-model of atmospheric pollutant dispersion in presence of plant canopy – application to forest canopies. Atmos Environ 10(2):89–98

Berthelsen BO, Olsen RA, Steinnes E (1995) Ectomycorrhizal heavy metal accumulation as a contributing factor to heavy metal levels in organic surface soils. Sci Total Environ 170: 141–149

Bertolotti G, Rada EC, Ragazzi M, Chiste A, Gialanella S (2014) A multi-analytical approach to the use of conifer needles as passive samplers of particulate matter and organic pollutants. Aerosol Air Qual Res 14(3):677–685

Blum A, Mayer J, Golan G (1983) Chemical desiccation of wheat plants as a simulator of post-anthesis stress. 2. Relations to drought stress. Field Crop Res 6(2):149–155

Bond JA, Bollich PK (2007) Effects of pre-harvest desiccants on rice yield and quality. Crop Prot 26(4):490–494

Botto L, Narayanan C, Fulgosi M, Lakehal D (2005) Effect of near-wall turbulence enhancement on the mechanisms of particle deposition. Int J Multiphase Flow 31(8):940–956
Boucher O (2015) Atmospheric aerosols – properties and climate impacts. Springer, Dordrecht, p 311
Boucher U, Balabane M, Lamy I, Cambier P (2005) Decomposition in soil microcosms of leaves of the metallophyte Arabidopsis halleri: effect of leaf-associated heavy metals on biodegradation. Environ Pollut 135:187–194
Brandt CJ, Rhoades RW (1972) Effects of limestone dust accumulation on composition of a forest community. Environ Pollut 3:217–225
Brandt CJ, Rhoades RW (1973) Effects of limestone dust accumulation on lateral growth of forest trees. Environ Pollut 4:207–213
Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW (2005) Regional vegetation die-off in response to global-change-type drought. Proc Natl Acad Sci U S A 102(42):15144–15148
Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong YL, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC, Whitsel L, Kaufman JD, American Heart Association Council on Epidemiology and Prevention, Council on the Kidney in Cardiovascular Disease, and Council on Nutrition, Physical Activity and Metabolism (2010) Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121(21):2331–2378
Bruck RI, Robarge WP, McDaniel A (1989) Forest decline in the boreal montane ecosystems of the southern Appalachian mountains. Water Air Soil Pollut 48(1-2):161–180
Burkhardt J (2010) Hygroscopic particles on leaves: nutrients or desiccants? Ecol Monogr 80(3): 369–399
Burkhardt J, Eiden R (1994) Thin water films on coniferous needles. Atmos Environ 28(12): 2001–2011
Burkhardt J, Hunsche M (2013) "Breath figures" on leaf surfaces-formation and effects of microscopic leaf wetness. Front Plant Sci 4:422. doi:10.3389/fpls.2013.00422
Burkhardt J, Pariyar S (2014) Particulate pollutants are capable to 'degrade' epicuticular waxes and to decrease the drought tolerance of Scots pine (Pinus sylvestris L.). Environ Pollut 184: 659–667
Burkhardt J, Pariyar S (2016) How does the VPD response of isohydric and anisohydric plants depend on leaf surface particles? Plant Biol 18:91–100
Burkhardt J, Peters K, Crossley A (1995) The presence of structural surface waxes on coniferous needles affects the pattern of dry deposition of fine particles. J Exp Bot 46(288):823–831
Burkhardt J, Kaiser H, Goldbach H, Kappen L (1999) Measurements of electrical leaf surface conductance reveal recondensation of transpired water vapour on leaf surfaces. Plant Cell Environ 22(2):189–196
Burkhardt J, Koch K, Kaiser H (2001) Deliquescence of deposited atmospheric particles on leaf surfaces. Water Air Soil Pollut Focus 1:313–321
Burkhardt J, Basi S, Pariyar S, Hunsche M (2012) Stomatal penetration by aqueous solutions – an update involving leaf surface particles. New Phytol 196(3):774–787
Camargo MCR, Toledo MCF (2003) Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control 14(1):49–53
Cape JN, Fowler D (1981) Changes in epicuticular wax of Pinus sylvestris exposed to polluted air. Silva Fenn 15:457–458
Carslaw KS, Boucher O, Spracklen DV, Mann GW, Rae JGL, Woodward S, Kulmala M (2010) A review of natural aerosol interactions and feedbacks within the Earth system. Atmos Chem Phys 10(4):1701–1737
Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO (1999) Changing sources of nutrients during four million years of ecosystem development. Nature 397(6719):491–497
Chamberlain AC (1967) Transport of *Lycopodium* spores and other small particles to rough surfaces. Proc R Soc Lond A Math Phys Sci 296(1444):45–70
Chamberlain AC (1975) The movement of particles in plant communities. In: Monteith JL (ed) Vegetation and the atmosphere. Academic, London, pp 155–203

Chameides WL, Yu H, Liu SC, Bergin M, Zhou X, Mearns L, Wang G, Kiang CS, Saylor RD, Luo C, Huang Y, Steiner A, Giorgi F (1999) Case study of the effects of atmospheric aerosols and regional haze on agriculture: an opportunity to enhance crop yields in China through emission controls? Proc Natl Acad Sci U S A 96(24):13626–13633

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature 326(6114):655–661

Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, Cafmeyer J, Guyon P, Andreae MO, Artaxo P, Maenhaut W (2004) Formation of secondary organic aerosols through photooxidation of isoprene. Science 303(5661):1173–1176

Cleugh HA, Miller JM, Bohm M (1998) Direct mechanical effects of wind on crops. Agroforestry Syst 41(1):85–112

Cray JA, Russell JT, Timson DJ, Singhal RS, Hallsworth JE (2013) A universal measure of chaotropicity and kosmotropicity. Environ Microbiol 15(1):287–296

Crépineau C, Rychen G, Feidt C, Le Roux Y, Lichtfouse E, Laurent F (2003) Contamination of pastures by polycyclic aromatic hydrocarbons (PAHs) in the vicinity of a highway. J Agric Food Chem 51:4841–4845

Croteau MN, Luoma SN, Stewart AR (2005) Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol Oceanogr 50:1511–1519

Da Silva LC, Oliva MA, Azevedo AA, De Araujo JM (2006) Responses of restinga plant species to pollution from an iron pelletization factory. Water Air Soil Pollut 175:241–256

Daresta BE, Italiano F, de Gennaro G, Trotta M, Tutino M, Veronico P (2015) Atmospheric particulate matter (PM) effect on the growth of Solanum lycopersicum cv. Roma plants. Chemosphere 119:37–42

Dässler H-G, Ranft H, Rehn K-H (1972) Zur Widerstandsfähigkeit von Gehölzen gegenüber Fluorverbindungen und Schwefeldioxid [The susceptibility of woody plants exposed to fluorine compounds and SO2]. Flora (Jena) 161:289–302

de Leeuw G, Andreas EL, Anguelova MD, Fairall CW, Lewis ER, O'Dowd C, Schulz M, Schwartz SE (2011) Production flux of sea spray aerosol. Rev Geophys 49

Derome J, Lindroos A-J (1998) Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni smelter, SW Finland. Environ Pollut 99:225–232

Despres VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Frohlich-Nowoisky J, Elbert W, Andreae MO, Poschl U, Jaenicke R (2012) Primary biological aerosol particles in the atmosphere: a review. Tellus B Chem Phys Meteorol 64

Dette HP, Koop T (2015) Glass formation processes in mixed inorganic/organic aerosol particles. J Phys Chem A 119(19):4552–4561

Deventer MJ, Griessbaum F, Klemm O (2013) Size-resolved flux measurement of sub-micrometer particles over an urban area. Meteorol Z 22(6):729–737

dos Santos AP, Diehl A, Levin Y (2010) Surface tensions, surface potentials, and the Hofmeister series of electrolyte solutions. Langmuir 26(13):10778–10783

Durga M, Bharathi S, Balakrishna Murty P, Devasena T (2015) Characterization and phytotoxicity studies of suspended particulate matter (SPM) in Chennai urban area. J Environ Biol 36:583–589

Dusek U, Frank GP, Hildebrandt L, Curtius J, Schneider J, Walter S, Chand D, Drewnick F, Hings S, Jung D, Borrmann S, Andreae MO (2006) Size matters more than chemistry for cloud-nucleating ability of aerosol particles. Science 312(5778):1375–1378

Dutcher CS, Wexler AS, Clegg SL (2010) Surface tensions of inorganic multicomponent aqueous electrolyte solutions and melts. J Phys Chem A 114(46):12216–12230

Dzierzanowski K, Popek R, Gawronska H, Saebo A, Gawronski SW (2011) Deposition of particulate matter of different size fractions on leaf surfaces and in waxes of urban forest species. Int J Phytoremediation 13(10):1037–1046

Eamus D, Boulain N, Cleverly J, Breshears DD (2013) Global change-type drought-induced tree mortality: vapor pressure deficit is more important than temperature per se in causing decline in tree health. Ecol Evol 3(8):2711–2729

Eamus D, Fowler D (1990) Photosynthetic and stomatal conductance responses to acid mist of Red Spruce seedlings. Plant Cell Environ 13(4):349–357

Eeva T, Lehikoinen E (2004) Rich calcium availability diminishes heavy metal toxicity in Pied Flycatcher. Funct Ecol 18:548–553

Ehn M, Thornton JA, Kleist E, Sipila M, Junninen H, Pullinen I, Springer M, Rubach F, Tillmann R, Lee B, Lopez-Hilfiker F, Andres S, Acir IH, Rissanen M, Jokinen T, Schobesberger S, Kangasluoma J, Kontkanen J, Nieminen T, Kurten T, Nielsen LB, Jorgensen S, Kjaergaard HG, Canagaratna M, Dal Maso M, Berndt T, Petaja T, Wahner A, Kerminen VM, Kulmala M, Worsnop DR, Wildt J, Mentel TF (2014) A large source of low-volatility secondary organic aerosol. Nature 506(7489):476–9

Eller BM (1977) Road dust induced increase of leaf temperature. Environ Pollut 13:99–107

Eller CB, Lima AL, Oliveira RS (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, Drimys brasiliensis (Winteraceae). New Phytol 199(1):151–162

Erickson BE (2003) Clear-cutting increases mercury in runoff. Environ Sci Technol 37(11): 200A–201A

Erisman JW, Draaijers G (2003) Deposition to forests in Europe: most important factors influencing dry deposition and models used for generalisation. Environ Pollut 124(3):379–388

Escobedo FJ, Nowak DJ (2009) Spatial heterogeneity and air pollution removal by an urban forest. Landsc Urban Plan 90(3-4):102–110

Eveling DW (1969) Effects of spraying plants with suspensions of inert dusts. Ann Appl Biol 64(1):139–151

Eveling DW (1972) Similar effects of suspensions of copper oxychloride and kaolin on sprayed leaves. Ann Appl Biol 70(3):245

Farmer AM (1993) The effects of dust on vegetation – a review. Environ Pollut 79(1):63–75

Feng J (2008) A size-resolved model and a four-mode parameterization of dry deposition of atmospheric aerosols. J Geophys Res Atmos 113(D12)

Feng MH, Shan XQ, Zhang SZ, Wen B (2005) Comparison of a rhizosphere-based method with other one-step extraction methods for assessing the bioavailability of soil metals to wheat. Chemosphere 59:939–949

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemyer R (1998) Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. Ecol Appl 8:706–733

Fernandez V, Eichert T (2009) Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. Crit Rev Plant Sci 28(1-2):36–68

Field JP, Belnap J, Breshears DD, Neff JC, Okin GS, Whicker JJ, Painter TH, Ravi S, Reheis MC, Reynolds RL (2010) The ecology of dust. Front Ecol Environ 8(8):423–430

Flückiger W, Flückiger-Keller H, Oertli JJ, Guggenheim R (1977) Pollution of leaf and needle surfaces near a highway and its effect on stomatal diffusive resistance. Eur J For Pathol 7(6): 358–364

Fowler D, Cape JN, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999) The global exposure of forests to air pollutants. Water Air Soil Pollut 116(1-2):5–32

Freer-Smith PH, Holloway S, Goodman A (1997) The uptake of particulates by an urban woodland: site description and particulate composition. Environ Pollut 95(1):27–35

Freer-Smith PH, El-Khatib AA, Taylor G (2004) Capture of particulate pollution by trees: a comparison of species typical of semi-arid areas (Ficus nitida and Eucalyptus globulus) with European and North American species. Water Air Soil Pollut 155(1-4):173–187

Freer-Smith PH, Beckett KP, Taylor G (2005) Deposition velocities to Sorbus aria, Acer campestre, Populus deltoides X trichocarpa 'Beaupre', Pinus nigra and X Cupressocyparis

leylandii for coarse, fine and ultra-fine particles in the urban environment. Environ Pollut 133(1):157–167
Frescholtz TF, Gustin MS, Schorran DE et al (2003) Assessing the source of mercury in foliar tissue of quaking aspen. Environ Toxicol Chem 22:2114–2119
Fritze H, Niini S, Mikkola K, Makinen A (1989) Soil microbial effects of a Cu-Ni smelter in southwestern Finland. Biol Fertil Soils 8(1):87–94
Fuzzi S, Baltensperger U, Carslaw K, Decesari S, van Der Gon HD, Facchini MC, Fowler D, Koren I, Langford B, Lohmann U, Nemitz E, Pandis S, Riipinen I, Rudich Y, Schaap M, Slowik JG, Spracklen DV, Vignati E, Wild M, Williams M, Gilardoni S (2015) Particulate matter, air quality and climate: lessons learned and future needs. Atmos Chem Phys 15(14): 8217–8299
Gallagher MW, Beswick KM, Duyzer J, Westrate H, Choularton TW, Hummelshoj P (1997) Measurements of aerosol fluxes to Speulder forest using a micrometeorological technique. Atmos Environ 31(3):359–373
Gao YZ, Zhu LZ (2004) Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55:1169–1178
Gawel JE, Ahner BA, Friedland AJ, Morel FMM (1996) Role for heavy metals in forest decline indicated by phytochelatin measurements. Nature 381:64–65
Gillette DA, Lawson RE, Thompson RS (2004) A "test of concept" comparison of aerodynamic and mechanical resuspension mechanisms for particles deposited on field rye grass (Secale cercele). Part 1. Relative particle flux rates. Atmos Environ 38(28):4789–4797
Gleason SM, Faucette DT, Toyofuku MM, Torres CA, Bagley CF (2007) Assessing and mitigating the effects of windblown soil on rare and common vegetation. Environ Manag 40(6): 1016–1024
Gmur NF, Evans LS, Cunningham EA (1983) Effects of ammonium sulfate aerosols on vegetation. 2. Mode of entry and responses of vegetation. Atmos Environ 17(4):715–721
Grace J, Wilson J (1976) Boundary layer over a Populus leaf. J Exp Bot 27(97):231–241
Grantz DA, Garner JHB, Johnson DW (2003) Ecological effects of particulate matter. Environ Int 29(2-3):213–239
Greenspan L (1977) Humidity fixed-points of binary saturated aqueous-solutions. J Res Nat Bur Stand 81(1):89–96
Guderian R (1986) Terrestrial ecosystems: particulate deposition. In: Legge AH, Krupa SV (eds) Air pollutants and their effects on the terrestrial ecosystem, vol 18: Advances in environmental science and technology. Wiley, New York, pp 339–363
Guha A (1997) A unified Eulerian theory of turbulent deposition to smooth and rough surfaces. J Aerosol Sci 28(8):1517–1537
Guha A (2008) Transport and deposition of particles in turbulent and laminar flow. Annu Rev Fluid Mech 40:311–341
Hageman KJ, Simonich SL, Campbell DH, Wilson GR, Landers DH (2006) Atmospheric deposition of current-use and historic-use pesticides in snow at national parks in the western United States. Environ Sci Technol 40:3174–3180
Hamilton DS (2015) Natural aerosols and climate: understanding the unpolluted atmosphere to better understand the impacts of pollution. Weather 70(9):264–268
Hamilton DS, Lee LA, Pringle KJ, Reddington CL, Spracklen DV, Carslaw KS (2014) Occurrence of pristine aerosol environments on a polluted planet. Proc Natl Acad Sci U S A 111(52): 18466–18471
Heinsoo K, Koppel A (1998) Minimum epidermal conductance of Norway spruce (Picea abies) needles: influence of age and shoot position in the crown. Ann Bot Fenn 35(4):257–262
Helmisaari H-S, Makkonen K, Olsson M, Viksna A, Mälkönen E (1999) Fine-root growth, mortality and heavy metal concentrations in limed and fertilized Pinus silvestris (L.) stands in the vicinity of a Cu-Ni smelter in SW Finland. Plant Soil 209:193–200
Herrett RA, Vlitos AJ, Crosby DG, Hatfield HH (1962) Leaf abscission induced by iodide ion. Plant Physiol 37(3):358–363

Hirano T, Kiyota M, Aiga I (1995) Physical effects of dust on leaf physiology of cucumber and kidney bean plants. Environ Pollut 89(3):255–261

Hoad SP, Jeffree CE, Grace J (1992) Effects of wind and abrasion on cuticular integrity in *Fagus sylvatica* L and consequences for transfer of pollutants through leaf surfaces. Agr Ecosyst Environ 42(3-4):275–289

Hofman J, Wuyts K, Van Wittenberghe S, Brackx M, Samson R (2014) On the link between biomagnetic monitoring and leaf-deposited dust load of urban trees: relationships and spatial variability of different particle size fractions. Environ Pollut 189:63–72

Hofmeister F (1887) Zur Lehre von der Wirkung der Salze. 2. Mittheilung. Ueber Regelmässigkeiten in der eiweissfällenden Wirkung der Salze und ihre Beziehung zum physiologischen Verhalten derselben. Archiv fuer experimentelle Pathologie und Pharmakologie XXIV:247–260

Hope AS, Fleming JB, Stow DA, Aguado E (1991) Tussock tundra albedos in the north slope of Alaska – effects of illumination, vegetation composition, and dust deposition. J Appl Meteorol 30(8):1200–1206

Howe TS, Billings S, Stolzberg RJ (2004) Sources of polycyclic aromatic hydrocarbons and hexachlorobenzene in spruce needles of eastern Alaska. Environ Sci Technol 38:3294–3298

Howsam M, Jones KC, Ineson P (2000) PAHs associated with the leaves of three deciduous tree species. I – Concentrations and profiles. Environ Pollut 108(3):413–424

Hoyt DV (1978) A model for the calculation of solar global insolation. Solar Energy 21:27–35

Huang C-W, Launiainen S, Gronholm T, Katul GG (2014) Particle deposition to forests: an alternative to K-theory. Atmos Environ 94:593–605

Huang CW, Lin MY, Khlystov A, Katul GG (2015) The effects of leaf size and microroughness on the branch-scale collection efficiency of ultrafine particles. J Geophys Res Atmos 120(8): 3370–3385

Huttunen S, Havas P, Laine K (1981) Effects of air pollutants on the wintertime water economy of the Scots pine *Pinus silvestris*. Holarctic Ecol 4(2):94–101

Jaenicke R (2005) Abundance of cellular material and proteins in the atmosphere. Science 308(5718):73

Jiao XC, Xu FL, Dawson R, Chen SH, Tao S (2007) Adsorption and absorption of polycyclic aromatic hydrocarbons to rice roots. Environ Pollut 148:230–235

Johnson D, Hale B (2008) Fine root decomposition and cycling of Cu, Ni, Pb, and Zn at forest sites near smelters in Sudbury, ON, and Rouyn-Noranda, QU, Canada. Hum Ecol Risk Assess 14:41–53

Joynt J, Bischoff M, Turco R, Konopka A, Nakatsu CH (2006) Microbial community analysis of soils contaminated with lead, chromium and petroleum hydrocarbons. Microb Ecol 51: 209–219

Kalinovic TS, Serbula SM, Radojevic AA, Kalinovic JV, Steharnik MM, Petrovic JV (2016) Elder, linden and pine biomonitoring ability of pollution emitted from the copper smelter and the tailings ponds. Geoderma 262:266–275

Kandeler E, Kampichler C, Horak O (1996) Influence of heavy metals on the functional diversity of soil microbial communities. Biol Fertil Soils 23:299–306

Kardel F, Wuyts K, Maher BA, Hansard R, Samson R (2011) Leaf saturation isothermal remanent magnetization (SIRM) as a proxy for particulate matter monitoring: inter-species differences and in-season variation. Atmos Environ 45(29):5164–5171

Katul GG, Gronholm T, Launiainen S, Vesala T (2010) Predicting the dry deposition of aerosol-sized particles using layer-resolved canopy and pipe flow analogy models: role of turbophoresis. J Geophys Res Atmos 115

Kaufman YJ, Tanre D, Boucher O (2002) A satellite view of aerosols in the climate system. Nature 419(6903):215–223

Kaupp H, McLachlan MS (1999) Gas/particle partitioning of PCDD/Fs, PCBs, PCNs and PAHs. Chemosphere 38:3411–3421

Kiikkilä O (2003) Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu-Ni smelter, in SW Finland. Silva Fenn 37:399–415

Kim CG, Bell JNB, Power SA (2003) Effects of soil cadmium on Pinus sylvestris L. seedlings. Plant Soil 257:443–449

Konrad W, Burkhardt J, Ebner M, Roth-Nebelsick A (2015) Leaf pubescence as a possibility to increase water use efficiency by promoting condensation. Ecohydrology 8(3):480–492

Kozlov MV, Haukioja E, Bakhtiarov AV, Stroganov DN, Zimina SN (2000) Root versus canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. Environ Pollut 107(3):413–420

Krajíková A, MejstÍík V (1984) The effect of fly-ash particles on the plugging of stomata. Environ Pollut 36:83–93

Krupa S, Booker F, Bowersox V, Lehmann CT, Grantz D (2008) Uncertainties in the current knowledge of some atmospheric trace gases associated with US agriculture: a review. J Air Waste Manage Assoc 58(8):986–993

Kuki KN, Oliva MA, Pereira EG, Costa AC, Cambraia J (2008) Effects of simulated deposition of acid mist and iron ore particulate matter on photosynthesis and the generation of oxidative stress in Schinus terebinthifolius Radii and Sophora tomentosa L. Sci Total Environ 403(1-3): 207–214

Kuki KN, Oliva MA, Costa AC (2009) The simulated effects of iron dust and acidity during the early stages of establishment of two coastal plant species. Water Air Soil Pollut 196:287–295

Kunz W, Henle J, Ninham BW (2004) 'Zur Lehre von der Wirkung der Salze' (about the science of the effect of salts): Franz Hofmeister's historical papers. Curr Opin Colloid Interface Sci 9(1-2):19–37

Lakzian A, Murphy P, Turner A, Beynon JL, Giller KE (2002) Rhizobium leguminosarum bv. viciae populations in soils with increasing heavy metal contamination: abundance, plasmid profiles, diversity and metal tolerance. Soil Biol Biochem 34:519–529

Lee TE, Miller SD, Turk FJ, Schueler C, Julian R, Deyo S, Dills P, Wang S (2006) The NPOESS VIIRS day/night visible sensor. Bull Am Meteorol Soc 87:191–199

Lei YD, Wania F (2004) Is rain or snow a more efficient scavenger of organic chemicals? Atmos Environ 38:3557–3571

Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A (2015) The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature 525(7569):367–371

Lequy E, Conil S, Turpault MP (2012) Impacts of Aeolian dust deposition on European forest sustainability: a review. For Ecol Manage 267:240–252

Lerman SL, Darley EF (1975) Particulates. In: Mudd JB, Kozlowski TT (eds) Responses of plants to air pollution. Academic, New York, pp 141–158

Lin ZQ, Schuepp PH (1996) Concentration and distribution of metal elements at the surface of balsam fir foliage by micro-proton induced X-ray emission. Environ Sci Technol 30(1): 246–251

Lingua G, Franchin C, Todeschini V, Castiglione S, Biondi S, Burlando B, Parravicini V, Torrigiani P, Berta G (2008) Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. Environ Pollut 153:137–147

Little P, Wiffen RD (1977) Emission and deposition of petrol engine exhaust Pb. 1. Deposition of exhaust Pb to plant and soil surfaces. Atmos Environ 11(5):437–447

Lo Nostro P, Ninham BW (2012) Hofmeister phenomena: an update on ion specificity in biology. Chem Rev 112(4):2286–2322

Lohmann U, Feichter J (2005) Global indirect aerosol effects: a review. Atmos Chem Phys 5: 715–737

Lovett GM (1994) Atmospheric deposition of nutrients and pollutants in North-America – an ecological perspective. Ecol Appl 4(4):629–650

Lovett GM, Lindberg SE (1993) Atmospheric deposition and canopy interactions of nitrogen in forests. Can J For Res 23:1603–1616

Maher BA, Ahmed IAM, Davison B, Karloukovski V, Clarke R (2013) Impact of roadside tree lines on indoor concentrations of traffic-derived particulate matter. Environ Sci Technol 47(23):13737–13744

Mahowald N, Ward DS, Kloster S, Flanner MG, Heald CL, Heavens NG, Hess PG, Lamarque JF, Chuang PY (2011) Aerosol impacts on climate and biogeochemistry. In: Gadgil A, Liverman DM (eds) Annual review of environment and resources, vol 36. Annual Reviews, Palo Alto, pp 45–74

Manktelow PT, Mann GW, Carslaw KS, Spracklen DV, Chipperfield MP (2007) Regional and global trends in sulfate aerosol since the 1980s. Geophys Res Lett 34(14)

Manso M, Castro-Gomes J (2015) Green wall systems: a review of their characteristics. Renew Sustain Energy Rev 41:863–871

Marchioli C, Picciotto M, Soldati A (2006) Particle dispersion and wall-dependent turbulent flow scales: implications for local equilibrium models. J Turbulence 7(60):1–12

Kummerova M, Krulova J, Zezulka S et al (2006) Evaluation of fluoranthene phytotoxicity in pea plants by Hill reaction and chlorophyll fluorescence. Chemosphere 65:489–496

Kummerova M, Zezulka S, Babula P et al (2013) Chemosphere 90:665–673

Martell EA (1974) Radioactivity of tobacco trichomes and insoluble cigarette-smoke particles. Nature 249(5454):215–217

Martin CE, Gravatt DA, Loeschen VS (1992) Photosynthetic responses of 3 species to acute exposures of nitrate-containing and sulfate-containing aerosols. Atmos Environ A General Top 26:381–391

Matzka J, Maher BA (1999) Magnetic biomonitoring of roadside tree leaves: identification of spatial and temporal variations in vehicle-derived particulates. Atmos Environ 33(28): 4565–4569

McDonald AG, Bealey WJ, Fowler D, Dragosits U, Skiba U, Smith RI, Donovan RG, Brett HE, Hewitt CN, Nemitz E (2007) Quantifying the effect of urban tree planting on concentrations and depositions of PM10 in two UK conurbations. Atmos Environ 41(38):8455–8467

McDowell NG, Allen CD (2015) Darcy's law predicts widespread forest mortality under climate warming. Nat Clim Change 5(7):669–672

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178(4):719–739

McLachlan MS (1999) Framework for the interpretation of measurements of SOCs in plants. Environ Sci Technol 33(11):1799–1804

Mengel K, Breininger MT, Lütz HJ (1990) Effect of simulated acidic fog on carbohydrate leaching, CO_2 assimilation and development of damage symptoms in young spruce trees (*Picea abies* L Karst). Environ Exp Bot 30(2):165–173

Mercado LM, Bellouin N, Sitch S, Boucher O, Huntingford C, Wild M, Cox PM (2009) Impact of changes in diffuse radiation on the global land carbon sink. Nature 458(7241):1014–1087

Meyers TP, Luke WT, Meisinger JJ (2006) Fluxes of ammonia and sulfate over maize using relaxed eddy accumulation. Agric For Meteorol 136(3-4):203–213

Michailides TJ, Morgan DP (1992) Effects of temperature and wetness duration on infection of pistachio by Botryosphaeria dothidea and management of disease by reducing duration of irrigation. Phytopathology 82:1399–1406

Mikhailov E, Vlasenko S, Martin ST, Koop T, Poschl U (2009) Amorphous and crystalline aerosol particles interacting with water vapor: conceptual framework and experimental evidence for restructuring, phase transitions and kinetic limitations. Atmos Chem Phys 9(24):9491–9522

Millhollen AG, Gustin MS, Obrist D (2006) Foliar mercury accumulation and exchange for three tree species. Environ Sci Technol 40:6001–6006

Mo L, Ma ZY, Xu YS, Sun FB, Lun XX, Liu XH, Chen JG, Yu XX (2015) Assessing the capacity of plant species to accumulate particulate matter in Beijing, China. PLoS One 10(10)

Mori J, Hanslin HM, Burchi G, Saebo A (2015a) Particulate matter and element accumulation on coniferous trees at different distances from a highway. Urban Forestry Urban Greening 14(1): 170–177

Mori J, Saebo A, Hanslin HM, Teani A, Ferrini F, Fini A, Burchi G (2015b) Deposition of traffic-related air pollutants on leaves of six evergreen shrub species during a Mediterranean summer season. Urban Forestry Urban Greening 14(2):264–273
Mulitza S, Heslop D, Pittauerova D, Fischer HW, Meyer I, Stuut JB, Zabel M, Mollenhauer G, Collins JA, Kuhnert H, Schulz M (2010) Increase in African dust flux at the onset of commercial agriculture in the Sahel region. Nature 466(7303):226–228
Murphy HJ (1968) Potato vine killing. Am Potato J 45(12):472–477
Muxworthy AR, Matzka M, Davila AF, Petersen N (2003) Magnetic signature of daily sampled urban atmospheric particles. Atmos Environ 37(29):4163–4169
Naidoo G, Chirkoot D (2004) The effects of coal dust on photosynthetic performance of the mangrove, Avicennia marina in Richards Bay, South Africa. Environ Pollut 127:359–366
Nanos GD, Ilias IF (2007) Effects of inert dust on olive (Olea europaea L.) leaf physiological parameters. Environ Sci Pollut Res 14(3):212–214
Nash TH III (1975) Influence of effluents from a zinc factory on lichens. Ecol Monogr 45:183–198
Neinhuis C, Barthlott W (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. Ann Bot 79(6):667–677
Neinhuis C, Barthlott W (1998) Seasonal changes of leaf surface contamination in beech, oak, and ginkgo in relation to leaf micromorphology and wettability. New Phytol 138(1):91–98
Nemitz E, Dorsey JR, Flynn MJ, Gallagher MW, Hensen A, Erisman JW, Owen SM, Dammgen U, Sutton MA (2009) Aerosol fluxes and particle growth above managed grassland. Biogeosciences 6(8):1627–1645
Newman EI (1995) Phosphorus inputs to terrestrial ecosystems. J Ecol 83(4):713–726
Nicholson KW (1993) Wind tunnel experiments on the resuspension of particulate material. Atmos Environ A General Top 27(2):181–188
Nicolas ME, Turner NC (1993) Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during postanthesis drought. Field Crop Res 31(1-2): 155–171
Nobel PS (1991) Physicochemical and environmental plant physiology. Academic, San Diego, p 635
Norton RA, Childers NF (1954) Experiments with urea sprays on the peach. Proc Am Soc Hortic Sci 63:23–31
Nowak DJ, Hirabayashi S, Bodine A, Hoehn R (2013) Modeled PM2.5 removal by trees in ten US cities and associated health effects. Environ Pollut 178:395–402
OECD (2012) OECD Environmental Outlook to 2050. OECD, Paris
Oikawa PY, Grantz DA, Chatterjee A et al (2014) Unifying soil respiration pulses, inhibition, and temperature hysteresis through dynamics of labile soil carbon and O-2. J Geophys Res Biogeosci 119:521–536
Oliva SR, Raitio H (2003) Review of cleaning techniques and their effects on the chemical composition of foliar samples. Boreal Environ Res 8(3):263–272
Oliva SR, Valdes B (2004) Influence of washing on metal concentrations in leaf tissue. Commun Soil Sci Plant Anal 35(11-12):1543–1552
Oliveira A, Pampulha ME (2006) Effects of long-term heavy metal contamination on soil microbial characteristics. J Biosci Bioeng 102:157–161
Olszyk DM, Bytnerowicz A, Takemoto BK (1989) Photochemical oxidant pollution and vegetation: effects of gases, fog, and particles. Environ Pollut 61:11–29
Otnyukova T (2007) Epiphytic lichen growth abnormalities and element concentrations as early indicators of forest decline. Environ Pollut 146:359–365
Ottele M, van Bohemen HD, Fraaij ALA (2010) Quantifying the deposition of particulate matter on climber vegetation on living walls. Ecol Eng 36(2):154–162
Ould-Dada Z, Baghini NM (2001) Resuspension of small particles from tree surfaces. Atmos Environ 35(22):3799–3809
Pajunoja A, Lambe AT, Hakala J, Rastak N, Cummings MJ, Brogan JF, Hao LQ, Paramonov M, Hong J, Prisle NL, Malila J, Romakkaniemi S, Lehtinen KEJ, Laaksonen A, Kulmala M,

Massoli P, Onasch TB, Donahue NM, Riipinen I, Davidovits P, Worsnop DR, Petaja T, Virtanen A (2015) Adsorptive uptake of water by semisolid secondary organic aerosols. Geophys Res Lett 42(8):3063–3068

Parker S, Foat T, Preston S (2008) Towards quantitative prediction of aerosol deposition from turbulent flows. J Aerosol Sci 39(2):99–112

Patankar NA (2004) Mimicking the lotus effect: influence of double roughness structures and slender pillars. Langmuir 20(19):8209–8213

Paulot F, Jacob DJ (2014) Hidden cost of U.S. agricultural exports: particulate matter from ammonia emissions. Environ Sci Technol 48(2):903–908

Pavlik M, Pavlıkova D, Zemanova V, Hnilicka F, Urbanova V, Szakova J (2012) Trace elements present in airborne particulate matter—stressors of plant metabolism. Ecotoxicol Environ Saf 79:101–107

Pegram LM, Record MT (2007) Hofmeister salt effects on surface tension arise from partitioning of anions and cations between bulk water and the air-water interface. J Phys Chem B 111(19): 5411–5417

Pennanen T, Frostegård Å, Fritze H, Bååth E (1996) Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. Appl Environ Microbiol 62:420–428

Peters K, Eiden R (1992) Modeling the dry deposition velocity of aerosol particles to a spruce forest. Atmos Environ A General Top 26(14):2555–2564

Petroff A, Mailliat A, Amielh M, Anselmet F (2008) Aerosol dry deposition on vegetative canopies. Part II: A new modelling approach and applications. Atmos Environ 42(16): 3654–3683

Petters MD, Kreidenweis SM (2007) A single parameter representation of hygroscopic growth and cloud condensation nucleus activity. Atmos Chem Phys 7(8):1961–1971

Pilinis C, Seinfeld JH, Grosjean D (1989) Water content of atmospheric aerosols. Atmos Environ 23(7):1601–1606

Pope CA, Dockery DW (2006) Health effects of fine particulate air pollution: lines that connect. J Air Waste Manage Assoc 56(6):709–742

Popek R, Gawronska H, Wrochna M, Gawronski SW, Saebo A (2013) Particulate matter on foliage of 13 woody species: deposition on surfaces and phytostabilisation in waxes – a 3-year study. Int J Phytoremediation 15(3):245–256

Pöschl U (2005) Atmospheric aerosols: composition, transformation, climate and health effects. Angew Chem Int Ed 44(46):7520–7540

Pöschl U, Shiraiwa M (2015) Multiphase chemistry at the atmosphere–biosphere interface influencing climate and public health in the anthropocene. Chem Rev 115(10):4440–4475

Pringle KJ, Tost H, Pozzer A, Poschl U, Lelieveld J (2010) Global distribution of the effective aerosol hygroscopicity parameter for CCN activation. Atmos Chem Phys 10(12):5241–5255

Prusty BAK, Mishra PC, Azeez PA (2005) Dust accumulation and leaf pigment content in vegetation near the national highway at Sambalpur, Orissa, India. Ecotoxicol Environ Saf 60 (2):228–235

Pryor SC, Gallagher M, Sievering H, Larsen SE, Barthelmie RJ, Birsan F, Nemitz E, Rinne J, Kulmala M, Groenholm T, Taipale R, Vesala T (2008) A review of measurement and modelling results of particle atmosphere-surface exchange. Tellus B Chem Phys Meteorol 60(1):42–75

Przybysz A, Saebo A, Hanslin HM, Gawronski SW (2014) Accumulation of particulate matter and trace elements on vegetation as affected by pollution level, rainfall and the passage of time. Sci Total Environ 481:360–369

Pueyo M, Sastre J, Hernandez E, Vidal M, Lopez-Sanchez JF, Rauret G (2003) Prediction of trace element mobility in contaminated soils by sequential extraction. J Environ Qual 32(6): 2054–2066

Pugh TAM, MacKenzie AR, Whyatt JD, Hewitt CN (2012) Effectiveness of green infrastructure for improvement of air quality in urban street canyons. Environ Sci Technol 46(14):7692–7699

Quayle BM, Mather TA, Witt MLI, Maher BA, Mitchell R, Martin RS, Calabrese S (2010) Application and evaluation of biomagnetic and biochemical monitoring of the dispersion and deposition of volcanically-derived particles at Mt. Etna, Italy. J Volcanol Geotherm Res 191(1-2):107–116

Rabalais NN (2002) Nitrogen in aquatic ecosystems. Ambio 31:102–112

Rai PK (2013) Environmental magnetic studies of particulates with special reference to biomagnetic monitoring using roadside plant leaves. Atmos Environ 72:113–129

Rapport DJ, Whitford WG (1999) How ecosystems respond to stress: common properties of arid and aquatic systems. BioScience 49:193–203

Räsänen JV, Holopainen T, Joutsensaari J, Ndam C, Pasanen P, Rinnan A, Kivimaenpaa M (2013) Effects of species-specific leaf characteristics and reduced water availability on fine particle capture efficiency of trees. Environ Pollut 183:64–70

Rauret G, Llaurado M, Tent J, Rigol A, Alegre LH, Utrillas MJ (1994) Deposition on holm oak leaf surfaces of accidentally released radionuclides. Sci Total Environ 157(1-3):7–16

Reinap A, Wiman BLB, Svenningsson B, Gunnarsson S (2009) Oak leaves as aerosol collectors: relationships with wind velocity and particle size distribution. Experimental results and their implications. Trees Struct Funct 23(6):1263–1274

Reisinger LM (1990) Analysis of airborne particles in the southern Appalachian mountains. Water Air Soil Pollut 50(1-2):149–162

Renner KA (1991) Chemical vine desiccation of 2 potato cultivars. Am Potato J 68(7):479–491

Reynolds AM (2000) Prediction of particle deposition on to rough surfaces. Agric For Meteorol 104(2):107–118

Reznik G, Schmidt E (2008) Separation of fine dust on plants by low current-speed. Chem Ing Tech 80(12):1849–1853

Ricks GR, Williams RJ (1974) Effects of atmospheric pollution on deciduous woodland. 2. Effects of particulate matter upon stomatal diffusion resistance in leaves of *Quercus petraea* (Mattuschka) Leibl. Environ Pollut 6(2):87–109

Ruijgrok W, Tieben H, Eisinga P (1997) The dry deposition of particles to a forest canopy: a comparison of model and experimental results. Atmos Environ 31(3):399–415

Saebo A, Popek R, Nawrot B, Hanslin HM, Gawronska H, Gawronski SW (2012) Plant species differences in particulate matter accumulation on leaf surfaces. Sci Total Environ 427:347–354

Salemaa M, Derome J, Helmisaari HS et al (2004) Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland. Sci Total Environ 324:141–160

Sanderson EG, Farant JP (2004) Indoor and outdoor polycyclic aromatic hydrocarbons in residences surrounding a Soderberg aluminum smelter in Canada. Environ Sci Technol 38: 5350–5356

Sant'Ovaia H, Lacerda MJ, Gomes C (2012) Particle pollution – an environmental magnetism study using biocollectors located in northern Portugal. Atmos Environ 61:340–349

Santos APM, Segura-Munoz SI, Nadal M, Schuhmacher M, Domingo JL, Martinez CA, Takayanagui AMM (2015) Traffic-related air pollution biomonitoring with Tradescantia pallida (Rose) Hunt. cv. purpurea Boom in Brazil. Environ Monit Assess 187(2):39

Sase H, Takamatsu T, Yoshida T, Inubushi K (1998) Changes in properties of epicuticular wax and the related water loss in Japanese cedar (Cryptomeria japonica) affected by anthropogenic environmental factors. Can J For Res 28(4):546–556

Sauter JJ, Voss JU (1986) SEM observations on the structural degradation of epistomatal waxes in *Picea abies* (L) Karst and its possible role in the Fichtensterben. Eur J For Pathol 16(7): 408–423

Schleppi P, Tobler L, Bucher JB, Wyttenbach A (2000) Multivariate interpretation of the foliar chemical composition of Norway spruce (Picea abies). Plant and Soil 219(1-2):251–262

Schönherr J, Bukovac MJ (1972) Penetration of stomata by liquids – dependence on surface tension, wettability, and stomatal morphology. Plant Physiol 49(5):813–819

Schuepp PH (1993) Leaf boundary layers. New Phytol 125(3):477–507

Sgrigna G, Saebo A, Gawronski S, Popek R, Calfapietra C (2015) Particulate matter deposition on Quercus ilex leaves in an industrial city of central Italy. Environ Pollut 197:187–194
Sharifi MR, Gibson AC, Rundel PW (1997) Surface dust impacts on gas exchange in Mojave Desert shrubs. J Appl Ecol 34(4):837–846
Sharifi MR, Gibson AC, Rundel PW (1999) Phenological and physiological responses of heavily dusted creosote bush (Larrea tridentata) to summer irrigation in the Mojave Desert. Flora 14:369–378
Sievering H, Rusch D, Marquez L (1996) Nitric acid, particulate nitrate and ammonium in the continental free troposphere: nitrogen deposition to an alpine tundra ecosystem. Atmos Environ 30:2527–2537
Simmleit N, Toth A, Szekely T, Schulten HR (1989) Characterization of particles adsorbed on plant surfaces. Int J Environ Anal Chem 36(1):7–11
Slinn WGN (1982) Predictions for particle deposition to vegetative canopies. Atmos Environ 16(7):1785–1794
Smith WH (1990) Forest nutrient cycling: toxic ions. In: Air pollution and forests: interactions between air contaminants and forest ecosystems. Springer, New York, pp 225–268
Snyder RL (2015) Drought tip: fog contribution to crop water use. University of California, Agriculture and Natural Resources, p 4
Soares DR, Siqueira JO (2008) Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. Biol Fertil Soils 44:833–841
Song YS, Maher BA, Li F, Wang XK, Sun X, Zhang HX (2015) Particulate matter deposited on leaf of five evergreen species in Beijing, China: source identification and size distribution. Atmos Environ 105:53–60
Spinka J (1971) Vliv zne‡i × têného ovzdu × í na ovocné stromy a zeleninu [Effects of polluted air on fruit trees and legumes]. Ziva 19:13–15
Srogi K (2007) Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett 5:169–195
Steffen W, Richardson K, Rockstrom J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, de Vries W, de Wit CA, Folke C, Gerten D, Heinke J, Mace GM, Persson LM, Ramanathan V, Reyers B, Sorlin S (2015) Planetary boundaries: guiding human development on a changing planet. Science 347(6223):1259855
Sternberg T, Viles H, Cathersides A, Edwards M (2010) Dust particulate absorption by ivy (Hedera helix L) on historic walls in urban environments. Sci Total Environ 409(1):162–168
Swap R, Garstang M, Greco S, Talbot R, Kallberg P (1992) Saharan dust in the Amazon basin. Tellus B Chem Phys Meteorol 44(2):133–149
Swietlik D, Faust M (1984) Foliar nutrition of fruit crops. Hortic Rev 6:287–355
Takamatsu T, Sase H, Takada J (2001) Some physiological properties of Cryptomeria japonica leaves from Kanto, Japan: potential factors causing tree decline. Can J For Res 31(4):663–672
Tao S, Jiao XC, Chen SH, Xu FL, Li YJ, Liu FZ (2006) Uptake of vapor and particulate polycyclic aromatic hydrocarbons by cabbage. Environ Pollut 140(1):13–15
Taulavuori K, Prasad MNV, Taulavuori E, Laine K (2005) Metal stress consequences on frost hardiness of plants at northern high latitudes: a review and hypothesis. Environ Pollut 135:209–220
Tremper AH, Agneta M, Burton S, Higgs DEB (2004) Field and laboratory exposures of two moss species to low level metal pollution. J Atmos Chem 49:111–120
Trimbacher C, Eckmüllner O (1997) A method for quantifying changes in the epicuticular wax structure of Norway spruce needles. Eur J For Pathol 27(2):83–93
Tunved P, Hansson HC, Kerminen VM, Strom J, Dal Maso M, Lihavainen H, Viisanen Y, Aalto PP, Komppula M, Kulmala M (2006) High natural aerosol loading over boreal forests. Science 312(5771):261–263
Turunen M, Huttunen S (1990) A review of the response of epicuticular wax of conifer needles to air pollution. J Environ Qual 19(1):35–45

Unsworth MH, Wilshaw JC (1989) Wet, occult and dry deposition of pollutants on forests. Agric For Meteorol 47(2-4):221–238
Urbat M, Lehndorff E, Schwark L (2004) Biomonitoring of air quality in the Cologne conurbation using pine needles as a passive sampler – Part I: magnetic properties. Atmos Environ 38(23): 3781–3792
USEPA (2004) Air Quality Criteria for Particulate Matter Final Report No. EPA 600/P-99/002aF-bF. U.S. Environmental Protection Agency, Washington
USEPA (2009) Final Report: Integrated Science Assessment for Particulate Matter EPA/600/R-08/139F, 2009. U.S. Environmental Protection Agency, Washington
Vaisvalavicius R, Motuzas A, Prosycevas I, Levinskaite L, Zakarauskaite D, Grigaliuniene K, Butkus V (2006) Effect of heavy metals on microbial communities and enzymatic activity in soil column experiment. Arch Agron Soil Sci 52:161–169
van Gardingen PR, Grace J, Jeffree CE (1991) Abrasive damage by wind to the needle surfaces of *Picea sitchensis* (Bong) Carr and *Pinus sylvestris* L. Plant Cell Environ 14(2):185–193
van Heerden PDR, Krüger GHJ, Kilbourn Louw M (2007) Dynamic responses of photosystem II in the Namib Desert shrub, Zygophyllum prismatocarpum, during and after foliar deposition of limestone dust. Environ Pollut 146:34–45
van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fule PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT (2009) Widespread increase of tree mortality rates in the western United States. Science 323(5913):521–524
Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman D (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7(3):737–750
Wagner EC (1939) Effects of certain insecticides and inert materials upon the transpiration rate of bean plants. Plant Physiol 14(4):717–735
Wang J, Cubison MJ, Aiken AC, Jimenez JL, Collins DR (2010) The importance of aerosol mixing state and size-resolved composition on CCN concentration and the variation of the importance with atmospheric aging of aerosols. Atmos Chem Phys 10(15):7267–7283
Wang L, Gong HL, Liao WB, Wang Z (2015) Accumulation of particles on the surface of leaves during leaf expansion. Sci Total Environ 532:420–434
Watmough SA, Hutchinson TC, Dillon PJ (2004) Lead dynamics in the forest floor and mineral soil in south-central Ontario. Biogeochemistry 71:43–68
Weinbaum SA, Neumann PM (1977) Uptake and metabolism of 15N-labeled potassium nitrate by French prune (*Prunus domestica* L.) leaves and the effects of two surfactants. J Am Soc Hortic Sci 102:601–604
Wesely ML, Hicks BB (2000) A review of the current status of knowledge on dry deposition. Atmos Environ 34(12-14):2261–2282
WHO (2013) Health effects of particulate matter, policy implications for countries in Eastern Europe, Caucasus and Central Asia. World Health Organization, WHO Regional Office for Europe, Copenhagen, p 20
Wild E, Dent J, Thomas GO, Jones KC (2006) Visualizing the air-to-leaf transfer and within-leaf movement and distribution of phenanthrene: further studies utilizing two-photon excitation microscopy. Environ Sci Technol 40(3):907–916
Wilson AR, Boyd AEW, Mitchell JG, Greaves WS (1947) Potato haulm destruction with special reference to the use of tar acid compounds. Ann Appl Biol 34(1):1–33
Wiman BLB (1986) Diurnal-variations of aerosol concentrations inside and above a young spruce stand – modeling and measurements. Water Air Soil Pollut 31(1-2):343–348
Wolterbeek B (2002) Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Environ Pollut 120(1):11–21
Wyers GP, Duyzer JH (1997) Micrometeorological measurement of the dry deposition flux of sulphate and nitrate aerosols to coniferous forest. Atmos Environ 31(3):333–343
Wyszkowska J, Boros E, Kucharski J (2007) Effect of interactions between nickel and other heavy metals on the soil microbiological properties. Plant Soil Environ 53:544–552

Wyttenbach A, Tobler L (1998) Effect of surface contamination on results of plant analysis. Commun Soil Sci Plant Anal 29(7-8):809–823

Yang J, Chang YM, Yan PB (2015) Ranking the suitability of common urban tree species for controlling PM2.5 pollution. Atmos Pollut Res 6(2):267–277

Yogui G, Sericano J (2008) Polybrominated diphenyl ether flame retardants in lichens and mosses from King George Island, maritime Antarctica. Chemosphere 73:1589–1593

Yu HB, Remer LA, Chin M, Bian HS, Tan Q, Yuan TL, Zhang Y (2012) Aerosols from overseas rival domestic emissions over North America. Science 337(6094):566–569

Yuangen Y, Campbell CD, Clark L, Camerson CM, Paterson E (2006) Microbial indicators of heavy metal contamination in urban and rural soils. Chemosphere 63:1942–1952

Zalud P, Szakova J, Sysalova J, Tlustos P (2012) Factors influencing uptake of contaminated particulate matter in leafy vegetables. Cent Eur J Biol 7(3):519–530

Zhang YJ, Cremer PS (2010) Chemistry of Hofmeister anions and osmolytes. In: Leone SR, Cremer PS, Groves JT, Johnson MA, Richmond G (eds) Annual review of physical chemistry, vol 61. Annual Reviews, Palo Alto, pp 63–83

Zhang LM, Gong SL, Padro J, Barrie L (2001) A size-segregated particle dry deposition scheme for an atmospheric aerosol module. Atmos Environ 35(3):549–560

Zhang Q, Jimenez JL, Canagaratna MR, Allan JD, Coe H, Ulbrich I, Alfarra MR, Takami A, Middlebrook AM, Sun YL, Dzepina K, Dunlea E, Docherty K, DeCarlo PF, Salcedo D, Onasch T, Jayne JT, Miyoshi T, Shimono A, Hatakeyama S, Takegawa N, Kondo Y, Schneider J, Drewnick F, Borrmann S, Weimer S, Demerjian K, Williams P, Bower K, Bahreini R, Cottrell L, Griffin RJ, Rautiainen J, Sun JY, Zhang YM, Worsnop DR (2007) Ubiquity and dominance of oxygenated species in organic aerosols in anthropogenically-influenced Northern Hemisphere midlatitudes. Geophys Res Lett 34(13)

Solar UV Irradiation-Induced Production of Greenhouse Gases from Plant Surfaces: From Leaf to Earth

Teis Nørgaard Mikkelsen, Dan Bruhn, and Per Ambus

Contents

T.N. Mikkelsen (✉)
Department of Environmental Engineering, Technical University of Denmark, Building 115, DK – 2800 Kgs., Lyngby, Denmark
e-mail: temi@env.dtu.dk

D. Bruhn
Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, DK-9220 Aalborg East, Denmark
e-mail: db@bio.aau.dk

P. Ambus
Department of Geosciences and Natural Resource Management, University of Copenhagen, Øster Voldgade 10, DK-1350 Copenhagen, Denmark
e-mail: peam@ign.ku.dk

Progress in Botany (2017) 78: 407–438, DOI 10.1007/124_2016_10,
Published online: 2 June 2016

Abstract During the past few decades it has been documented that the ultra-violet (UV) component of natural sunlight alone or in combination with visible light can instantaneously stimulate aerobic plant production of a range of important trace gases: CH_4, CO_2, CO, short-chain hydrocarbons/ non-methane volatile organic compounds (NMVOC), NO_x and N_2O. This gas production, near or at the plant surface, is a new discovery and is normally not included in emission budgets (e.g. by the Intergovernmental Panel on Climate Change, IPCC) due to a lack of information with respect to validation and upscaling. For CH_4 it is known that the light dose controls emission under ambient and artificial light conditions, but the atmospheric gas composition and other environmental factors can influence gas production as well. Several plant components, including pectin and leaf wax, have been suggested as a precursor for CH_4 production, but underlying mechanisms are not fully known. For other gases such generating processes have not been established yet and mechanisms remain hypothetical. Field measurements of UV-induced emissions of the gases under natural light conditions are scarce. Therefore, realistic upscaling to the ecosystem level is uncertain for all gases. Nevertheless, based on empirical response curves, we propose the first global upscaling of UV-induced N_2O and CO to illustrate emission ranges from a global perspective and as a contribution to an ongoing quantification process. When scaled to the global level, the UV-induced emission of CO by vegetation surfaces amounts to up to 22 Tg yr^{-1}, which equals 11–44% of all the natural terrestrial plant sources accounted for so far. The total light-driven N_2O emissions amount to 0.65–0.78 Tg yr^{-1}, which equals 7–24% of the natural terrestrial source strength accounted for (range 3.3–9 Tg N yr^{-1}). In this review, we summarize current knowledge, based on experimental work with sunlight and artificial light, and estimate potential emission ranges and uncertainties, placing the available data into perspective. We discuss the state of the art in proposed mechanisms, precursors and environmental relationships, we consider the relevance of measured emission rates, and we also suggest a range of future research topics. Furthermore we propose and describe methods and techniques that can be used for future research.

1 Introduction

For decades it has been recognized that sunlight plays significant roles in atmospheric chemistry and that the UV component is the driving force for tropospheric photochemical processes. For instance, UV-photolysis of ozone generates excited state oxygen atoms, which react with water vapour to constitute the primary source of hydroxyl radical (OH). OH is the major component for the overall oxidising capacity of the troposphere (Isaksen et al. 2009). Lately, a number of trace gases are reported to be released by plants in instantaneous responses to UV-radiation, such as methane (CH_4) (Bruhn et al. 2007, 2009, 2012, 2014a; Röckmann et al. 2007; Vigano et al. 2007, 2008, 2009; McLeod et al. 2008; McLeod and Keppler 2010; Messenger et al. 2009; Fraser et al. 2015), carbon monoxide (CO) (Derendorp et al. 2011a; Bruhn et al. 2013), short-chain hydrocarbons (Derendorp et al. 2011b; Fraser et al. 2015), mono-nitrogen oxides (NO_x and NO_y) (Hari et al. 2003; Raivonen et al. 2006, 2009) and nitrous oxide (N_2O) (Bruhn et al. 2014b).

These UV-driven gas productions are in principle newly revealed terrestrial sources that remain to be considered in greenhouse gas accounting. Production of all gases stimulated by UV involves emissions at relatively low rates, and the group of gases are diverse and have different impacts and interactions with the atmosphere. However, several of the gases are potent greenhouse gases or interact with the turnover of atmospheric greenhouse gases.

We describe current knowledge of methods and techniques for measuring these gases, with a particular focus on the special requirements needed to accomplish flux measurements under controlled UV-exposures and at generally very low rates. The distinction between laboratory and field measurements is addressed with respect to techniques and deductions. We also report on current understanding of the possible mechanisms and sources behind these gas productions, comment on current upscaling attempts, and present the first upscaling and quantification of UV stimulated CO and N_2O emissions. Finally, we highlight the perspectives of the newly discovered UV stimulated gas sources with respect to research needs and impact on current research.

2 Light-Induced Gases at the Plant Surface

Here we summarize the important features of most of the gases that are known to be formed at the plant surface during exposure to UV light. The gases can be divided into two groups: (1) well-mixed greenhouse gases (CO_2, CH_4 and N_2O) and (2) short-lived gases (CO, NO_x and non-methane volatile organic compounds (NMVOC)).

2.1 Well-Mixed Greenhouse Gases (CO_2, CH_4 and N_2O)

The atmospheric concentrations of the greenhouse gases carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have all increased since 1750 due to human activity. In 2011 the concentrations of these greenhouse gases were 391 ppm, 1803 ppb and 324 ppb, and exceeded preindustrial levels by about 40%, 150% and 20%, respectively (Table 1). Concentrations of CO_2, CH_4, and N_2O now substantially exceed the highest concentrations recorded in ice cores during the past 800,000 years (Masson-Delmotte et al. 2013). The mean rates of increase in atmospheric concentrations over the past century are unprecedented in the last 22,000 years (IPCC 2013). In Table 1 we present the Global Warming Potential (GWP) that integrates radiative forcing (RF) out to a particular time horizon, in this case 100 years. The GWP can be interpreted as an index of the total energy added to the climate system by a component in question relative to that added by CO_2 (Myhre et al. 2013). There are multiple sources of all three gases, which can be divided into two main groups: anthropogenic and natural (Table 1). Quantification of the various source strengths from both groups remain uncertain and global budgets remain unclear (Ciais et al. 2013).

The main anthropogenic CO_2 sources are burning of fossil fuels (coal, oil and gas), deforestation and production of cement (Ciais et al. 2013). The removal of anthropogenic CO_2 from the atmosphere by natural processes will take a few hundred thousand years (Ciais et al. 2013). The natural CO_2 sources are autotrophic and heterotrophic respiration, decomposition of plant tissues (litter and soil carbon that is released back into the atmosphere) and additional disturbance processes (e.g. natural fires). The natural source strength is 20 times higher than the anthropogenic emission, but is counterbalanced by natural CO_2 uptake from the atmosphere by plant photosynthesis (Beer et al. 2010).

Massive increases in the number of domestic ruminants, natural gas extraction and use, expansion of rice paddy agriculture and establishment of urban landfills and waste dumps represent the dominant anthropogenic CH_4 sources (Stocker et al. 2013). Wetlands are the dominant natural source of atmospheric CH_4 (EPA 2010). During the last 2 decades, natural sources of CH_4 have accounted for 35–50% of the decadal mean global emissions (Ciais et al. 2013).

The anthropogenic N_2O sources are primarily agricultural, and the anthropogenic part accounts for approximately 40% of the total emission (Ciais et al. 2013). Natural sources are constituted by upland soils and riparian areas together with oceans, estuaries and rivers (EPA 2010). Human-induced perturbations of the nitrogen cycle, in addition to interactions with CO_2 sources and sinks, affect emissions of N_2O both on land and from the ocean (Stocker et al. 2013). It is likely that N_2O emissions from soils will increase due to the increased demand for feed/

Table 1 Important features of gases that are known to be formed at the plant surface during exposure to UV light

							Source				
Gas	Atm. Conc.	References	Lifetime (years)	References	GWP 100 year	References	Antropogenic	References	Natural	References	Units
CH_4	1803 ppb	Hartmann et al. (2013)	9.1	Hartmann et al. (2013)	28	Myhre et al. (2013)	354 ± 45	Ciais et al. (2013)	202 ± 35	Ciais et al. (2013)	Tg CH_4 yr^{-1}
CO_2	391 ppb	Hartmann et al. (2013)	n.a.	–	1	Myhre et al. (2013)	8.3 ± 0.7	Ciais, et al. (2013)	n.a.	Ciais et al. (2013)	PgC yr^{-1}
N_2O	324 ppb	Hartmann et al. (2013)	131	Hartmann et al. (2013)	265	Myhre et al. (2013)	6.9 (2.7–11.1)	Ciais, et al. (2013)	11.0 (5.4–19.6)	Ciais et al. (2013)	TgN yr^{-1}
CO	80 ppb	IPCC (2001)	Months	Myhre et al. (2013)	5.3[b] ± 2.3	Shindell et al. (2009)	608	Ciais, et al. (2013)	50–200[c]	Tarr et al. (1995)	TgC yr^{-1}
NO_x	5–999 ppb	IPCC (2001)	Hours	Hartmann et al. (2013)	−159[b] ± 79	Shindell et al. (2009)	37.5	Dentener et al. (2006)	11.3	Dentener et al. (2006)	TgN yr^{-1}
NMVOC	n.a.	–	Hours – Months	Hartmann et al. (2013)	n.a.	–	126.9	Boucher et al. (2013)	440–720[a]	Boucher et al. (2013)	TgC yr^{-1}

[a]Only isoprene and monoterpenes
[b]Direct and indirect aerosol effects included
[c]Only from plants

food and the reliance of agriculture on nitrogen fertilizers. Climate warming will likely amplify agricultural and natural terrestrial N_2O sources (Ciais et al. 2013).

2.2 Short-Lived Gases (CO, NO_x and NMVOC)

Emissions of CO, NMVOCs and NO_x ($NO + NO_2$) do not have a direct effect on RF, but affect climate indirectly as precursors to tropospheric O_3 and aerosol formation, and their impacts on hydroxyl-concentrations and CH_4 lifetime. NMVOCs include aliphatic, aromatic and oxygenated hydrocarbons (e.g. aldehydes, alcohols and organic acids), and have atmospheric lifetimes ranging from hours to months. Global coverage of NMVOC measurements is poor, except for a few compounds (Hartmann et al. 2013). Emissions of CO and NMVOC are virtually certain to have induced a positive RF via production of the climatic drivers CO_2, CH_4 and O_3, while emissions of NO_x are likely to have induced a net negative RF (Table 1; IPCC 2013). With its lifetime of 2–3 months, the effect of CO emission is less dependent on location than is the case for NO_X (Myhre et al 2013). Due to their short atmospheric lifetime (hours), NO_x concentrations are highly variable in time and space. Solomon et al. (2007) described the potential of satellite observations of NO_2 to verify and improve NO_x emission inventories and their trends, and reported NO_2 increases of 50% over the industrial areas of China from 1996 to 2004. An extension of this analysis reveals increases between of 1.7× and 3.2× over parts of China, while over Europe and the USA NO_2 has decreased by 30–50% between 1996 and 2010 (Hilboll et al. 2013).

The major sources of atmospheric CO are in situ production by oxidation of hydrocarbons (mostly CH_4 and isoprene) and direct emission resulting from incomplete combustion of biomass and fossil fuels. The anthropogenic CO emission is estimated to be 608 TgC yr^{-1} (Table 1); natural sources have been estimated to account for up to half of the global CO emissions (Khalil and Rasmussen 1990), and direct emissions from plants are estimated to be 50–200 TgC yr^{-1} (Tarr et al. 1995). An analysis of Measurements of Pollutants in the Troposphere (MOPITT) and Atmospheric Infrared Sounder (AIRS) satellite data suggest a clear and consistent decline of CO columns for 2002–2010 over a number of polluted regions in Europe, North America and Asia, with a global trend of about -1% yr^{-1} (Yurganov et al. 2010; Fortems-Cheiney et al. 2011; Worden et al. 2013; Hartmann et al. 2013).

Reports on trends in a range of NMVOCs generally indicate a decline over urban and rural regions of North America and Europe, on the order of a few percent to more than 10% yr^{-1} (Hartmann et al. 2013). The anthropogenic emission is between 15 and 22% of the total NMVOC emissions (Table 1).•

3 Methods and Techniques

UV-induced gaseous emissions from specific substances, plant organs, whole plants or whole ecosystems have classically been studied under controlled environmental conditions by employment of sealed enclosures ranging in complexity from simple commercial test tubes to highly advanced plant cuvettes (plant parts) or whole chamber enclosures (plants or plant and soil communities).

Generally the studied gas components are emitted at very low rates from plant surfaces, and in order to achieve detectable levels of gas accumulation the use of enclosures is required. A static enclosure that is operated by manual sampling or connected in a closed gas sampling loop to the analyzer (e.g. Bruhn et al. 2009; 2014b) provides high analytical sensitivity for determining changes in gas mixing ratios, but may require appropriate meticulous techniques to control undesired changes in other gas components, e.g. moisture, CO_2 and O_3. Alternatively an open flow-through design may be used whereby the enclosure is continuously purged with ambient- or zero-air (e.g. Vigano et al. 2008). Meanwhile, deployment of enclosures is associated with multiple challenges that may affect the experimental conditions in uncontrolled and undesired directions, leading to experimental flaws and artefacts.

Environmental controls of temperature, humidity and air composition are crucial for work with biological materials in enclosures and to reveal important abiotic controllers for the investigated processes. Complications by uncontrolled changes in the environment may further be augmented by the fact that relatively long enclosure times are needed in order to uncover low reaction constants. It is beyond the scope of this article to provide a detailed protocol of principles and methodologies for the use of sealed enclosures to study gas exchange from surfaces. Instead, the reader is referred to literature providing detailed descriptions on flux-chamber design and applications (e.g. de Klein and Harvey 2015; Altimir et al. 2002; Skiba et al. 1992). In Appendix 1, we expand methods and techniques with respect to temperature, chamber material, surface reactions and reactive species, analysis of gas mixing ratios and light sources.

4 Mechanisms, Sources and Emissions

Sunlight can induce trace gas emission from plants by several mechanisms from different precursors. Here we focus on direct effects of UV-radiation on trace gas emission from terrestrial plant surfaces as these are largely ignored in global budgets. Indirect effects of UV on trace gas emission and other physiological functions are reviewed elsewhere (Caldwell et al. 1995, 1999; Björn 1996; Rozema et al. 1997; Bruhn et al. 2012).

4.1 UV-Radiation Penetration Through the Canopy

Leaves throughout a canopy are affected not only by the PAR and IR-spectrum of solar radiation but also by UV-radiation. Leaves do not transmit UV-radiation but reflect some (up to ca. 6%) UV-radiation (Grant 1997), and the high energy light still penetrates the canopy. Canopy structure, leaf area index (LAI), the extent of direct and diffuse radiation all influence UV penetration through the canopy (Brown et al. 1994; Shulski et al. 2004) and leaves. The penetration by UV-B varies less than that of PAR with leaf inclination due to the higher diffuse component of UV light than PAR (Caldwell 1981; Deckmyn et al. 2001). For example, canopy UV-B transmittance (τ) may vary with LAI between $\tau = \exp(-1.01\ \mathrm{LAI})$ and $\tau = \exp(-0.17(\mathrm{LAI} - 1))$ depending on species and degree of clear sky (Shulski et al. 2004).

4.2 UV-Radiation Penetration Through the Leaf

UV-B absorption of adaxial leaf cuticles caused by pigments (chromophores, e.g. flavonoids and other phenolic compounds covalently bound to cutin) ranges from very high in some species with $<3\%$ transmittance to $>64\%$ transmittance in other species (Baur et al. 1998). The highest absorption is typically in evergreen species (Baur et al. 1998). Some evergreen species also contain fluorophores in the cuticular wax, which may convert solar UV irradiation into blue light that can be harnessed for photosynthesis. However, the epicuticular wax per se (fatty acyl chains) can also absorb significant amounts of UV-B and thus protect against UV-B (Long et al. 2003). Further, trichome layers also protect against UV-B (Karabourniotis and Bornman 1999). UV-A radiation penetrates deeper into the mesophyll than UV-B in all examined species (Liakoura et al. 2003). Whereas pigment changes in leaves during seasonal changes result in varying degrees of reflectance and transmittance of PAR and IR, there appear to be no seasonal changes in degrees of reflectance and transmittance in the UV region (Yoshimura et al. 2010), despite strong seasonal fluctuations in the leaf concentration of UV-absorbing compounds (Liakoura et al. 2001).

4.3 UV-Photochemistry Mechanism in and on Plant Surfaces

Photochemical reactions are typically complex. UV-radiation can excite various molecules and this may result in a change in molecular orbital occupancy, an increase in energy, and changes in local bonding and charge distribution. Upon return to a lower energy state of the molecules, the released energy or the energy

transfer to a neighbouring molecule triggers reactions almost instantaneously. The radiation energy is inversely proportional to the wavelength. Thus, UV-B radiation causes the cleavage of more chemical bonds than does, for instance, UV-A and PAR. Following this, a multitude of radical reactions may take place and thus greatly increase the quantum yield.

From the lab there is plenty of evidence that artificial UV-radiation induces an almost instantaneous, i.e. photochemically induced, trace gas emission from plants or plant components, such as carbon-based molecules including CH_4 (McLeod et al. 2008; Keppler et al. 2008; Vigano et al. 2008, 2009; Bruhn et al. 2009, 2014a, b; Messenger et al. 2009; Fraser et al. 2015), CO (Tarr et al. 1995; Schade et al. 1999; Brandt et al. 2009; Derendorp et al. 2011a; Bruhn et al. 2013), CO_2 (McLeod et al. 2008), and hydrocarbons (McLeod et al. 2008; Derendorp et al. 2011b; Fraser et al. 2015) and nitrogen-based molecules including N_2O (Bruhn et al. 2014b) and NO_x/NO_y (Hari et al. 2003; Raivonen et al. 2006, 2009). Conversely, from the field distinct evidence that natural UV-radiation induces an almost instantaneous, i.e. photochemically induced, trace gas emission from plants is far less common, but is documented for CO (Bruhn et al. 2013), N_2O (Bruhn et al. 2014b) and NO_x/NO_y (Hari et al. 2003; Raivonen et al. 2006, 2009). With respect to CH_4 and isoprene, however, there are only indirect indications that natural UV-radiation induces an almost instantaneous emission (Keppler et al. 2006; Tiiva et al. 2007).

4.3.1 Action Spectra

Additional evidence pointing towards direct UV-induced trace gas emission from plants or plant components being an abiotic rather than a biotic process is that in most cases the higher energy UV-B results in higher emission rates than does UV-A at a given irradiance intensity. This is observed in the lab for plant emission of CH_4 (McLeod et al. 2008; Bruhn et al. 2009), CO (Tarr et al. 1995; Schade et al. 1999; Bruhn et al. 2013) and N_2O (Bruhn et al. 2014b). Only McLeod et al. (2008) have conducted a detailed analysis of the action spectrum of direct UV-induced trace gas emission, finding the CH_4 efflux rate from citrus pectin-impregnated glass fibre sheets to scale linearly with an idealized spectral UV weighting function. The function weighted CH4 efflux is an order of magnitude lower for each 80 nm increase in wavelength. This spectral weighting function differs from other processes where metabolic activity intrinsically is involved, such as the erythema function (see further discussion in McLeod et al. 2008).

The crude indication of an action spectrum of CH_4 emission from pectin in aqueous solution (Fig. 1) resembles the detailed action spectrum of citrus pectin-impregnated glass fibre sheets (Fig. 1). Importantly, though, the crude indication of action spectra of trace gas emission appears to be highly dependent on the precursor as well as the condition of that precursor (Fig. 1). For example, leaves of two *Brassica* species appear to emit relatively far more CH_4 in response to UV-B than to UV-A, as would be expected from the results of a single component such as pectin.

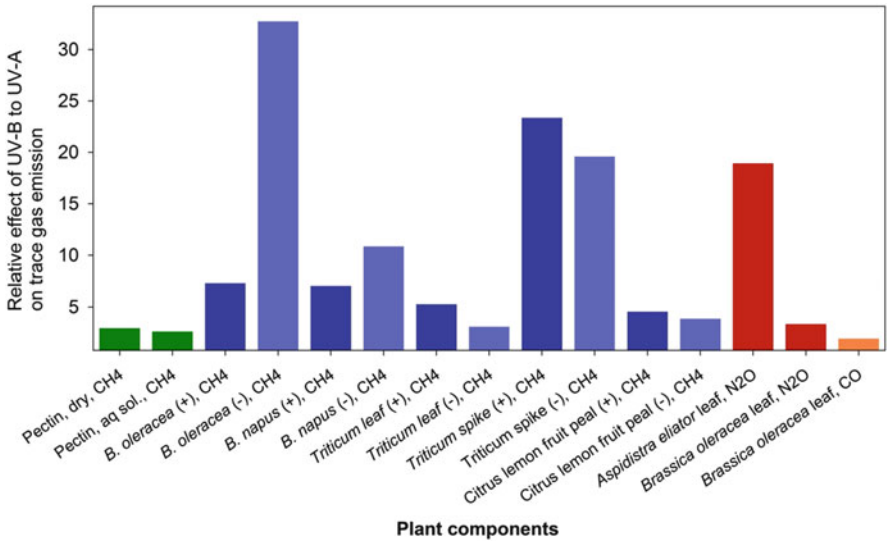

Fig. 1 Relative effect of UV-B to UV-A on trace gas emission from different plant components and surfaces. The relative effect of UV-B to UV-A is here defined as emission rate in response to UV-B (312 nm) relative to that in response to UV-A (375 nm) when adjusted according to irradiance intensity. CH_4: Pectin, dry (McLeod et al. 2008), Pectin, aq. sol. (Bruhn et al. 2009), *Brassica oleracea* leaves, *Brassica napus* leaves, *Triticum* leaves, *Triticum ears*, and *citrus lemon* fruit peal (Rolsted MMM, Bruhn D, Mikkelsen TN, Ambus P, unpublished); N_2O: Bruhn et al. (2014b); CO: Bruhn et al. (2013). (+) designates that natural surface wax is present, (−) designates that natural surface wax is either removed mechanically or not present at all (in the case of Triticum spikes). *Green* = CH_4 from pectin, *Dark blue* = CH_4 from leaf with nat. wax., *Light blue* = CH_4 from leaf without nat. wax, *Red* = N_2O, *Orange* = CO

Furthermore, in the case of the leaves of the two *Brassica* species, it appears that the removal of leaf surface wax results in a relatively higher effect of UV-B to that of UV-A in terms of CH_4 emission. This may reflect a combination of UV-A radiation penetrating deeper into the mesophyll than UV-B in all examined species (Liakoura et al. 2003) when the wax is intact. However this depends on species (Baur et al. 1998) and perhaps several plant components (incl. surface wax per se) are potential precursors to UV-induced trace gas emission (Table 2).

4.3.2 UV-Response Functions

UV-induced trace gas emission is commonly reported to exhibit a near linear response function from both intact organs/tissues as well as from single plant components (McLeod et al. 2008; Bruhn et al. 2009, 2013, 2014a; Derendorp et al. 2011a, b). This, together with the fact that direct UV-induced trace gas emission often occurs at constant rates over long periods (Bruhn et al. 2009), strongly indicates photochemical reactions from plentiful precursors.

Table 2 Potential precursors for trace gas formation in direct response to UV-radiation examined and/or suggested in the literature

Gas	Suggested source (plant or surface deposit)	References	Positive $[O_2]$ or O-radicals-dependence	References
CH_4	Pectin (methyl groups)	Keppler et al. (2006), Keppler et al. (2008), Vigano et al. (2008), Vigano et al. (2009), McLeod et al. (2008), Bruhn et al. (2009) and Messenger et al. (2009)	Yes	Keppler et al. (2008), Messenger et al. (2009) and Messenger et al. (2009)
	Wax (15-nonacosanone & 2-hexadecanone)	Bruhn et al. (2014a)	Yes	Bruhn et al. (2014a)
	Cellulose	Vigano et al. (2008), Vigano et al. (2009)	[a]	
	Lignin	Vigano et al. (2008), Vigano et al. (2009)	[a]	
	Methionine	Bruhn et al. (2012)	[a]	
	Ascorbic acid	Althoff et al. (2010)	Yes	Althoff et al. (2010)
CO	Cellulose	Schade and Crutzen (1999)	Yes	Tarr et al. (1995), Yonemura et al. (1999) and Derendorp et al. (2011a, b)
CO_2	Lignin	Rozema et al. (1997), Day et al. (2007)		
C2–C5 hydrocarbons	[a]	Schade and Crutzen (1999), Fraser et al. (2015)	Yes	Schade and Crutzen (1999)
N_2O	Wax Mesophyll Surface bound N_2O NO_3^- NH_4NO_3	Bruhn et al. (2014b) Bruhn et al. (2014b) Kim et al. (2010) Rubasinghege and Grassian (2009) Bruhn et al. (2014b), Rubasinghege et al. (2011)	Yes	Rubasinghege and Grassian (2009), Prasad (2002) and Prasad and Zipf (2008)
NOx,y	Needle surfaces, HNO_3 or NO_3^-	Hari et al. (2003), Raivonen et al. (2006) and Raivonen et al. (2009)		

[a]Equals unknown

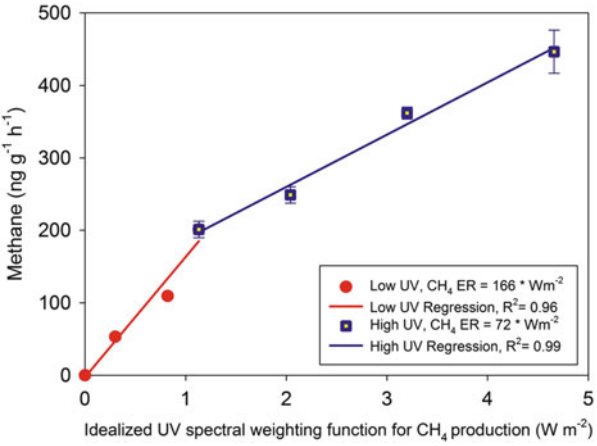

Fig. 2 Pectin CH_4 production (ng g^{-1} h^{-1}) as a function of spectrally weighted UV-intensity (W m^{-2}). The CH_4 production decays 1 decade when the spectrum increases 80 nm, e.g. the CH_4 emission is 1 at 300 nm and 0.1 at 380 nm: spectral weighting function from Fig. 1a and data from Table 1 in McLeod et al. (2008). Data is from the UV313 lamp filtered with 125-μm cellulose diacetate which filters UV wavelengths <290 nm. Linear regressions functions shown in box. Values are means of three replicates and standard errors are less than symbol size except where visible. *ER* emission rate

However, further examination (Fig. 2) of the only published dataset on UV-induced pectin-based CH_4 productions at very low UV intensities indicates release to be more responsive (by a factor of 2) than under higher intensities. The role of self-shading in this context remains to be fully investigated. Thus, even for simple linear responses in UV-induced trace gas emission, there is reason to believe that the underlying photochemical mechanisms are complex. Only Raivonen et al. (2009) have reported on an analysis of the potential linearity of the direct response function of any trace gas (NO_x) emission to natural temporal variation in UV intensity (UV-A).

4.3.3 Temperature Interactions

In most cases trace gas emission from plant material is also observed in darkness (PAR and UV absent) and with a positive response to temperature increases, although with sensitives too low to infer underlying abiotic processes (Derendorp et al. 2011a). However, reliable indications are lacking of interactions between the photochemical reactions and temperature with respect to trace gas emission from plant material.

4.3.4 [O_2] or O-Radicals Dependency

It caused quite a surprise (Kirschbaum et al. 2006, 2007) when Keppler et al. (2006) first reported an aerobic plant CH_4 emission in response to solar radiation. In all examined cases of UV-induced trace gas emission from plant materials, there is a positive dependency on [O_2] or O-radicals (Table 2). Further, this confirms a combination of instantaneous photochemical reactions and subsequent radical reactions in most cases. UV-radiation can therefore in theory act as a stimulus via an increased reactive oxygen species (ROS) reaction, and consequently the actual precursor of the emitted gas does not itself need to be a good absorber of UV-radiation for the process to occur. However, Lee et al. (2012) provide evidence that photo-oxidation may only be one of several photo degradation processes, as they observed the process occurring in the absence of O_2. They speculated that the direct breakdown of chemical groups such as carboxyl, carbonyl and methoxyl groups may result in CO_2, CO and CH_4 release.

4.4 Precursors

In Table 2 we have compiled current knowledge on the potential precursors for trace gas formation in *direct* response to UV-radiation examined and/or suggested in the literature. For the C-based trace gases there are many structural components, which may act as precursors. In contrast, for the N-based trace gases the potential precursors appear to be more dependent on surface deposited molecules (Table 2).

It seems to be the consensus that the polysaccharide pectin is the most important precursor for UV-induced plant CH_4 emission due to its content of methyl groups (Keppler et al. 2006; Keppler et al. 2008; Vigano et al. 2008, 2009; McLeod et al. 2008; Bruhn et al. 2009; Messenger et al. 2009; Bloom et al. 2010; Fraser et al. 2015). Whereas we agree that pectin is one of the potential precursors, we are currently not convinced that it necessarily is the most important one. Pectin is laid down in primary plant cell walls. For pectin to be reached by UV irradiation in nature, UV irradiation has to first penetrate the outer surface wax layer naturally occurring on plant organs. In Fig. 3 we show different pairs of plant organs with natural surface wax or without surface wax, respectively.

As described in Sect. 4.2, UV irradiation is to some degree screened at the surface of plant tissues and organs. Thus, an approach to illustrate whether pectin is the most important precursor for CH_4 formation is to measure and compare the UV-induced CH_4 production from material of pairs of plant organs with more or less natural UV-screening surface wax (Fig. 3) as well as in samples of plant leaves with or without the natural wax removed. We did this and found that UV-B induced CH_4 formation in both *citrus limon* peels and *Cydonia oblonga* peels as well as in *Brassica oleracea capitata f. alba* leaves was halved upon removal of the surface wax. This evidently contradicts the notion that in nature pectin is the most

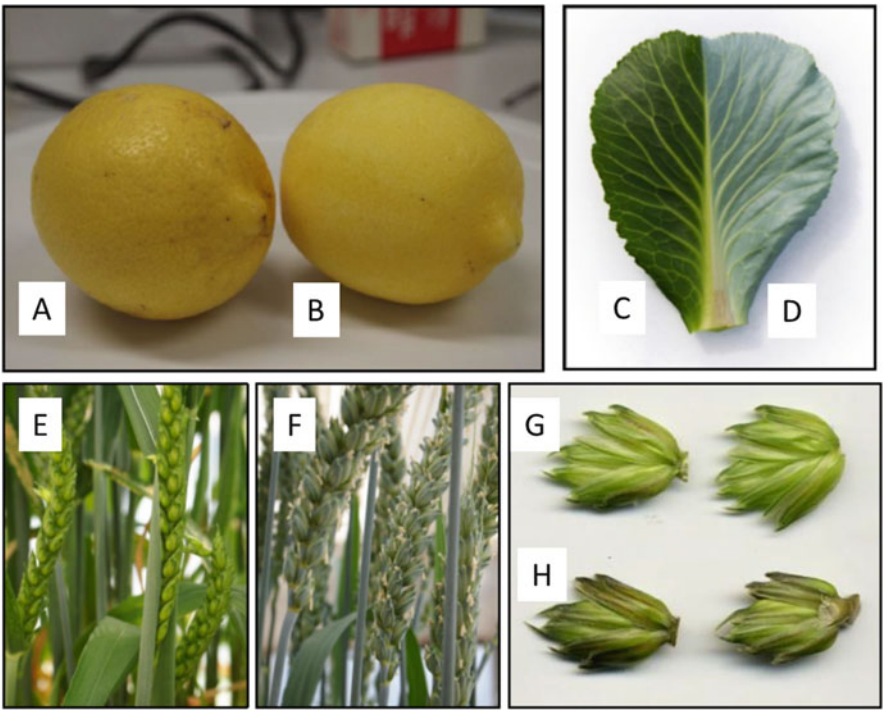

Fig. 3 Different pairs of plant material with different amounts of surface wax. (*A+B*) Organically grown *Citrus limon* fruit with natural surface wax (*A*) or with wax mechanically removed (*B*) by gentle scrubbing with a kitchen sponge. (*C+D*) *Brassica oleracea capitata f. alba* leaf with surface wax removed (*C*) or intact (*D*). (*E+F*) *Triticum aestivum* "Tähti" (*E*, with almost no surface wax) and *Triticum aestivum* "Vinjett" (*F*, with normal surface wax) ears. (*G+H*) Detailed parts of *Triticum aestivum* "Tähti" (*G*) and *Triticum aestivum* "Vinjett" (*H*) ears. Rolsted MMM, Bruhn D, Mikkelsen TN, Ambus P, unpublished. Photos: Rolsted MMM

important precursor for UV-induced CH_4 formation, especially because both *citrus limon* peels and *Cydonia oblonga* peels are chosen for industrial pectin extraction due to the high pectin content. Conversely, there was no difference in UV-B induced CH_4 formation between *Triticum aestivum* "Tähti" (with almost no surface wax) ears and *Triticum aestivum* "Vinjett" (with normal surface wax) ears, or between leaves of *Triticum aestivum* when irradiated from either adaxial side (with almost no wax) or from the abaxial side (with much natural surface wax). Removal of surface wax should, in theory, increase UV exposure of the pectin, but in no case did wax removal result in a higher rate of CH_4 formation. Furthermore, we recently demonstrated that the surface wax per se is resulting in CH_4 formation upon UV irradiation (Bruhn et al. 2014a). In conclusion, we are still far from having a clear understanding of the relative contribution of different precursors in any UV-induced trace gas formation and emission. Additionally, given that UV-radiation of different wavelengths reaches different depths in the plant tissue

(Liakoura et al. 2003) in a species-dependent manner (Baur et al. 1998), it seems most likely that each of the different precursors (Table 2) are affected differently with respect to wavelengths of UV irradiation. Consequently, we cannot with any certainty extrapolate an action spectrum for one precursor to that of an entire tissue or organ (Fig. 1).

5 Upscaling

When an unaccounted natural source is discovered there is an urgent demand for extrapolating observed rates to a global scale, so the magnitude of the new source can be put into perspective. However, if mechanisms are unknown, there is a high risk in an upscaling exercise, because driving forces and controlling factors unintentionally might be ignored, leading to the wrong outcome. On the other hand, if some factors are known, response curves can be constructed and upscaling can be conducted under defined premises, even though there are still unknown factors. Results can then be treated as a platform for understanding and as a contribution to an ongoing knowledge improvement process. Based on current knowledge, we suggest a simple global upscaling and source strength for sunlight-induced emission of the gases CH_4, CO and N_2O at the plant surface.

5.1 Upscaling of Methane, CH_4

The discovery of aerobic CH_4 emissions from plants became breaking news in 2006 (Keppler et al. 2006), mainly because their global upscaling suggested a source strength of 62–236 Tg yr^{-1}, which represented approximately 10–40% of the annual total of methane entering the modern atmosphere, and approximately 30–100% of annual methane entering the preindustrial (0–1700 AD) atmosphere (Ferretti et al. 2007). Four independent research groups subsequently revised the global magnitude of this potential CH_4 source by different approaches, and jointly suggested emissions in the lower end compared to the pioneering study by Keppler et al. (2006). Based on carbon stable isotope analysis (Ferretti et al. 2007), standing leaf biomass (Parsons et al. 2006), leaf-mass-based estimation and photosynthesis-based estimation (Kirschbaum et al. 2006), and extrapolation from initially reported chamber measurements (Butenhoff and Khalil 2007), aerobic CH_4 emissions from vegetation were respectively estimated at 0–176 Tg yr^{-1}, 42 Tg yr^{-1}, 10–60 Tg yr^{-1} and 20–69 Tg yr^{-1}. None of the studies revealed underlying mechanisms for aerobic CH_4 emission.

At the American Geophysical Union (AGU) fall meeting in 2007, three groups presented a major driving factor, UV-radiation, for the aerobic CH_4 emission (Bruhn et al. 2007; Röckmann et al. 2007; Vigano et al. 2007), and in the following years UV generated CH_4 emission was confirmed in several publications (Vigano

et al. 2008, 2009; McLeod et al. 2008; Keppler et al. 2008; Bruhn et al 2009; Messenger et al. 2009). McLeod and Keppler (2010) concluded in a review that the proposed formation of CH_4 under aerobic conditions in plants is robust, but the magnitude and significance for the global CH_4 budget remained unresolved.

After the discovery of UV as a driving factor, only one group has tried to upscale aerobic plant generated CH_4; Bloom et al. (2010) provided a putatively low global estimate of 0.2–1.0 Tg y^{-1} plant-produced CH_4. The upscaling was only based on UV-induced CH_4 emission measured from purified pectin. Bloom et al. (2010) assumed that UV-induced CH_4 emission measured in purified pectin is representative of UV-induced leaf CH_4 emission when taking leaf pectin content into account. However, we believe that there is good evidence in the literature to indicate that this is not the case, since Vigano et al. (2008), for example, showed that, at a certain UV irradiation, the CH_4 emission by commercially purified pectin was ca. 80 ng CH4 g^{-1} DW h^{-1}, whereas that of dried perennial ryegrass leaf was almost threefold higher at ca. 200 ng CH_4 g^{-1} DW h^{-1} – see Bruhn et al (2012) for further discussions. Therefore, the current upscaling by Bloom et al. (2010) must be seen as a preliminary attempt to evaluate global significance from the basis of limited information, and it is important to gain more knowledge for modelling of the UV driven CH_4 from plants at a global level. We suggest that future modelling of the UV-driven CH_4 from plants must include data obtained under field conditions with respect to plant growth and development, and exposure to natural sunlight. Such data are currently not available, and therefore methane upscaling is not included in this review.

5.2 Upscaling of Carbon Monoxide, CO

All natural terrestrial direct CO emissions, in the range of 50–200 Tg CO yr^{-1}, have hitherto been ascribed by the IPCC (1995, 2001) to photo-induced CO emission by living plants (cf. Tarr et al. 1995). However, in studies on underlying photo-induced CO emission by living plants (Seiler and Giehl 1977; Seiler et al. 1978), which were incorporated into global CO budgets in the early IPCC assessment reports (IPCC 1995, 2001), the UV component of (sun)light was not considered (Bruhn et al. 2013). Therefore, we still await a proper global estimate of UV radiance-induced CO emission by living vegetation. Bruhn et al. (2013) provides the first in situ measurements of ecosystem CO emission by living plants in response to natural solar UV irradiation. Importantly, Bruhn et al. (2013) find that in the studied natural grass field the photo-induced CO emission due to natural solar UV-radiation is more than half of the value of that due to total solar spectrum at the Earth's surface. This may imply that the previous global estimate of photo-induced CO emission from living plants of 50–200 Tg CO yr^{-1} (cf. Tarr et al. 1995) should perhaps be doubled. Thus, future global budgets need to include CO emission caused by natural UV irradiance.

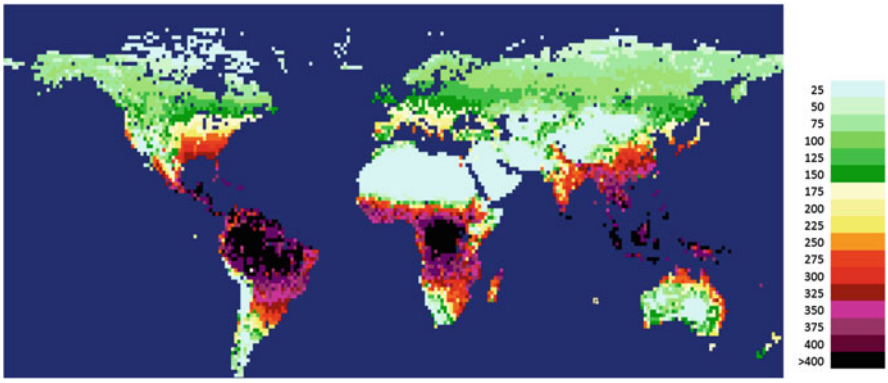

Fig. 4 Estimated annual global CO emissions (mg CO m^{-2}) from terrestrial vegetation surfaces induced by temperature and natural UV-radiation

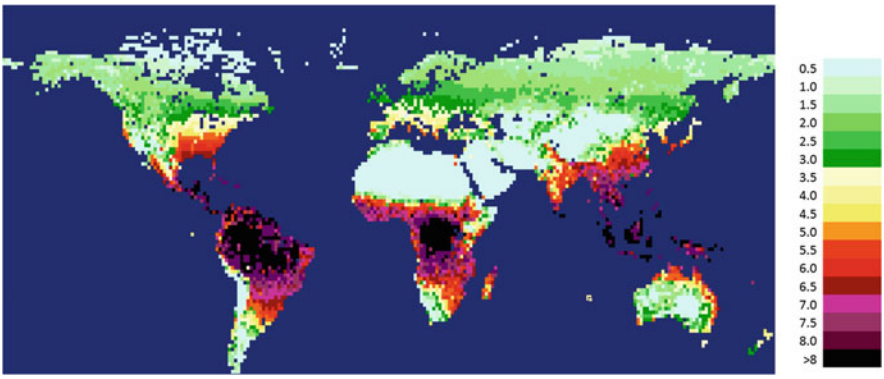

Fig. 5 Estimated annual global N_2O emissions (mg N_2O m^{-2}) from terrestrial vegetation surfaces, induced by temperature and natural UV-radiation

Here, we use the results from Bruhn et al. (2013), and estimate the global UV-driven CO emissions. The upscaling is based on in situ ecosystem–atmosphere CO exchange measurements from natural vegetation and under ambient UV-B conditions in September and October of 2011 at DTU Risø campus (55°41'12"N, 12°05'52"E) in combination with laboratory experiments with artificial UV (Fig. 4). For materials and methods, see Bruhn et al. (2013), and for the upscaling procedure, see Appendices 2 and 3. There is substantial geographical variation in source strength (Fig. 4), which is mostly caused by the geographical variation in surface UV-radiation intensity, similar to the responsiveness of N_2O (Fig. 5). The emission of CO in response to the UV-component of natural solar radiation was also evident at the ecosystem scale. When scaled to the global level, the UV-induced emission of CO by vegetation surfaces amounts up to 22 Tg yr^{-1}, which equals 11–44% of all the natural terrestrial living plant sources hitherto accounted for, which range between 50 and 200 Tg CO yr^{-1} (IPCC 1995, 2001; Tarr et al. 1995).

5.3 Upscaling of Nitrous Oxide, N_2O

In order to evaluate the global significance of our new discovery of a terrestrial UV-driven N_2O source, we attempted to scale the processes of temperature- and UV-induced N_2O emission rates by vegetation to the global level (Fig. 5) – for materials and methods, see Bruhn et al. (2014a, b) and upscaling procedure, see Appendices 2 and 3. The upscaling was feasible because the magnitude of measured N_2O emission rates in response to natural sunlight, including low intensities of UV-radiation ranging from 280 to 400 nm, was similar to the magnitude of measured N_2O emission rates in response to high intensities of artificial UV-radiation within the 309–314-nm narrow range (Bruhn et al. 2014b). The total of these radiation-driven N_2O sources amounts to 0.65–0.78 Tg yr^{-1}, which equals 7–24% of all the natural terrestrial N_2O sources hitherto accounted for, which range between 3.3 and 9 Tg N yr^{-1} (Solomon et al. 2007). There is substantial geographical variation in the source strength (Fig. 5), which is mostly caused by the geographical variation in surface UV-radiation intensity – similar to the responsiveness of CO (Fig. 4). Importantly, the irradiance responses of N_2O emissions across all examined wave length ranges (UV-B, UV-A, PAR) is steepest at low irradiance intensities (Bruhn et al. 2014b). This intensity-dependent sensitivity is not taken into account in our linear scaling of the UV-induced N_2O emission rates to the global level, and it is therefore likely that we underestimate the N_2O source strength.

6 Perspectives and Conclusions

6.1 Realistic Emission Rates

Despite the many reports on directly UV-induced trace gas emission (CH_4, CO, CO_2, C2–C5 hydrocarbons/NMVOC, N_2O and NO_x) from plant materials, there are very few studies with replicated measurements of plant trace gas emission in response to natural solar radiation including UV (NO_y, Raivonen et al. 2009; CO, Bruhn et al. 2013; N_2O, Bruhn et al. 2014b). In all three examples there were indications that measured realistic emission rates were substantial compared to those of other emission/uptake processes at the ecosystem level. At this stage it is unfortunately not possible to say anything in general about realistic emission rates from a wider perspective.

6.2 Future Studies

From the evidence listed above it becomes apparent that much research is necessary for a more comprehensive understanding of mechanism, precursors and indeed in situ emission rates. Therefore we suggest that future experiments include tests of:

- Action spectra and linearity in response function at low UV levels at intact tissues in many more species
- Responses to natural variation in UV intensities in the field
- Effect of deposition of especially N-precursors
- Direct responses to UV after the plants previously have been exposed to variable UV exposures during growth
- Investigation if stomatal conductance has any effects on the UV-induced gas emission

6.3 Known Gas Emission Stimulated by UV

It is well established that sunlight and UV in particular stimulate the production of several gases at the surface or near the surface of living plants. Currently there is documented evidence for production of the following gas species: CH_4, CO, CO_2, NMVOCs, NO_x and N_2O. The number of gases produced by UV stimulation is probably greater, but further gas screening studies are needed to assess this. Independent of gas species, the UV-induced gas emission rates documented until now are very low, and as a consequence it is very challenging with respect to equipment and experimental setup to investigate these processes. Most records concern CH_4 production, but there are still many unanswered questions for this gas with regards to dose responses and production under natural conditions. For the other mentioned gases there are even more unanswered questions, nevertheless we have enough information to provide the first attempt at a global budget of UV-induced CO and N_2O emissions based on measurements from natural vegetation under field conditions. The result indicates that UV-driven CO production may contribute as much as 11–44% of all the natural terrestrial plant sources. The UV-induced N_2O source equals 7–24% of the natural terrestrial source strength. These global estimates should be regarded as a contribution to an ongoing quantification process, but this high global share emphasizes the urgent need for more work. In order to establish reliable global estimates and enable future predictions, it is apparent that much research is necessary to elucidate mechanisms, precursors, environmental relationships and establishment of relevant and realistic emission rates.

6.4 Perspectives

This newly discovered light-associated aspect of trace gas emission from living vegetation may have significant consequences for our understanding of exchange processes between the global biosphere and atmosphere. It is very likely a global phenomenon occurring on all leaf surfaces exposed to sunlight in both managed and natural ecosystems. Our global estimates for CO and N_2O under the current environmental conditions evidently show that radiation-driven processes are significant natural sources, and this could also be true for the other gases. An important feature is that the gas production is occurring at or just under the leaf surface, resulting in periodic high gas concentration within the boundary layer surrounding the leaves. This could, for instance, reduce the gas uptake of ozone in leaves since CO accelerates the reaction of O_3 with ethylene (Horie and Moortgat 1998), a process that has so far not been considered in ozone effect research. Through geological eras, the radiation-driven greenhouse gas (direct: CO_2, CH_4, N_2O and indirect: CO) impact must have fluctuated with UV-radiation and other processes producing or consuming greenhouse gases (e.g. soil respiration, denitrification and methanogens is in wetlands, and methane oxidation in upland soils), and therefore the development of climate on Earth.

Acknowledgements We are thankful to Prof Ary Hoffmann for reading the manuscript and providing helpful suggestions for corrections and NordGen (The Nordic Genetic Resource Center) for providing seeds for the experiments. This work was supported by the FP7 EU Eclaire project.

Appendix 1

Methods and Techniques

This appendix expands three methods and techniques in the text with respect to environmental factors and other important issues related to measurements of UV-induced gases.

Temperature

Particularly challenging is temperature control inside confinements made of transparent materials and at the same time exposed to intense irradiation by lamps or natural sunlight that may lead to severe scorching of plants. Under laboratory conditions, experiments may be run in well vented and temperature controlled growth cabinets (Bruhn et al. 2009) or enclosures equipped with heating tape (Vigano et al. 2008) to maintain stable temperature conditions. Direct temperature

control of enclosures may include simple and inexpensive means such as ice blocks (M. Drösler, pers. comm.) or more advanced applications in the form of Peltier cooling technology (Mikkelsen and Ro-Poulsen 2002; Bruhn et al. 2014a, b; Sundqvist et al. 2012). Temperature can be determined directly at the leaf surface with a micro-thermocouple attached to the material (Keppler et al. 2006) or air temperature detected by thermocouples or conventional thermometers situated in the enclosure. Exterior surface temperature of enclosures can be measured by using a heat conducting steel probe connected to a high precision temperature meter (Bruhn et al. 2014a, b).

Chamber Material

Chambers should be made of materials that allow transmittance of UV-radiation without filtering. Commonly used materials in transparent chamber or plant cuvettes include UV-transparent synthetic quartz-glass, tradename Suprasil®, that offers optimal UV-transmissions (Vigano et al. 2008; Rosenqvist et al. 2012; Bruhn et al. 2014a, b), alternatively UV-transparent acrylic materials (Rosenqvist et al. 2012; Bruhn et al. 2014a, b) can be used. Controlled transmittance of UV in experimental setups can be achieved by the application of filters to reduce or filter out specific UV-wavelengths reaching surfaces being investigated. Many commercial acrylic materials, with trade names such as Plexiglas or Perspex, will attenuate UV penetration and can be deployed to manipulate UV intensity (Bruhn et al. 2014a, b). For specific and controlled filtering of UV-radiation, various filters can be applied either at the light source or covering the enclosure windows; a comprehensive review of UV manipulation is given by Aphalo et al. (2012).

Surface Reactions and Reactive Species

Synthetic soft plastic and rubber materials used in growth cabinets, such as hoses, tubes, pots, sealants and wire insulators, provide potential complications if exposed in experimental setups to study UV-induced gas emissions. Firstly, these materials may release phytotoxic compounds, leading to plant growth problems or plant death; for a review see Rosenqvist et al. (2012). Secondly, photochemical reactions on the surface of synthetic materials when exposed to UV-radiation can produce gases like methane (Bruhn D, unpublished) or N_2O (Bruhn et al. 2014) that may confound experimental results. For this reason, it is strongly recommended to include empty/blank controls in the experimental protocol (Bruhn et al. 2014a, b; Sundqvist et al. 2012; Fig. 6). Presence of synthetic materials in the experimental units should be minimized, and materials shielded with (e.g.) PFTE replaced with inert materials (glass, metal) where appropriate or pre-conditioned by heating (Sundqvist et al. 2012).

Fig. 6 Plant leaves inserted in UV-B transparent vials. Note vials without leaves are used as blank controls

Analysis of Gas Mixing Ratios

Analysis of mixing ratios of target gases in the enclosures can be achieved principally by two different approaches. These are (1) manual grab sampling by syringe where a subsample of enclosure headspace is transferred to the analyzer or a storage vial for subsequent analysis; incubation may also take place in vials that can be mounted directly in the analytical unit such as a GC-autosampler, avoiding the need for manual sample transfer (Bruhn et al. 2014a, b). Alternatively, (2) the headspace gas concentrations can be observed in real-time where the test unit is connected to an appropriate gas analyzer in a sealed gas loop for continuous or cyclic analysis (Sundqvist et al. 2012).

Gas-chromatography: Conventional GC-instrumentation equipped with Flame-Ionization-Detection [FID] for CH_4 (e.g. Vigano et al. 2008), methanizer-FID for CO and CO_2 (Ueta et al. 2013) and Electron-Capture-Detection [ECD] for N_2O (e.g. Bruhn et al. 2014a, b) are applicable for grab sample analysis in order to cross-check the optical techniques, and where experiments with small vials prevent measurements with optical systems that require additional sample volume. The reproducibility with GC-analysis is typically ± 10 ppb although the micro-GC system tested by Ueta et al. (2013) for combined CO and CO_2 analysis exhibited detection limits of 3–5 ppm. Trace-gas GC analysis at ambient concentrations require sample volumes of typically 0.5–1 ml.

Laser spectroscopy: During the last couple of decades laser spectrometers for sensitive, accurate and fast analysis of air trace gas constituents have become available at affordable pricing. In their work with UV-induced CH_4 dynamics, Vigano et al. (2008) and Sundqvist et al. (2012) used an off-axis integrated cavity output spectrometer (Los Gatos Inc.) for real-time monitoring of CH_4 mixing ratios. With use of laser spectroscopy, it is necessary to pay attention to cross-interference from other gas species; Vigano et al. (2008) verified this for plant emission of

abundant methanol (CH_3OH). For studies on UV-induced emissions of carbon monoxide (Bruhn et al. 2013) and nitrous oxide (Bruhn et al. 2014a, b), a Los Gatos laser N_2O/CO spectrometer was applied following proper correction for cross-sensitivity with water and direct cross-interference between CO and N_2O. The sensitivity of laser spectrometers is several fold higher compared with GC analysis, and allows reproducibility in the range of ±1 ppb.

Stable isotope analysis: GC combined with stable isotope (SI) analysis provides a powerful tool to study source partitioning and reaction pathways of trace gases emitted from surfaces. Isotope-ratio-mass-spectrometry (IRMS) in combination with proper pre-concentration (e.g. cryo-trapping) and chromatographic separation of analytical compounds has been used for studying carbon (^{13}C), hydrogen (deuterium; D) in CH_4 (Keppler et al. 2006, 2008) and nitrogen (^{15}N) in N_2O (Bruhn et al. 2014a, b). The reproducibility of gas mixing ratios by GC-IRMS is diminished (±20–30 ppb at ambient concentrations) compared with conventional GC analysis and the sample amount required for proper analysis is in the range of tens of milliliters. Dueck et al. (2007) analyzed the concentration of ^{13}C-methane in CH_4 emitted from fully ^{13}C-labelled plant material using photo-acoustic spectroscopy in combination with a continuous-wave, optical parametric oscillator (OPO) and reported a detection limit of 3 ppb. Whereas the work by Dueck et al. (2007) demonstrated only negligible emissions of CH_4 based on the spectrometric method, later work by Vigano et al. (2008) with the same plant material showed a contrasting result with significant emissions of ^{13}C–CH_4, supposedly due to different analytical sensitivities (Vigano et al. 2008).

SI analysis encompasses recognition of the isotopic composition in gases emitted from materials with isotopic abundances at natural levels (e.g. Keppler et al. 2006) as well as from isotopically enriched materials (e.g. Bruhn et al. 2014a, b). Isotopic variations arise from mass-dependent isotope fractionation in biological and chemical processes, and natural abundance analysis of the trace gases, may add information about the nature and origin of precursors. Application of the rare (heavy) isotope is valuable not only for revealing information on specific precursor substances but also for providing a tool to study consumption processes.

The recent development of isotopic laser spectrometers (e.g. instruments offered by Picarro, Aerodyne, Los Gatos) provides new opportunities to investigate mechanisms and processes in UV-induced trace gas emissions. However, so far no work taking advantage of these instruments has been reported in literature.

Light Sources

Work on UV-induced trace gas emissions inevitably requires selection of a proper light source and establishment of associated irradiation intensities, wavelengths and action spectra. The selection of a light source is application driven and depends on the requirements imposed by the study. The main requirements concern the intensity and spectral distribution of radiant output of the lamp. The geometry of the

setup, including the source-target-distance and area of exposure, sets certain limits not only on the light source but also on characteristics of the monochromator if this is applied (Aphalo et al. 2012).

In this context, it is important to stress that the current review addresses works investigating the direct photolytic effect of UV-light exposure for plant-derived trace gas emissions. In this sense, translocation studies where plants are grown under natural or controlled conditions with attenuated or enhanced UV-exposure and subsequently examined for historical UV effects are not considered.

It is beyond the limits of this manuscript to present a detailed review and recommendation on selection of proper light source equipment for UV studies. Instead, the reader is referred to comprehensive reviews on usage of artificial light sources in UV photobiology given by Aphalo et al. (2012), and UV quantification reviewed by Björn et al. (2012). A number of potential light sources can be selected for UV work, either as single light sources, or more often in combination to achieve desired optical conditions. A brief list is shown below; for a detailed discussion we refer to Aphalo et al. (2012).

Fluorescent lamps and tubes are low pressure mercury vapour lamps that emit radiation at specific spectral lines, mostly in the UV region of the spectrum.

Xenon arc lamps are specialized light sources that produce intense visible and UV-radiation. High intensity water-cooled deuterium lamps (150 W) have a fairly flat radiant intensity curve in the UV-B region that is appropriate for mechanistic plant UV photobiology studies.

Spectrographs composed of a light source and a monochromator may be used in applications requiring spectrally-resolved UV-radiation exposure of biological specimens. Lasers usually produce very narrow and intense beams of monochromatic light. For the purpose of UV photobiology, tuneable optical parametric oscillator (OPO) pump lasers (pump wavelength 355 nm) are especially useful.

Specific experimental setups for studying UV-effects on plant gas emissions are described in detail in literature cited above, and illustrate the complexity and experimental precautions associated with such studies. As an example, Vigano et al. (2008) used 6 types of lamps, one PAR lamp, four UV-A and UV-B lamps, and one UV-C lamp. The UV content (UV-A and UV-B separately) was determined with a Waldmann UV meter calibrated for each individual UV lamp, except for the UV-C lamp. These authors did not report on the action spectrum for CH_4 release from biomass upon UV irradiation, and the UV strength was reported as the non-weighted integral over the UV-A range (400–320 nm), UV-B range (320–280 nm) or total UV range (400–280 nm). By choosing this approach (using unfiltered, non-weighted UV-radiation) the authors neglected a possible wavelength dependence of the biologically effective dose (Vigano et al. 2008). Bruhn et al. (2009), in addition to PAR lamps, used four different lighting sources to obtain desired UV-B and UV-A irradiance; the UV sources were placed at varying distances to yield the reported irradiances. The irradiance spectra of the experimental tubes and the transmission spectra of the glass vials used were further established in order to reveal wavelength dependent responses in the experiments.

Appendix 2

Global Drivers for CO and N_2O Contribution

Global CO and N_2O upscaling was based on the parameterized response equations to UV-irradiation and temperature (see Bruhn et al. 2013, 2014b; Appendix 3).

The drivers were driven by geospatial satellite information on UV-B temperature and land surface classifications derived from normalized difference vegetation index (NDVI) and snow cover (SC). The UV310 nm data was obtained from the Giovanni OMI/Aura Online Visualization and Analysis Daily Level 3 Global Gridded Products (http://giovanni.sci.gsfc.nasa.gov/giovanni/), the temperature data from MODIS (Moderate Resolution Imaging Spectroradiometer, NASA Earth Observations, http://neo.sci.gsfc.nasa.gov) and NDVI, LAI, and SC data from NASA Earth Observations (http://neo.sci.gsfc.nasa.gov/) and handled in a global longitude/latitude grid (250×150). The effect of UV was scaled with the global UV Irradiance at 310 nm at surface level (averaged across the years 2005, 2007, 2009, Local Noon Time). Temperature dependence was scaled based on daytime land surface temperatures averaged per month (over the 10 years 2001–2010) of available data. The temperature response parameterization did not include temperatures below 0°C, while the CO/N_2O emission from grids with a temperature below 0°C were set to zero in the upscaling. Land surface area was determined from satellite land dataset information and the area was calculated from longitude/latitude information. Snow cover (SC) was averaged per month (2009) and we excluded areas covered by snow. The remaining land surface area was classified as being vegetation covered or vegetation free based on the NDVI. The upscaling approach was conservative in the way that the surface area did not include topography. Further, most of the UV-radiation received by leaf surfaces are indeed screened (absorbed or reflected) by the surface wax (Cen and Bornman 1993; Liakoura et al. 2003; Jacobs et al. 2007). Therefore, for the global estimate of the UV-effect on CO/N_2O emission by vegetation, we assumed an effective Leaf Area Index (LAI) of unity. The proportions of vegetation and sand area were determined by NDVI classification. Response functions for plants were applied to surface covered by vegetation. The NDVI were averaged per month (2009). Correlations between LAI satellite measurement and NDVI from 2009 showed that for instance NDVI around 0.3, 0.5, 0.7 and 0.75 reflects vegetation with 0.4, 0.9, 2.0 and 4.0 layers of leaves per ground area (LAI) respectively (data not shown). This information was used to construct four groups with different distributions among vegetation and vegetation free surfaces. The groups are: (1) NDVI < 0.2, 100% sand; (2) $0.2 <$ NDVI < 0.4, 60% sand and 40% vegetation; (3) $0.4 <$ NDVI < 0.6, 20% sand and 80% vegetation; and (4) NDVI > 0.6, 100% vegetation. These vegetation cover values are lower than a derivation from the NDVI LAI relationship would indicate, but since vegetation cover is clustered by nature with LAI values up to over 5, this must result in more vegetation free areas than an average estimate would produce. For upscaling, any LAI above 1 would give the same values. This

division into group categories decides the percentage area of vegetation and vegetation-free combination in each of the 250 × 150 grid cells.

Appendix 3

Response Functions for Global CO and N2O Contribution

For each of 250 × 150 grid cells (Appendix 2) we estimated the CO or N_2O emission rate (*ER*) on a monthly basis as

$$ER = \alpha \times e^{\beta \overline{T_{day}}} \times \frac{\overline{UV}}{50} \overline{days \times DL} \times area \times \left(1 - \frac{area_{SC}}{area}\right) \times prop_{cat}$$

where α is a base *ER* of ecosystem CO or N_2O emission (nmol m^{-2} h^{-1}) measured at 21.4°C and 50 mW UV-B (see Bruhn et al. 2013, 2014b). We assumed a response to temperature, $(T) = \alpha \times e^{\beta T}$, as measured at leaf level (Bruhn et al. 2013, 2014b) when exposed to UV-B. In the upscaling we substituted T with a mean daytime temperature for the respective grid cell, $\overline{T_{day}}$. As we have demonstrated near-linear relationships between irradiance of both UV-B and UV-A and CO and N2O emissions, respectively (Bruhn et al. 2013, 2014b), we scaled the base *ER* of ecosystem CO or N_2O emission with the mean UV Irradiance (mW) at 310 nm at surface level for the grid cell. Further, *ER* for grid cell was adjusted according to the monthly average day length per month, $\overline{DL}$, number of days per month, area and category of land vegetation.

References

Althoff F, Jugold A, Keppler F (2010) Methane formation by oxidation of ascorbic acid using iron minerals and hydrogen peroxide. Chemosphere 80:286–292

Altimir N, Vesala T, Keronen P, Kulmala M, Hari P (2002) Methodology for direct field measurements of ozone flux to foliage with shoot chambers. Atmos Environ 36(1):19–29

Aphalo PJ, Albert A, McLeod A, Heikkilä A, Gómez I, Figueroa FL, Robson TM, Strid A (2012) Manipulating UV radiation. In: Aphalo PJ, Albert A, Björn LO, McLeod A, Robson TM, Rosenqvist E (eds) Beyond the visible: a handbook of best practice in plant UV photobiology. COST Action FA0906 UV4growth. University of Helsinki, Helsinki, p 176

Baur P, Stulle K, Schonherr J, Uhlig B (1998) Absorption of UV-B to blue light radiation by leaf cuticles of selected crop plants. Gartenbauwissenschaft 63:145–152

Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Rödenbeck C, Arain MA, Baldocchi D, Bonan GB, Bondeau A, Cescatti A, Lasslop G, Lindroth A, Lomas M, Luyssaert S, Margolis H, Oleson KW, Roupsard O, Veenendaal E, Viovy N, Williams C, Woodward FI, Papale D (2010) Terrestrial gross carbon uptake: global distribution and covariation with climate. Science 329:834–838

Björn LO (1996) Effects of ozone depletion and increased UV-B on terrestrial ecosystems. Int J Environ Stud 51:217–243

Björn LO, McLeod A, Aphalo PJ, Albert A, Lindfors AV, Heikkilä A, Kolarž P, Ylianttila L, Zipoli G, Grifoni D, Huovinen P, Gómez I, Figueroa FL (2012). Quantifying UV radiation. In: Aphalo PJ, Albert A, Björn LO, McLeod A, Robson TM, Rosenqvist E (eds) Beyond the visible: a handbook of best practice in plant UV photobiology. COST Action FA0906 UV4growth. University of Helsinki, Helsinki, p 176

Bloom AA, Lee-Taylor J, Madronich S, Messinger DJ, Palmer PI, Reay DS, McLeod AR (2010) Global methane emission estimates from ultraviolet irradiation of terrestrial plant foliage. New Phytol 187:417–425

Boucher O, Randall D, Artaxo P, Bretherton C, Feingold G, Forster P, Kerminen V-M, Kondo Y, Liao H, Lohmann U, Rasch P, Satheesh SK, Sherwood S, Stevens B and Zhang XY (2013) Clouds and aerosols. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York

Brandt LA, Bohnet C, King JY (2009) Photochemically induced carbon dioxide production as a mechanism for carbon loss from plant litter in arid ecosystems. J Geophys Res 114:G02004. doi:10.1029/2008JG000772

Brown MJ, Parker GG, Posner NE (1994) A survey of ultraviolet-B radiation in forests. J Ecol 82:843–854

Bruhn D, Mikkelsen TN, Ambus P (2007) Aerobic emission of methane by terrestrial plant material in response to UV-irradiance. Eos Trans. Am Geophys Union 88(52), Fall Meet. Suppl., Abstract, B53A-0940

Bruhn D, Mikkelsen TN, Obro J, Willats WGT, Ambus P (2009) Effects of temperature, ultraviolet radiation and pectin methyl esterase on aerobic methane release from plant material. Plant Biol 11:43–48

Bruhn D, Moller IM, Mikkelsen TN, Ambus P (2012) Terrestrial plant methane production and emission. Physiol Plant 144:201–209

Bruhn D, Albert KR, Mikkelsen TN, Ambus P (2013) UV-induced carbon monoxide emission from living vegetation. Biogeosciences 10:7877–7882

Bruhn D, Mikkelsen TN, Rolsted MMM, Egsgaard H, Ambus P (2014a) Leaf surface wax is a source of plant methane formation under UV radiation and in the presence of oxygen. Plant Biol 16:512–516

Bruhn D, Albert KR, Mikkelsen TN, Ambus P (2014b) UV-induced N_2O emission from plants. Atmos Environ 99:206–214

Butenhoff CL, Khalil MAK (2007) Global methane emissions from terrestrial plants. Environ Sci Technol 41:4032–4037

Caldwell MM (1981) Plant response to solar ultraviolet-B radiation. In Lange et al. (ed) Physiological plant ecology. Springer, Berlin, pp 170–186

Caldwell MM, Teramura AH, Tevini M, Bornman JF, Bjorn LO, Kulandaivelu G (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio 24:166–173

Caldwell MM, Searles PS, Flint SD, Barnes PW (1999) Terrestrial ecosystem responses to solar UV-B radiation mediated by vegetation, microbes and abiotic chemistry. In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell Science, Oxford, pp 241–262

Cen YP, Bornman JF (1993) The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of Brassica-napus. Physiol Plant 87:249–255

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Le Quéré C, Myneni, Piao S, Thornton P (2013). Carbon and other biogeochemical cycles. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis.

Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York

Day TA, Zhang ET, Ruhland CT (2007) Effects of ultraviolet radiation accelerates mass and lignin loss of *Larrea tridentate* litter in the Sonoran Desert. Plant Ecol 193:185–194

de Klein C, Harvey M (2015) Nitrous oxide chamber methodology guidelines. Version 1.1 ISBN 978-0-478-40585-9 (online). Ministry for Primary Industries, Wellington

Deckmyn GE, Cayenberghs E, Ceulemans R (2001) UV-B and PAR in single and mixed canopies grown under different UV-B exclusions in field. Plant Ecol 154:125–133

Dentener F, Drevet J, Lamarque JF, Bey I, Eickhout B, Fiore AM, Hauglustaine D, Horowitz LW, Krol M, Kulshrestha UC, Lawrence M, Galy-Lacaux C, Rast S, Shindell D, Stevenson D, Van Noije T, Atherton C, Bell N, Bergman D, Butler T, Cofala J, Collins B, Doherty R, Ellingsen K, Galloway J, Gauss M, Montanaro V, Müller JF, Pitari G, Rodriguez J, Sanderson M, Solmon F, Strahan S, Schultz M, Sudo K, Szopa S, Wild O (2006) Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation, Global Biogeochem. Cycles 20:GB4003. doi:10.1029/2005GB002672

Derendorp L, Holzinger R, Röckmann T (2011a) UV-induced emissions of C2-C5 hydrocarbons from leaf litter. Environ Chem 8:602–611

Derendorp L, Quist JB, Holzinger R, Röckmann T (2011b) Emissions of H_2 and CO from leaf litter of Sequoiadendron giganteum and their dependence on UV radiation and temperature. Atmos Environ 45:7520–7524

Dueck TA, de Visser R, Poorter H, Persijn S, Gorissen A, de Visser W, Schapendonk A, Verhagen J, Snel J, Harren FJM, Ngai AKY, Verstappen F, Bouwmeester H, Voesenek LACJ, van der Werf A (2007) No evidence for substantial aerobic methane emission by terrestrial plants: a ^{13}C-labelling approach. New Phytol 175:29–35

EPA (2010) Methane and nitrous oxide emissions from natural sources. Office of Atmospheric Programs (6207J), Washington, EPA 430-R-10-001

Ferretti DF, Miller JB, White JWC, Lassey KR, Lowe DC, Etheridge DM (2007) Stable isotopes provide revised global limits of aerobic methane emissions from plants. Atmos Chem Phys 7:237–241

Fortems-Cheiney A, Chevallier F, Pison I, Bousquet P, Szopa S, Deeter MN, Clerbaux C (2011) Ten years of CO emissions as seen from Measurements of Pollution in the Troposphere (MOPITT). J Geophys Res 116:D05304

Fraser WT, Blei E, Fry SC, Newman MF, Reay DS, Smith KA, Mcleod AR (2015) Emission of methane, carbon monoxide, carbon dioxide and short-chain hydrocarbons from vegetation foliage under ultraviolet irradiation. Plant Cell Environ 38:980–989

Grant RH (1997) Partitioning of biologically active radiation in plant canopies. Int J Biometeorol 40:26–40

Hari P, Raivonen M, Vesala T, Munger JW, Pilegaard K, Kulmala M (2003) Ultraviolet light and leaf emission of NO_x. Nature 422:134

Hartmann DL, Klein Tank AMG, Rusticucci M, Alexander LV, Brönnimann S, Charabi Y, Dentener FJ, Dlugokencky EJ, Easterling DR, Kaplan A, Soden BJ, Thorne PW, Wild M, Zhai PM (2013). Observations: atmosphere and surface. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York

Hilboll A, Richter A, Burrows JP (2013) Long-term changes of tropospheric NO2 over megacities derived from multiple satellite instruments. Atmos Chem Phys 13:4145–4169. doi:10.5194/acp-13-4145-2013

Horie O, Moortgat GK (1998) The effect of the addition of CO on the reaction of ozone with ethene. Chem Phys Lett 288:464–472

IPCC: Radiative Forcing of Climate Change, Ch 2. Climate Change 1995, The Science of Climate Change, Contribution of WGI to the Second Assessment Report of the Intergovernmental

Panel on Climate Change, edited by: Schimel D, Alves D, Enting I, Heimann M, Joos R, Raynaud D, Wigley T, Prather M, Derwent R, Ehhalt D, Eraser R, Sanhueza E, Zhou X, Jonas R, Charlson R, Rodhe H, Sadasivan S, Shine KR, Fouquart Y, Ramaswamy V, Solomon S, Srinivasan J, Albritton D, Derwent R, Isaksen L, Lal M, Wuebbles D, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 51–64, 1995

IPCC (2001) In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds) Climate change 2001: the scientific basis, contribution of working group I to the third assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York, p 881

IPCC (2013) In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York

Isaksen I, Granier C, Myhre G, Berntsen T, Dalsren S, Gauss M, Klimont Z, Benestad R, Bousquet P, Collins W, Cox T, Eyring V, Fowler D, Fuzzi S, Jckel P, Laj P, Lohmann U, Maione M, Monks P, Prevot A, Raes F, Richter A, Rognerud B, Schulz M, Shindell D, Stevenson D, Storelvmo T, Wang WC, van Weele M, Wild M, Wuebbles D (2009) Atmospheric composition change: climate chemistry interactions. Atmos Environ 43:5138–5192

Jacobs JF, Koper GJM, Ursem WNJ (2007) UV protective coatings: a botanical approach. Prog Org Coat 58:166–171

Karabourniotis G, Bornman JF (1999) Penetration of UV-A, UV-B and blue light though the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. Physiol Plant 105:655–661

Keppler F, Hamilton JTG, Brass M, Rockmann T (2006) Methane emissions from terrestrial plants under aerobic conditions. Nature 439:187–191

Keppler F, Hamilton JTG, McRoberts WC, Vigano I, Brass M, Rockmann T (2008) Methoxyl groups of plant pectin as a precursor of atmospheric methane: evidence from deuterium labelling studies. New Phytol 178:808–814

Khalil MAK, Rasmussen RA (1990) The global cycle of carbon monoxide: trends and mass balance. Chemosphere 20:227–242

Kim KH, Watanabe K, Menzel D, Freund H-J (2010) UV photo-dissociation and photodesorption of N_2O on Ag(111). J Phys Condens Matter 22:084012

Kirschbaum MUF, Bruhn D, Etheridge DM, Evans JR, Farquhar GD, Gifford RM, Paul KI, Winters AJ (2006) A comment on the quantitative significance of aerobic methane release by plants. Funct Plant Biol 33:521–530

Kirschbaum MUF, Niinemets Ü, Bruhn D, Winthers AJ (2007) How important is aerobic methane release by plants? Funct Plant Sci Biotech 1:138–145

Lee H, Rahn T, Throop HL (2012) An accounting of C-based trace gas release during abiotic plant litter degradation. Glob Chang Biol 18:1185–1195

Liakoura V, Manetas Y, Karabouniotis G (2001) Seasonal fluctuations in the concentration of UV-absorbing compounds in leaves of some Mediterranean plants under field conditions. Physiol Plant 111:491–500

Liakoura V, Bornmann JF, Karabourniotis G (2003) The ability of abaxial and adaxial epidermis of sun and shade leaves to attenuate UV-A and UV-B radiation in relation to the UV absorbing capacity of the whole leaf methanolic extracts. Physiol Plant 117:33–43

Long LM, Patel HP, Cory WC, Stapleton AE (2003) The maize epicuticular wax layer provides UV protection. Funct Plant Biol 30:75–81

Masson-Delmotte V, Schulz M, Abe-Ouchi A, Beer J, Ganopolski A, González Rouco JF, Jansen E, Lambeck K, Luterbacher J, Naish T, Osborn T, Otto-Bliesner B, Quinn T, Ramesh R, Rojas M, Shao X, Timmermann A (2013) Information from paleoclimate archives. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of

working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York
McLeod A, Keppler F (2010) Vegetation. In: Reay D, Smith P, van Amstel A (eds) Methane and climate change. Earthscan, London/Washington, pp 74–96
McLeod AR, Fry SC, Loake GJ, Messenger DJ, Reay DS, Smith KA, Yun BW (2008) Ultraviolet radiation drives methane emissions from terrestrial plant pectins. New Phytol 180:124–132
Messenger DJ, McLeod AR, Fry SC (2009) The role of ultraviolet radiation, photosensitizers, reactive oxygen species and ester groups in mechanisms of methane formation from pectin. Plant Cell Environ 32:1–9
Mikkelsen TN, Ro-Poulsen H (2002) In situ autumn ozone fumigation of mature Norway spruce - effects on net photosynthesis. Phyton 42:97–104
Myhre G, Shindell D, Bréon F-M, Collins W, Fuglestvedt J, Huang J, Koch D, Lamarque J-F, Lee D, Mendoza B, Nakajima T, Robock A, Stephens G, Takemura T, Zhang H (2013) Anthropogenic and natural radiative forcing. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York
Parsons AJ, Newton PCD, Clark H, Kelliher FM (2006) Scaling methane emissions from vegetation. Trends Ecol Evol 21:423–424
Prasad SS (2002) A new model of N2O quantum yield in the UV photolysis of O-3/O-2/N-2 mixtures: contributions of electronically excited O-3 and O-3 center dot N-2. J Chem Phys 117:10104e10108
Prasad SS, Zipf EC (2008) Atmospheric production of nitrous oxide from excited ozone and its potentially important implications for global change studies. J Geophys Res Atmos 113, D15307
Raivonen M, Bonn B, Sanz MJ, Vesala T, Kulmala M, Hari P (2006) UV-induced NOy emissions from Scots pine: could they originate from photolysis of deposited HNO3? Atmos Environ 40:6201–6213
Raivonen M, Vesala T, Pirjola L, Altimir N, Keronen P, Kulmala M, Hari P (2009) Compensation point of NO_x exchange: net result of NO_x consumption and production. Agric For Meteorol 149:1073–1081
Röckmann T, Vigano I, Holzinger R, van Weelden H, Keppler F (2007) News about methane emission from plant matter. Eos Trans. Am Geophys Union 88(52), Fall Meet. Suppl., Abstract, B51F-05
Rosenqvist E, Figueroa FL, Gómez I, Aphalo PJ (2012) Plant growing conditions. In: Aphalo PJ, Albert A, Björn LO, McLeod A, Robson TM, Rosenqvist E (eds) Beyond the visible: a handbook of best practice in plant UV photobiology. COST Action FA0906 UV4growth. University of Helsinki, Helsinki, p 176
Rozema J, van de Staaij J, Björn LO, Caldwell M (1997) UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol Evol 12:22–28
Rubasinghege G, Grassian VH (2009) Photochemistry of adsorbed nitrate on aluminum oxide particle surfaces. J Phys Chem A 113:7818–7825
Rubasinghege G, Spak SN, Stainer CO, Carmichael GR, Grassian VH (2011) Abiotic mechanism for the formation of atmospheric nitrous oxide from ammonium nitrate. Environ Sci Technol 45:2691–2697
Schade GW, Crutzen PJ (1999) CO emissions from degrading plant matter (II) estimate of a global source strength. Tellus B 51:909–918
Schade GW, Hofmann R-F, Crutzen PJ (1999) CO emissions from degrading plant matter, (I) measurements. Tellus B 51:889–908
Seiler W, Giehl H (1977) Influence of plants on the atmospheric carbon monoxide. Geophys Res Lett 8:329–332

Seiler W, Giehl H, Bunse G (1978) The influence of plants on atmospheric carbon monoxide and dinitrogen oxide. Pure Appl Geophys 116:439–451

Shindell DT, Faluvegi G, Koch DM, Schmidt GA, Unger N, Bauer SE (2009) Improved attribution of climate forcing to emissions. Science 326:716–718

Shulski MD, Walter-Shea EA, Hubbard KG, Yuen GY, Horst G (2004) Penetration of photosynthetic active radiation and ultra violet radiation into Alfalfa and Tall Fescue canopies. Agron J 96:1562–1571

Skiba U, Hargreaves KJ, Fowler D, Smith KA (1992) Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. Atmos Environ 26:2477–2488

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New york

Stocker TF, Qin D, Plattner G-K, Alexander LV, Allen SK, Bindoff NL, Bréon F-M, Church JA, Cubasch U, Emori S, Forster P, Friedlingstein P, Gillett N, Gregory JM, Hartmann DL, Jansen E, Kirtman B, Knutti R, Krishna Kumar K, Lemke P, Marotzke J, Masson-Delmotte V, Meehl GA, Mokhov II, Piao S, Ramaswamy V, Randall D, Rhein M, Rojas M, Sabine C, Shindell D, Talley LD,. Vaughan DG Xie S-P (2013) Technical summary. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge/New York

Sundqvist E, Crill P, Molder M, Vestin P, Lindroth A (2012) Atmospheric methane removal by boreal plants. Geophys Res Lett 39, L21806

Tarr MA, Miller WL, Zapp RG (1995) Direct carbon monoxide photoproduction from plant matter. J Geophys Res 100:11403–11413

Tiiva P, Rinnan R, Faubert P, Rasanen J, Holopainen T, Kyro E, Holopainen JK (2007) Isoprene emission from a subarctic peatland under enhanced UV-B radiation. New Phytol 176:346–355

Ueta I, Mizuguchi A, Tani K, Kawakubo S, Saito Y (2013) Rapid temperature-programmed separation of carbon monoxide and carbon dioxide on a packed capillary column in gas chromatography: application to the evaluation of photocatalytic activity of TiO_2. Anal Sci 29:673–676

Vigano I, Holzinger R, Röckman T (2007) The isotope signature of methane emitted from plant matter upon irradiation with UV light. Eos Trans. Am Geophys Union 88(52), Fall Meet. Suppl., Abstract, B53A-0938

Vigano I, van Weelden H, Holzinger R, Keppler F, McLeod A, Röckmann T (2008) Effect of UV radiation and temperature on the emission of methane from plant biomass and structural components. Biogeosciences 5:937–947

Vigano I, Röckmann T, Holzinger R, van Dijk A, Keppler F, Greule M, Brand WA, Geilmann H, van Weelden H (2009) The stable isotope signature emitted from plant material under UV irradiation. Atmos Environ 43:5637–5646

Worden HM, Deeter MN, Frankenberg C, George M, Nichitiu F, Worden J, Aben I, Bowman KW, Clerbaux C, Coheur PF, de Laat ATJ, Detweiler R, Drummond JR, Edwards DP, Gille JC, Hurtmans D, Luo M, Martínez-Alonso S, Massie S, Pfister G, Warner JX (2013) Decadal record of satellite carbon monoxide observations. Atmos Chem Phys 13:837–850

Yonemura S, Morokuma M, Kawashima S, Tsuruta H (1999) Carbon monoxide photoproduction from rice and maize leaves. Atmos Environ 33:2915–2920

Yoshimura H, Zhu H, Wu Y, Ma R (2010) Spectral properties of plant leaves pertaining to urban landscape design of broad-spectrum solar ultraviolet radiation reduction. Int J Biometeorol 54:179–191

Yurganov L, McMillan W, Grechko E, Dzhola A (2010) Analysis of global and regional CO burdens measured from space between 2000 and 2009 and validated by ground-based solar tracking spectrometers. Atmos Chem Phys 10:3479–3494