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Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration

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Preface

Arbuscular mycorrhizal fungi are ubiquitous soil organisms that form associations with roots of almost all plant species. They facilitate acquisition of nutrients by plants, contribute to processes associated with soil aggregation, and play understated roles in ecosystem function at various scales. They also participate in rhizosphere processes that protect plants against disease and improve access to water during periods of temporary or persistent water deficit. The effective management of mycorrhizal fungi is often an unrecognised component of sustainable agricultural production that contributes to the profitability of farming systems. During the restoration of disturbed lands, arbuscular mycorrhizal fungi contribute with ectomycorrhizal fungi to re-establishing effective nutrient cycling processes and other essential soil biological functions in ecologically significant plant communities.

At a fundamental level, recent advances in the taxonomy and techniques for recognising and assessing the diversity of arbuscular mycorrhizal fungi offer opportunities for reinvigorating research on the management of mycorrhizas in agricultural and natural ecosystems, including evaluation of their economic value. These advances provide the incentive for promoting knowledge of plant–mycorrhizal interactions in debates about soil and land management, fertiliser decision-making, implications for selection of crop rotations, choice of plant cultivars, maintenance of pastures and grasslands for animal production, and environmental impacts of intensive horticultural production. Although it is difficult to quantify the economic benefits of mycorrhizas, ignoring their roles will lead to failure in capturing their benefits. This will be even more important when the challenges of sustaining agricultural production using limited resources with low environmental impacts are highlighted in the coming years.

Appreciation of arbuscular mycorrhizal fungi as dynamic communities in the very contrasting environments of soil and roots is essential to managing their contributions through agronomic practices or inoculation. Competitive interactions among these fungi during colonisation of roots will influence dominance and function of both naturally occurring and introduced fungi as well as the survival from season to season of those which are most effective. Thus, inclusion of

arbuscular mycorrhizal fungi in biofertiliser formulations needs to be based on detailed knowledge of biotic and environmental interactions in space and time. A critical evaluation of the selection, technical production, and the use of inoculant arbuscular mycorrhizal fungi—in addition to the marketing of products containing these fungi—needs to be underpinned by sound comprehension of ecological concepts and principles.

Arbuscular mycorrhizas have the potential to mitigate nutrient loss by soil erosion and leaching, as well as increasing nutrient use efficiency. Renewed evaluations of dominant fertiliser inputs of both phosphorus and nitrogen require consideration of mycorrhizal associations, including avoidance of, or compensation for, negative effects of crop management on these associations. This extends to the role of arbuscular mycorrhizas in acquisition of zinc by plants. Furthermore, as arbuscular mycorrhizas can enhance plant survival and growth in extreme environments, research that highlights the potential for acclimation versus adaptation of mycorrhizal fungi will better inform management decisions in disturbed sites or in sites subject to temporary water deficit, salinity, or heavy metal toxicity.

Finally, an understanding of how roots are colonised by communities of these common soil fungi is essential for capturing their benefits. Predictive models that include spatial variability and soil mapping offer the potential for calibrating the impacts of soil properties and land use practices in sustaining the colonising potential of effective communities of mycorrhizal fungi. The role of mycorrhizas in soil carbon sequestration is of increasing interest, as is the potential for moderating their soil and rhizosphere environment by application of ameliorants such as biochar. However, for communities of arbuscular mycorrhizal fungi, their ubiquity and potential are generally hidden from the majority of land managers and thus overlooked. The intensification of agriculture for food production in the coming decades will benefit from the application of knowledge of molecular, physiological, and ecological function of arbuscular mycorrhizas via practical solutions to their use in sustainable agriculture and land restoration.

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Chapter 1
Use of Mycorrhiza in Sustainable Agriculture and Land Restoration

Zakaria M. Solaiman and Bede Mickan

1.1 Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with over 80 % of terrestrial plant species (Brundrett 2002). Arbuscular mycorrhizas are ancient and ubiquitous symbioses formed between a relatively small group of soil fungi and higher plant roots which has been traced back 460 million years (Redecker 2002). The symbiosis is primarily characterised by its association with phosphorus (P) uptake by host plants and the enhancement of water uptake through the extraradical fungal hyphal networks. This symbiosis can also trigger physiological and molecular signals at subcellular levels, alter plant community structure and increase plant tolerance to various abiotic and biotic stresses. Mycorrhizal hyphal networks link plants of the same and different species below ground and are able to transfer resources between plants and release signal molecule defence-related proteins, lipochitooligosaccharides and strigolactones. There have been significant recent advances in the understanding of physiological processes and taxonomy of these fungi (Kohout et al. 2014; Saito 2000). They are obligate symbionts belonging to the phylum Glomeromycota (Redecker et al. 2000). Their activity in agricultural ecosystems is well documented (Abbott and Gazey 1994; Bedini et al. 2013; Pellegrino and Bedini 2014) as is their presence during rehabilitation of forest ecosystems (Brundrett and Nanjappa 2013; Solaiman and Abbott 2003, 2008). The distribution of ectomycorrhizal (ECM) fungi is also widespread, but they form associations with only 3 % of terrestrial plant families (Smith and Read 2008). ECM fungi are members of the phyla Ascomycota and Basidiomycota (Hibbett et al. 2000; Siddiqui et al. 2008). Unlike the ECM fungi, AM fungi are dependent

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on plants for their carbon (C) and when a symbiosis is formed, both ECM and AM fungi can demand 20–40 % of photosynthetically fixed plant C (McNear 2013).

1.2 Molecular Identification and New Taxonomic Classification of AM Fungi

Molecular techniques are applied to the identification of AM fungi under both greenhouse and field conditions, but there are many limitations in relation to the detection methods used as these are multinuclear organisms (Kohout et al. 2014; Schüßler et al. 2001). The diversity of AM fungi identified using molecular techniques has been studied at an ecosystem level (Sanders et al. 1995), for particular plant species (Jansa et al. 2003; Kjøller and Rosendah 2001), during seasonal variation in colonisation of roots of arable crops (Daniell et al. 2001), for coexisting species (Gollotte et al. 2004; Vandenkoornhuyse et al. 2003), and in relation to the influence of agricultural practices (Brito et al. 2013).

Despite rapid advances in methodologies, molecular techniques are still considered difficult to use for the identification of AM fungi because DNA extraction from soils typically yields low quantity and quality compared to plant roots (Renker et al. 2003; Olsson et al. 2010). There are also methodological and biological challenges to accurately assess AM fungi community composition which arises from the vast differences in DNA quantity from spores and hyphae in soil used for template DNA (Schüßler et al. 2001). Moreover, the higher quantity of DNA in multi-nucleic AM fungal spores compared to hyphae which contain significantly fewer nuclei which can skew community composition data (Gamper et al. 2008). This leads to potential bias in estimates of relative abundance where AM fungal morphotypes during sporulation will have a greater relative abundance than other morphotypes (Saks et al. 2013). The recent progress of next-generation sequencing techniques will open up a detail examination of fungal communities, and the reduction of cost of analyses will lead to greater accessibility, but there will be challenges.

Kohout et al. (2014) recently used different primers to compare four routinely used AM fungal-specific primer systems for nuclear ribosomal DNA such as (1) the partial small subunit (SSU), (2) the partial large subunit (LSU), (3) the partial SSU and internal transcribed spacer (ITS; “Redecker”) and (4) the partial SSU-ITS-partial LSU region (“Krüger”). They also included a new primer combination (5) the ITS2 region in the comparison. They concluded that “Krüger” primers tended to yield the highest AM fungal diversity and higher Shannon diversity index than did SSU primers. They confirmed a strong bias towards the Glomeraceae in the LSU and SSU primer systems and differences in the composition of AM fungal communities based on the “Redecker” primer system.

Willis et al. (2013) considered aspects of AM fungal ecology emphasising past and present importance of the phylum and concluded that it is essential to include

the AM symbiosis in studies of higher plants in order to provide a more holistic view of the ecosystems. Concomitant morphological and molecular analyses have led to major breakthroughs in the taxonomic organisation of the phylum Glomeromycota (Oehl et al. 2011). Fungi in this phylum are known to form arbuscular mycorrhizas, and so far the phylum has 3 classes, 5 orders, 14 families and 29 genera described. They present the current classification developed in several recent publications and provide a summary to facilitate the identification of taxa from genus to class level. The history and complexity of the taxonomy and systematics of these obligate biotrophs has been addressed by recognising four periods (Stürmer 2012). The initial discovery period (1845–1974) was categorised mainly by description of sporocarp-forming species and the proposal of a classification for these fungi. The following alpha taxonomy period (1975–1989) established a solid morphological basis for species identification and classification, resulting in report of many new species and the need to standardise the nomenclature of spore subcellular structures. The cladistics period (1990–2000) stated the first classification of AM fungi based only on phenotypic characters. At the end of this period, genetic characters played a role in defining taxa and elucidating evolutionary relationships within the group. The most recent phylogenetic fusion period (2001 to present) started with the proposal of a new classification based on genetic characters using sequences of the multicopy rRNA genes.

Opik et al. (2010) have offered a new database, MaarjAM, that summarises publicly available Glomeromycota DNA sequence and associated metadata. These data have been made accessible in an open-access database (http://maarjam.botany.ut.ee). Two hundred and eighty-two SSU rRNA gene virtual taxa were described based on a comprehensive phylogenetic analysis of all collated Glomeromycota sequences showing limited distribution ranges in most Glomeromycota taxa and a positive relationship between the width of a taxon's geographical range and its host taxonomic range.

Based on morphological and molecular characteristics, 19 genera, viz. *Acaulospora*, *Ambispora, Archaeospora, Cetraspora, Dentiscutata, Diversispora, Entrophospora, Fuscutata, Geosiphon, Gigaspora, Glomus, Intraspora, Kuklospora, Otospora, Pacispora, Paraglomus, Racocetra, Scutellospora* and *Quatunica*, comprising more than 200 species are documented in AM fungi (Manoharachary et al. 2010). Liu et al. (2009) stated that the AM fungal taxonomy position has moved forward to a phylum with 214 species belonging to 19 genera, 13 families, 4 orders and 1 class now reported. It was suggested that molecular techniques would be the key approaches in the future study of AM fungal species diversity. However, the intraspecific diversity of *Glomus mosseae* in a survey of 186 publications reporting the occurrence of *G. mosseae* from at least 474 different sites from 55 countries resulted in a geographical map of their distribution based on both morphological and molecular techniques (Liu et al. 2009).

While the taxonomy of AM fungi is based on morphological characters of the asexual resting spores, molecular approaches to community ecology have revealed a previously unknown diversity from colonised roots (Rosendahl 2008). The long asexual evolution of the fungi has resulted in considerable genetic diversity within

morphologically recognisable species which challenges concepts of individuals and populations. The fossil record and molecular data show that the evolutionary history of AM fungi goes back at least to the Ordovician (460 million years ago), coinciding with the colonisation of the terrestrial environment by the first land plants (Redecker 2002). The fast-growing number of available DNA sequences for molecular identification of the Glomales within roots has been designed and tested. These detection methods have opened up entirely new perspectives for studying the ecology of AM fungi.

The AM fungal phylogeny of the genus *Glomus* of the family Glomaceae has been analysed based on full-length SSU rRNA gene sequences and shows that *Glomus* is not monophyletic (Schwarzott et al. 2001). The separation of *Archaeospora* and *Paraglomus* from *Glomus* was reported. Two ancestral families of AM fungal species such as Archaeosporaceae and Paraglomaceae were discovered from deeply divergent ribosomal DNA sequences in the past (Morton and Redecker 2001). Each family is phylogenetically distant from each other and from other glomalean families despite similarities in mycorrhizal morphology. Morphological characters once considered unique clearly are distributed in phylogenetically distant groups. The spores of some species in *Glomus* have considerable divergence at the molecular level. It is the combination of DNA sequences and mycorrhizal morphology which provides the basis for recognising *Archaeospora* and *Paraglomus*. These investigations reinforce the value of molecular data sets in providing a clearer understanding of phylogeny which in turn can lead to a more robust taxonomy.

1.3 Mechanisms of Nutrient Exchange in Arbuscular Mycorrhizas

In AM associations, nutrient exchange between fungi and host is one of the most important functions. Recent advances in biochemical and molecular biological techniques have revealed a great amount of new information on this topic (Smith and Read 2008; Harrison 1999; Saito 2000; Smith and Smith 2012). This has largely reinforced the ideas concerning symbiotic C and P transfer proposed by classical works in the 1970s and 1980s (Tinker 1975). However, the biochemical mechanisms of nutrient exchange in this symbiosis are still unclear. The following view of phosphorus transfer from fungus to host plant has been widely accepted (Smith and Read 2008). Phosphate in the soil solution is absorbed in the extraradical hyphae via a P transporter (Harrison and van Buuren 1995). The absorbed P is condensed into polyphosphate (poly-P) in the extraradical hyphae and translocated by protoplasmic streaming into the intraradical hyphae (Cox et al. 1975; Cooper and Tinker 1981; Solaiman et al. 1999). At the end, the poly-P may be hydrolysed and released as P across the fungal membrane, probably at the arbuscule. Solaiman and Saito (2001) observed that a substantial proportion of P in

mycorrhizal roots was of fungal origin, while the proportion of fungal biomass to root biomass was <2 %. Phosphate efflux and the decrease in poly-P content in the hyphae were both improved by the addition of glucose and 2-deoxyglucose, an analogue of glucose. The rates of enhancement for P efflux and poly-P decrease were analogous, suggesting that P efflux from intraradical hyphae was coupled with poly-P hydrolysis. Based on these findings, the translocation of P from fungus to host was estimated in relation to the distribution of hyphal P in extraradical and intraradical parts of the AM fungus (Solaiman and Saito 2001). By applying the technique of separating intraradical hyphae from host roots, they were able to clarify the translocation and transfer of P in AM symbiosis in a quantitative manner. Further investigation of P efflux using the present experimental system will shed light on the mechanisms of nutrient exchange.

AM fungi rely on the photosynthetic C supplied by their host plants to complete their life cycle. In return, the fungi supply nutrients to the plant especially P, N and Zn. The site of nutrients exchange is primarily the arbuscule (Solaiman and Saito 1997; Harrison 1999; Balestrini and Bonfante 2005). The molecular and structural organisations of arbuscules facilitate and regulate the processes of nutrient exchange. Plant N and P transporter proteins have been reported (Ellerbeck et al. 2013; Javot et al. 2007b). Gene expression studies also showed that specific members of these protein families are expressed in the roots of colonised plants (Harrison et al. 2002; Javot et al. 2007a). Phosphate acquisition via the mycorrhizal pathway begins with the uptake of available P from soil by fungal extraradical hyphae (Bucher 2007). These hyphae extend beyond the host root zone, allowing a greater soil volume to be exploited for P uptake. Uptake at the soil hypha interface is mediated by fungal high-affinity P transporters (Harrison and van Buuren 1995). Following fungal uptake, phosphate is transferred to the fungal vacuole where it is polymerised to form polyphosphate chains and translocated through the vacuolar compartment to the intraradical hyphae (Solaiman et al. 1999). The poly-P is then hydrolysed and phosphate released to the interfacial apoplast from where plant mycorrhizal Pht1 transporters guide the phosphate across the periarbuscular membrane for delivery to other parts of the plant. The extraradical hyphae of AM fungi also absorb ammonium, nitrate and amino acids (Hodge et al. 2001), and the role of mycorrhiza on N delivery is becoming better understood (Chalot et al. 2006; Ellerbeck et al. 2013). The majority of N is thought to be taken up in the form of ammonia via the fungal-encoded AMT1 family transporters such as the protein GintAMT1 characterised from *Glomus intraradices* (Lopez-Pedrosa et al. 2006). There is no evidence for fungal translocation of either ammonium or nitrate and it is thought that nitrogen transport occurs in the form of the amino acid arginine (Govindarajulu et al. 2005), and amino acids may be delivered directly to the interfacial apoplast for plant absorption. However, there is also evidence for an alternative route whereby arginine is broken down by ornithine aminotransferase and urease to release free ammonium. It has been proposed that ammonium is exported by protein-mediated mechanisms and a candidate fungal AMT transporter has been identified that is highly expressed in the internal hyphae (Govindarajulu et al. 2005). Gene expression analyses of medic and rice have identified

mycorrhiza-induced transcripts that putatively encode ammonium transporters that are candidates for this function. There is also the possibility for passive ammonia uptake across the periarbuscular membrane, perhaps facilitated by the presence of aquaporin proteins (Uehlein et al. 2007). The use of radiolabeled substrates has demonstrated that AM fungi take up plant carbohydrates in the form of hexose (Solaiman and Saito 1997). The route of this transport is specific to the arbuscule. There is little molecular evidence for the presence of hexose export proteins in the periarbuscular membrane; although a number of mycorrhiza-responsive sugar transporter genes have been identified in medic, they are thought to act as proton-sugar symporters in sugar import rather than export, possibly in support of high metabolic activity in arbuscules (Harrison 1996).

AM fungi deliver both P and N to the root through arbuscules. Previously MtPT4, a *Medicago truncatula* phosphate transporter located in the periarbuscular membrane that is essential for symbiotic P transport and for maintenance, was identified (Javot et al. 2011). In mtpt4 mutants arbuscule degeneration occurs prematurely and symbiosis fails (Javot et al. 2011). The MtPT4 arbuscule phenotype is strongly correlated with shoot N levels. On the other, the transport mechanism of sugars to the apoplast is passive movement. For example, when hexose reached in the apoplast is absorbed by the fungus via specific transport proteins. The characterisation of the GpMST1 hexose transporter from *Geosiphon pyriformis*, a Glomeromycotan fungus, provides a promising direction for further investigation (Schussler et al. 2006). In the intraradical hyphae, much of the C is changed to storage lipids, predominantly as triacylglycerides. Lipids not only act as storage C but also are the main form of C translocated from intra- to extraradical hyphae where they provide the major respiratory substrate.

AM fungi provide benefits to their plant hosts by enhancing mineral nutrition, increasing tolerance to water stress, inducing greater resistance to pathogens and reduce sensitivity to toxic substances present in the soil. However, the cost of colonisation can be ~20 % of the host's fixed C being consumed by the microbial symbiont. Nonetheless, under experimental conditions when nutrients are limiting, mycorrhizal crop plants typically exhibit a better performance over non-mycorrhizal plants in high-input agricultural systems, the relative advantages are reduced while the C costs remain same and the performance of colonised plants can fall below that of non-colonised plants (Janos 2007). Phillips et al. (2013) proposed a framework for considering how tree species and their mycorrhizal symbionts differentially couple C and nutrient cycles in temperate forests. Given that tree species predominantly colonise by a single type of AM fungi or ECM fungi and that the two types of fungi differ in their modes of nutrient acquisition, the abundance of AM and ECM trees in a paddock may provide an integrated index of biogeochemical transformations relevant to C cycling and nutrient retention.

AM fungi can obtain the photosynthates from host plants and can promote N uptake by host plants via the absorption of various N sources by mycorrhizal hyphae leading to improvement in nutrition and stress tolerance of host plants (Li et al. 2013). The symbiont absorbs and transfers N, but the mechanisms behind the N metabolism and translocation from AM fungi to host plants are still in debate.

The roles of AM fungi in N allocation in host plants and the ecological significance at community and ecosystem levels need to be studied in more detail because they vary widely (Hodge and Storer 2014).

1.4 Role of AM Fungi in Sustainable Agriculture

Sustainable agricultural systems use natural processes to achieve satisfactory levels of productivity and food quality while decreasing fertiliser use, dropping input costs and preclude environmental pollution and its impacts (Siddiqui et al. 2008; Harrier and Watson 2004). It should also be ecologically feasible and socially responsible. Several soil factors contribute to sustainable agriculture through control of soil-borne diseases and increased soil microbial activity leading to increased antagonism and parasitism within the rhizosphere level (Jawson et al. 1993; Knudsen et al. 1995). Research approaches are presently focused on the search for suitable alternatives to the use of commercial artificial pesticides. However, progress has also been accomplished in exploring the use of microorganisms for improvement of soil fertility and ultimately increased crop productivity. Greater emphasis is being placed on enhancing exploitation of indigenous soil microbes which will contribute to soil fertility and increase plant growth as well as plant protection.

AM fungi have been difficult to study (Hamel and Strullu 2006), but they are now recognised as key components of soil ecosystems rather than only a plant root component. Recent advances in knowledge conveyed by new techniques for soil microbiology research open the way to AM fungi management in crop nutrition and production. AM fungi can influence crop growth, nutrition and production even in phosphorus-rich soils (Balzergue et al. 2013; Solaiman and Hirata 1997). However, growing crops in soil with lower levels of fertility could enhance the multiple beneficial effects of AM fungi in agroecosystem including decreased nutrient loss to the environment. Inclusion of mycorrhizal bioassays in soil testing protocol (Djuuna et al. 2009) for use in fertilisation recommendations and development of improved inoculants to manipulate AM fungi (Abbott et al. 1987) and screening of crop cultivars with improved symbiotic abilities (Smith et al. 1992) could contribute to agroecosystem stability and sustainability (Hamel and Strullu 2006).

In agriculture, several factors influence plant response and plant benefits from mycorrhizas such as host crop dependency on mycorrhizal colonisation, tillage, fertiliser application and the potential of mycorrhizal fungi inocula. Interest in AM fungal inoculation for sustainable agriculture is based on their roles in the improvement of plant growth through nutrient and water uptake (Augé 2004) and improvements in soil fertility as well as soil aggregate stability (Rillig et al. 2007; Rillig 2004).

1.5 Role of AM and ECM Fungi in Protection of Soil-Borne Diseases

Biological control of soil-borne diseases is currently accepted as a key practice in sustainable agriculture. AM fungi have shown potential in protecting host plants from soil-borne pathogens. While few AM isolates have been tested against these soil-borne pathogens, some appear to be more effective than others (Cameron et al. 2013; Azćon-Aguilar and Barea 1996). The degree of protection varies with the pathogen species involved and can be moderated by soil and other microclimatic conditions of the rhizosphere. Only weak responses to AM fungi colonisation have been found in some activities like lignification, production of phytoalexins and peroxidises and expression of genes encoding for PR proteins, indicating that AM fungi do not elicit typical defence responses (Azćon-Aguilar and Barea 1996). However, these compounds could make roots sensitive to the presence of pathogens and enhance defence mechanisms to subsequent pathogen infection (Benhamou et al. 1994). In this study, responses of AM and non-mycorrhizal transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *Chrysanthemi* were investigated in mycorrhizal roots; the growth of the pathogen was usually restricted to the epidermis and cortical tissues, whereas in non-mycorrhizal roots the infection of pathogen reached at depth even up to the vascular stele. The *Fusarium* hyphae inside mycorrhizal roots exhibited a high level of structural disorganisation, probably induced by a strong reaction of the host cells characterised by the accumulation of phenolic-like compounds and the production of hydrolytic enzymes such as chitinases. This strong reaction was not induced by non-mycorrhizal roots, suggesting that the activation of plant defence responses by mycorrhiza formation provides a protection at a certain level against the pathogen. In contrast to the weak defence response towards AM fungi found in AM hosts, it is noteworthy that in myc$^-$ pea mutants, AM fungi trigger a strong resistance reaction. This suggests that the AM fungi are able to elicit a defence response but that symbiosis-specific genes have mechanism to control the expression of the genes related to plant defence during AM establishment (Gianinazzi-Pearson et al. 1996). The expression of several PRs in tobacco plants did not affect the level of colonisation by *Glomus mosseae* which was only reduced in plants constitutively expressing an acidic isoform of tobacco PR-2, a glucanase (Vierheilig et al. 1996).

Ectomycorrhizas (ECM) have a positive effect on the performance of seedlings due to the beneficial relationship between plants and mycorrhizal fungi (Guerin-Laguette et al. 2004; Machón et al. 2006; Minchin et al. 2012). They are also effective against various plant root rot diseases (Duchesne 2000). Many studies have observed the protective role of ECM not only against fungal pathogens (Morin et al. 1999) but also against nematodes (Machón et al. 2006). Pine wilt disease is a globally severe forest disease and demonstrates the importance of ECM relationships (Akema and Futai 2005). In this study, the abundant ECM found in the upper slope enhanced water uptake by the pines, mitigated drought stress and thereby decreased the mortality of pine trees from pine wilt disease.

1.6 Role of AM and ECM Fungi in Restoration of Native Forest Ecosystems

Land clearing of terrestrial ecosystems claims several million hectares annually in Australia (Warren et al. 1996) which causes loss of essential physicochemical and biological soil properties (Skujins and Allen 1986). These properties largely determine soil quality and fertility that supports plant establishment and productivity. Soil degradation limits the potential for restoration of native plants (Agnew and Warren 1996), and erosion and desertification are accelerated. Desertification reduces the inoculum potential of mutualistic symbionts such as AM fungi that are key ecological factors in governing the cycles of major plant nutrients and hence in sustaining vegetative covers in natural habitats.

AM fungi improve the ability of plants to establish and cope in stressful conditions including nutrient deficiency, drought and soil disturbance (Schreiner et al. 1997). The fungal hyphae contribute to the formation of water-stable aggregates necessary for good soil tilth (Jeffries and Barea 2000). Loss of mycorrhizal propagules from degraded ecosystems can overcome either by natural or artificial revegetation where an increase of inoculum may be needed in these ecosystems (Requena et al. 1996). Inoculation of plants with mycorrhizal fungi in revegetation schemes should not only help plant establishment (Herrera et al. 1993) but also improve soil biological, chemical and physical properties thus contributing to soil quality (Carrillo-García et al. 1999). The introduction of a plant species along with a known AM fungus is a successful biotechnological tool to aid the recovery of desertified ecosystems (Azćon-Aguilar et al. 2003).

Many forest trees are dependent on a symbiotic association of their roots with ECM fungi and mobilise minerals from soil and transfer them to the plant. In exchange the trees deliver assimilated C to the fungi. The hyphae of ECM fungi are the source of C to soil microbes and depend on their tree hosts for their energy needs. In return, they take up P, N, S and Zn from soil and translocate them to their host and greatly extend the functional root system of the host plants (Allen 1991). An ECM fungus can connect roots of several trees by fungal hyphae network. Most ECM fungi are basidiomycetes such as *Amanita*, *Cortinarius*, *Lactarius*, *Russula* and *Suillus*, among the best-known ECM genera (Hacskaylo 1972). ECM are widespread particularly in temperate regions where many of the ecologically important tree species involve such as species of *Abies*, *Betula, Fagus, Picea*, *Pinus*, *Pseudotsuga*, *Quercus* and *Salix*.

Below-ground biodiversity is essential for the maintenance of forest growth and ecosystem functions as well as for reforestation of disturbed lands due to mining activities. ECM fungi are economically symbiotic soil fungi forming a sheath around the root tip and form a special structure called Hartig net (Smith and Read 2008). They gain C from the tree and in return support the tree in taking up nutrients, water and metabolites. The fungus also protects plants from parasites, nematodes and soil pathogens. The importance of ECM in forest plantations has received much attention when it was observed that trees often fail to establish at

new sites if the ECM symbiont was absent (Menkis et al. 2012). This effect has been found in exotic pine transplantation in different parts of the world. In Western Australia, *Pinus radiata* and *P. pinaster* failed to establish in nursery beds in the absence of ECM fungi (Lakhanpal 2000). Pine seedlings are known to be tolerant to environmental stresses such as acid mist when colonised with ECM fungi (Asai and Futai 2001). Tropical rainforests harbour the highest known tree diversity on the planet, and many ecological studies have attempted to explain the familiar symbiotic association of so many co-occurring species (Leigh et al. 2004; Valencia et al. 1994).

1.7 Conclusions

Mycorrhizal fungi are well known to have a wide range of benefits to their host plants. They can enhance nutrient uptake especially P, N and Zn. They can also suppress soil pathogens, enhance tolerance to drought stress and reduce sensitivity to toxic substances contaminated to the soil. The suitability of conditions needs to be managed for indigenous fungi to colonise hosts in their natural habitat or to minimise loss of these fungi with high-input farming/disturbance. Highly mycorrhizal host crop cultivars should be selected for use in crop rotations. Conventional plant breeding in soils with high nutrient contents may select against the most efficient fungal communities or even against the mycorrhizal association. Many efforts have been made in recent years to get benefits from mycorrhizas for agriculture, horticulture, forestry, land restoration and contaminated site remediation. The results have generally been consistently positive under controlled conditions, with some difficulties due to complications from diverse variables under field conditions. Mycorrhizal interactions between plants, fungi and the environment are complex and often indivisible. Mycorrhizas are an essential below-ground component in the establishment and sustainability of plant communities, but thorough knowledge is required to achieve maximum benefits from these microorganisms and their associations.

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Chapter 2
Assessing Economic Benefits of Arbuscular Mycorrhizal Fungi as a Potential Indicator of Soil Health

L.K. Abbott and S. Lumley

2.1 Introduction to Soil Health Indicators

Arbuscular mycorrhizal (AM) fungi have the potential to influence the economic benefits of agricultural systems through both direct and indirect processes related to plant nutrition (e.g. Smith and Smith 2012), access to moisture in water-limiting situations (e.g. Manoharan et al. 2010), building soil structure (e.g. Rillig and Mummey 2006), protection of soil carbon in aggregates (e.g. Jastrow et al. 1998) and strengthening plant resilience to disease (e.g. Azćon-Aguilar and Barea 1996). In some situations, AM fungi may have negative influences, particularly in relation to carbon transfer (Smith and Smith 2012). However, despite the demonstrated potential for AM fungi to contribute to soil physical, chemical and biological processes under controlled conditions, their contributions can be overridden in farming systems by management decisions that do not take them into account.

Although contributions of mycorrhizas are well documented (Smith and Read 2008), it is generally difficult to quantify their economic benefits (Miller et al. 1994). This is because there has been little work done either to identify systematically all such benefits or to identify how variables that influence mycorrhizal function might interact with each other to influence overall benefits. To complicate matters further, it is possible that the nature and magnitude of such benefits might be site specific, requiring all possible mycorrhizal impacts for defined rotations in a specific location to be considered. The emphasis in this overview is to determine the relevance of AM fungi in '*normal agricultural field*

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conditions', including field inoculation with AM fungi where this is demonstrated to be commercially practical.

Factors known to affect the magnitude of mycorrhizal influence under field conditions (both positive and negative) include the availability of soil phosphorus in relation to the requirements of the plant, the diversity and abundance of the AM fungi present, the plant host species growing in the farming system (either within rotations or in continuous planting of one crop or communities of pasture species), the size of the plant and its stage of development, and the levels of soil carbon and nitrogen. Other issues likely to influence mycorrhizal colonisation include plant stressors such as disease (Azćon-Aguilar and Barea 1996), soil constraints such as salinity or acidity (Evelyn et al. 2009; Juniper and Abbott 1993; Sano et al. 2002), heat and water limitation (Manoharan et al. 2010), and the presence of other soil organisms which interact directly with mycorrhizal hyphae such as soil mesofauna (Endlweber and Scheu 2007). In addition, the various factors that influence AM fungi may interact with one another, leading to negative, synergistic or additive effects (Pearson et al. 1993, 1994; Thonar et al. 2014).

2.2 Introduction to Economic Evaluation of Environmental Contributions

Arbuscular mycorrhizas are but one element of soil biodiversity, which strongly influences soil health. As well as being influenced by the presence of other soil biota, such as saprophytic fungi (Albrechtova et al. 2012), the abundance and role of AM fungi are in turn influenced by soil treatments such as tillage and soil amendments (Brito et al. 2012; Lehmann and Joseph 2009). While their complete range of impacts on agricultural and natural ecosystems is yet to be fully appreciated, their potential for beneficial effects in all types of ecosystems has been acknowledged (Chaurasia 2004). However, the need for inoculation is controversial (Schwartz et al. 2006) and cannot be determined without clear understanding of the benefits of AM fungi present in the soil and the suitability of inoculants (Abbott et al. 1992).

A problem with valuing any aspect of biodiversity is that it is generally held to be an economic intangible, that is, it has no market price (Baker and Ruting 2014; Bishop 2013; Pearce 1995). In common with many other environmental goods and services that have vast overall intangible benefits to society, biodiversity itself cannot be bought and sold, making its value very difficult to quantify (Martinez-Alier 1987). This is unlike goods and services for which a market, and therefore a price, exists (Baker and Ruting 2014). Environmental and ecological economists have long attempted to develop methodologies for valuing intangibles because without some measure of their economic benefit, these valuable resources tend to be ignored or neglected in a world where the market and its attendant prices are treated with an almost religious reverence (Dobell 1995; Loy 1997; Pearce 2002;

Lumley 2013). Thus, it is difficult to make financial comparisons of their worth in comparison with resources like minerals and timber which have tangible values. This reverence strongly influences policy globally, and decision makers have come to rely on comparative financial values to prioritise budget allocations and other important determinations (Bishop 2013; Lumley 2013).

Various attempts have been made to quantify biodiversity value because its benefits are known amongst biologists and other scientists to be far-reaching and because biodiversity loss can have long-term, sometimes catastrophic, consequences for the human economy. Pimental et al. (1997) conducted an economic analysis of the benefits of biodiversity in which they concluded that they were worth $300 billion annually to the US economy alone. In their article, the authors disaggregated various biodiversity services into 21 activities while trying to place a financial value on each activity. One of the activities they identified was 'soil formation' about which they stated (Pimental et al. 1997, p. 748): 'Diverse soil biota facilitate soil formation and improve it for crop production'. They estimated the biodiversity value of soil formation to be worth $5 billion to the US economy and $25 billion to the world economy annually. Given that this estimate was in 1997 US dollars, it will now be substantially higher. Arbuscular mycorrhizas constitute a significant subset of soil fungi, and while it is not possible to extrapolate the value of mycorrhizas alone from the figures for soil biota estimated by Pimental et al., it is likely that their economic benefits are globally significant. Schulz (2001, p. 111) while investigating the effect of arbuscular mycorrhizas on the development of micropropagated oil palms noted that: 'While the economic benefits of arbuscular mycorrhizas per se have not been calculated, it has long been recognised that they do indeed have substantial worth and overall significance to soil health. In recent years the interest in mycorrhizas has increased, partly due to economic benefits because most of the economically important plants in agriculture, horticulture and forestry have been found to be mycorrhizal'. Delian et al. (2011) claimed that the presence of mycorrhizas in soil can increase economic profitability and it is widely recorded that mycorrhizas influence crop productivity (e.g. Smith and Read 2008; Gazey et al. 2004), although Ryan and Kirkegaard (2012) question their benefits. In a modelling study of the apparent diversity of mycorrhizal effects, Veresoglou and Malley (2012) claimed that potentially beneficial versus damaging relationships between plants and mycorrhizal fungi depended upon the number and nature of mycorrhizal species that colonised the plant. In response to a suggestion that mycorrhizal colonisation might be damaging in some Australian cropping situations, Smith and Smith (2011, p. 73) state 'We know of no convincing evidence for deleterious effects in the field that can confidently be ascribed to AM symbiosis'.

The potential breadth of contributions of AM fungi to important aspects of plant health and soil quality, underlying the notion that they might be used as an indicator of soil health, 'have received less emphasis than increases in production, probably because the economic benefits are less easily quantified or appreciated' (Smith and Read 1996, p. 454). Smith and Read (1996, ibid) also state that 'The possible economic benefits of managing mycorrhizal populations in agriculture and horticulture need to be critically assessed in the context of the ecology of the systems,

not simply in the growth of the crops'. Acknowledging the difficulties inherent in such an analysis, we propose a framework as a means of assessing the economic benefits of arbuscular mycorrhizas in the context of agro-ecosystems (e.g. Smith and Smith 2011) while recognising their broader ecological and global context (e.g. Chaurasia 2004). Furthermore, the same roles that are exhibited in agricultural soils extend into diverse natural ecosystems, and some of these environmental resources indirectly benefit agricultural ecosystems (Ryan and Kirkegaard 2012). Indeed, as Ryan and Kirkegaard (2012, p. 50) state, 'the role of AMF in restoration of native plants and ecosystems on agricultural lands in Australia may merit investigation. Plants in Australian native ecosystems are colonised by AMF; although there may be a significant nonmycorrhizal component in some instances'.

In order to estimate economic values of mycorrhizas at either paddock or farm level, factors affecting the life cycle of AM fungi, especially the colonisation of roots by communities of these fungi, need to be quantified. However, there are risks to making such estimates if they are based on (1) inaccurate measurement of mycorrhizal hyphae in soil and in roots including discrepancies associated with measurement of root density and/or root architecture (see Gutjahr and Paszkowski 2013); (2) misunderstanding of the behaviour and measurement of colonisation of roots by AM fungi according to the method of identification of species, 'strain' or morphotype (see Shi et al. 2012); (3) inaccurate measurement of mycorrhizal function, including estimation of variation in contributions of different AM fungi throughout stages of the plant growth cycle (see Mickan et al. submitted); (4) inaccurate assessment of benefits and disbenefits due to failure to account for mycorrhizal variation within crop rotations (see Koide and Peoples 2012); (5) not recognising the discreet effects of C and N cycles on mycorrhizas and their interactions with P cycles through plant uptake and use (see Johnson 2010); (6) lack of recognition of effects of other soil organisms which may be both under- and overstated (see Lewandowski et al. 2013; Steinaker and Wilson 2008); (7) lack of recognition of effects of plant disease and other stressors leading to distorted quantification of mycorrhizal contributions (Hilou et al. 2014; Singh et al. 2013); and (8) inaccurate assessment associated with independent and interrelated climate or environmental attributes.

Risk minimisation strategies can be taken into account to deal with some or all of the factors that impede realistic economic valuation of mycorrhizas. Some of the risks apply widely, but others are more farm or paddock specific. Without even rudimentary local knowledge of AM fungi in agricultural ecosystems, there is potential that management practices will be used that fail to consider and consequently fail to capture potential benefits. Within the rhizosphere, AM fungi occur at the interface of soil biophysical and biochemical processes, and this central position warrants clarification of their role as an indicator of soil health.

AM fungi occur ubiquitously in agricultural systems and have a close affiliation with roots of most agricultural plants (Smith and Read 2008). Therefore, factors which influence their distribution, abundance, diversity, infectivity and longevity in roots and soil have the potential to be incorporated into an integrated indicator of soil health.

2.3 Arbuscular Mycorrhizal Measurement

Most demonstrations of benefits of AM fungi have been made in terms of increased early plant vigour associated with mycorrhizal function under controlled conditions, including inoculation in the field. In parallel, detrimental impacts have been widely reported, particularly during early stages of plant growth (Graham and Abbott 2000; Johnson and Graham 2013). It is more difficult to demonstrate mycorrhizal function under field conditions (Ryan and Angus 2003; Watts-Williams and Cavagnaro 2012). Gazey et al. (2004) demonstrated mycorrhizal benefits in terms of P uptake and growth of subterranean clover under field conditions in southwestern Australia using a phosphorus response curve approach that included an inoculation control. Ryan and Kirkegaard (2012) concluded there was little evidence of benefits of mycorrhizas in agricultural production systems commonly used in Australia, and indeed they found that some of these agronomic practices may reduce colonisation of roots by AM fungi. Given that the practices involved are based on considerable research to identify the best agronomic practices for sustaining production, there is an opportunity to explore whether this level of production uses practices that do not capture some components of soil biological fertility (Abbott and Murphy 2003) and that further investigation of the basis of 'sustainable production' that does not maximise contributions of soil flora and fauna is required. Generally, claims of mycorrhizal benefits in agricultural soils that relate to improving profitability rather than maximising productivity, as well as their possible role in the decontamination of soils polluted by residual organophosphates and their contribution to sustainability of crop production (Smith and Read 2008; Gazey et al. 2004; Delian et al. 2011; Albrechtova et al. 2012; Brito et al. 2012), are all in need of investigation within a framework that highlights intangible economic benefits.

Overall, while it is relatively easy to demonstrate mycorrhizal benefits under controlled conditions, including controlled field experiments, it is not easy to extend this to assessment of their benefits under '*normal agricultural field conditions*' because the fungi are ubiquitous. Even though different fungi have been shown to differ in their effectiveness (e.g. Smith et al. 2000; Graham and Abbott 2000), the extent to which this is translated into field soils where competition between fungi leads to differences in relative abundance in roots and in infectivity (based on relative inoculum potential) is difficult to measure. However, despite their ubiquity, the contributions of different AM fungi during plant growth stages under 'normal' agricultural field conditions are not well established. While it is known that different AM fungi have different capabilities to scavenge for P under P-limited conditions for plant growth (Schweiger et al. 2007; Thonar et al. 2011), the extent to which this plays out during stages of plant development is not clarified in 'normal' agricultural field conditions. Diversity in the life cycles of AM fungi in association with plants leads to changes in their relative abundance in root systems and in soil over time. For example, Pearson and Schweiger (1993, 1994) showed how understanding the life cycles of AM fungi in both roots and soil helped identification of the mechanism of competition between two fungi that occur

Table 2.1 Risks in assessing economic value of arbuscular mycorrhizas and potential remedies for overcoming such risks

Risks in assessing economic value of mycorrhizas	Remedy for overcoming risks in assessing economic value of mycorrhizas
Inaccurate measurement of mycorrhizal hyphae in soil and in roots associated with variation in root density and/or root architecture	Understand the relationship between root growth and mycorrhizal colonisation throughout the life cycle of plants in agricultural rotations
Inaccurate measurement of mycorrhizal function, including varying contributions of different fungi throughout the stages of the plant growth cycle	Understand the extent to which different mycorrhizal fungi colonise roots during the plant growth cycle and how this affects mycorrhizal contributions at different stages
Misunderstanding of the behaviour and measurement of colonisation of roots by species, strains and/or morphotypes of mycorrhizal fungi	Understand how communities of mycorrhizal fungi interact with one another in roots and whether this affects their ability to access P and water, and the ramification of hyphae in soil
Inaccurate assessment of benefits and disbenefits due to failure to account for mycorrhizal variation according to crop rotation	Understand how mycorrhizas contribute in sequences of crop and pasture species so that benefits can be accounted for seasonally
Not recognising the discreet effects of C and N cycles on mycorrhizas and their interactions with P	Understand interrelationships between mycorrhizas and C and N cycles in soil to calculate P and N fertiliser requirements that do not override potential mycorrhizal contributions
Lack of recognition of effects of other soil organisms which may be under or overstated	Understand how other soil organisms interact with mycorrhizal fungi
Lack of recognition of effects of plant disease and other stressors leading to distorted quantification of mycorrhizal contributions	Understand how mycorrhizal fungi interact with plant pathogens either to alleviate plant disease or to influence quantification of their abundance
Inaccurate assessment associated with independent and interrelated environmental and/or climate attributes	Understand how soil conditions such as salinity, acidity, compaction and waterlogging influence the life cycles of mycorrhizal fungi

commonly within roots of agricultural plants in southwestern Australia. Factors of significance were the dynamics of colonisation of roots associated with changes in sporulation and soluble carbohydrates. Given this degree of complexity, measurement of mycorrhizal fungi as '% root colonised' at one point in time may be of little relevance to estimation of the potential contribution of mycorrhizal fungi over an entire plant production cycle. Examples of the limitations in measurement of mycorrhizas and their function in 'normal' agricultural field conditions are illustrated below.

If mycorrhizas are not accurately measured, there will be risks in assessment of their potential contributions (Table 2.1). The measurement most commonly used is the proportion of root length colonised. However, there can be large variation in the density of root colonised and fungal structures within roots (McGonigle et al. 1990) and in the diameter of roots, all of which influence the total biomass of fungi present

both inside the root and in the surrounding soil (Abbott and Robson 1984, 1985). Furthermore, these differences are not usually recorded (Gazey et al. 1992) and change with time (McGonigle 2001).

2.4 Mycorrhizal Benefits and Costs

Field studies of benefits of AM fungi are fewer than are glasshouse studies primarily because of the difficulties in establishing and monitoring experiments (McGonigle 1988). However, another factor in assessing the benefit of mycorrhizas in agricultural systems is that their contribution may be diffusely distributed amongst a number of areas, none of which reaches a threshold level, but when considered together, there is a benefit. Most studies focus on one aspect, and quantification relevant to assessing a wider suite of contributions can be prohibitive in terms of time and cost (Schnepf et al. 2008).

Where AM fungi contribute to P uptake, the benefit can be measured in terms of savings in fertiliser (e.g. Schweiger et al. 2007). There has been little consideration of potential savings in nitrogen fertiliser, but the close links between P and N cycles (Johnson 2010) mean that such attention is warranted. Evaluation of phosphorus-use efficiency of plants in crop rotations, continuous cropping or pasture production could include estimations of contributions of AM fungi. If this were done, there will be a clearer estimation of nitrogen fertiliser needs in agricultural systems. While there has been in-depth analysis of P and N fertiliser requirements for agricultural production (according to crop or pasture species for particular rotations and tillage practices), little attention has been paid to the potential roles that effective communities of AM fungi might contribute to these calculations. Where such contributions are not considered, there is a greater chance for potentially useful contributions of AM fungi to be overlooked. A logical stepwise process for N and P fertiliser recommendations could include first an estimate of P requirements that takes into account the potential benefit of AM fungi that are present. This would form a baseline for estimation of N fertiliser requirements. Where AM fungi were demonstrated to be likely to provide a benefit (because the 'right' fungi were present in the 'right' amounts for the crop/pasture sequence), then this could be taken into account. Where AM fungi were demonstrated to be unlikely to provide a benefit (because the 'wrong' fungi were present in the 'wrong' amounts for the crop/pasture sequence), then this could also be taken into account in terms of remediation required through agricultural management to restore mycorrhizal communities to a state where they can make close to their potential contribution (i.e. a state of equilibrium). Thus, understanding the state of the existing community of AM fungi underpins decisions about N and P fertiliser use for a given agricultural sequence. Clearly, AM fungi will have less to contribute under some circumstances than others, but the emphasis needs to be on the extent to which they are achieving their potential in a given situation.

Other benefits of AM fungi such as (1) facilitating plant access to moisture under drying soil conditions, (2) increasing retention of soil carbon by protecting it from

microbial degradation via enhanced aggregation of soil particles and (3) creating a soil and rhizosphere environment that is more resilient to development of plant disease may be co-benefits of more effective supply of nutrients to plants, but they can also stand alone in situations where the AM fungi have no particular role in nutrient-use efficiency. This could occur in soils that are already well supplied with P and N for plant growth.

2.5 Is There a Link Between Mycorrhiza Measurement and Benefit?

The only way to obtain an idea of the economic benefits of arbuscular mycorrhizas is to ascertain the link between their presence and function and the impacts that they have on agricultural ecosystems or, more particularly in this instance, on productivity and/or profitability. In some cases, there may be a negative impact, or disbenefit, on plant growth, although Smith and Smith (2011) disputed this, and Veresoglou and Malley (2012) suggested that any potential disbenefits depended on the number and type of colonising mycorrhizal fungi. This is necessarily a complex process because of the number of variables involved.

Table 2.2 Variables, impacts and risks of assessing the economic benefits of mycorrhizas: fungal factors

Variable	Potential effect	Impact	Risk	Risk minimisation strategy
Growth rate of mycorrhizal fungi in roots and in soil	Mycorrhizal fungi promote soil aggregation and plant growth	Positive	May use an inaccurate measure of mycorrhizal growth and function	Use both proportion of root colonised and absolute amount and quantify mycorrhizal biomass
Type of mycorrhizal fungi present	Different species or subspecies might grow at different rates and have differing benefits to plant and soil	Positive	Misunderstanding behaviour of individual species or subspecies could cause inaccurate assessment of their benefits	Identify growth attributes and behaviour of species and subspecies present and their interactions
Number of mycorrhizal species of subspecies present	There may be several species or subspecies present in varying amounts and they might interact competitively or synergistically	Positive or negative	Ignorance of how mycorrhizal species or subspecies interact could result in ignorance of competition or synergism	Identify the way mycorrhizal species or subspecies interact and give value for synergistic or competing effects

Table 2.3 Variables, impacts and risks of assessing the economic benefits of mycorrhizas: soil and plant variables

Variable	Potential effect	Impact	Risk	Risk minimisation strategy
Level of soil phosphorus	Promotes plant and mycorrhizal growth but needs to be balanced	Positive or negative	P see-saw effect. Both too much and too little P inhibit mycorrhizal growth	Assume ~40 ppm is optimal level of soil P for mycorrhizal growth and soil quality
Plant characteristics (e.g. size and growth stage)	Plant attributes such as size and growth stage affect mycorrhizal colonisation and function	Positive or negative	The role of plant size and growth stage might lead to inaccurate assessment of number and size of hyphae	Identify impact of plant attributes such as size and growth stage on measure of hyphae
Crop cycle characteristics	Attributes of plant type and rotation type could affect mycorrhiza activity	Positive or negative	May lead to inaccurate assessment of benefits and disbenefits due to failure to account for mycorrhizal variation according to crops in cycle	Account for mycorrhizal attributes and association for each plant in a rotation
Soil carbon and nitrogen levels	Levels of soil carbon and N affect soil quality and may interact with P	Positive or negative	Not recognising the discreet effects of C and N cycles on mycorrhizas and interaction with P	Account for carbon and nitrogen cycles and interaction with phosphorus

Table 2.4 Variables, impacts and risks of assessing the economic benefits of mycorrhizas: other environmental or climatic factors

Variable	Potential effect	Impact	Risk	Risk minimisation strategy
Presence of other key soil organisms	Other soil organisms may have a positive or negative effect on mycorrhizal function	Positive or negative	If possible effects of other soil organisms are not recognised, the effects of mycorrhizas might be under or overstated	Identify any organisms that affect soil quality, plant growth and mycorrhizal function and quantify impact if possible
Presence of plant diseases and disease vectors	The presence of plant diseases and their spread by vectors will inhibit plant growth and may affect mycorrhizal function	Negative	If presence of plant diseases and other stressors is not recognised, their impact on plant growth and/or mycorrhizal function may distort mycorrhizal benefit assessment	Identify the impacts of plant diseases on plant growth and mycorrhizal function

(continued)

Table 2.4 (continued)

Variable	Potential effect	Impact	Risk	Risk minimisation strategy
Climate attributes	Variation in temperature, sunlight and rainfall might influence plant growth and mycorrhizal function	Positive or negative	Independent and interrelated climate attributes might lead to inaccurate assessment of mycorrhizal benefits	Identify independent and interrelated climate impacts on plant growth and mycorrhizal function
Interaction of variables	Identified variables might have a linear or exponential effect on mycorrhizal function	Positive or negative	Lack of recognition of interaction of variables might lead to inaccurate assessment of mycorrhizal benefits	Identify the extent and nature of all possible interactions between variables

In order to estimate economic values of mycorrhizas at paddock or farm level, various factors affecting mycorrhizal influences on plants and soil need to be assessed, characterised and quantified (Tables 2.2, 2.3 and 2.4).

2.6 Risk Minimisation Strategies

A simplistic way to obtain an estimate of the economic benefits of mycorrhizas is to estimate the value of crop production with and without mycorrhizas present, although this is difficult to do under field conditions (see Gazey et al. 2004). Given the wide range of variables influencing either production outcomes or profitability, as well the difficulties associated with accurate measurement of the mycorrhizas themselves, it is important to employ risk minimisation strategies and to monitor and control, as far as possible, the conditions under which such an estimate is made.

Risk minimisation strategies can be taken into account to deal with some or all of the factors that impede realistic economic valuation of mycorrhizas. Some of the risks apply widely, but others are more farm specific or even paddock specific. Clearly the range of crops, soil, disease and climate conditions is almost limitless although we have attempted to identify the risks and variables inherent in this type of assessment. In the first instance, case studies should be implemented on a farm-by-farm basis whereby the independent variables associated with cropping regime, climate, soil conditions, disease organisms and vectors can be held reasonably constant with the presence and nature of mycorrhizas being characterised. While it may not be possible to cultivate a plot devoid of mycorrhizas if, within the same vicinity, a plot with a significantly different mycorrhizal profile can be identified, then any difference in productivity can be attributed to the difference in

mycorrhizal profile (see Gazey et al. 2004). A dollar value can then be calculated for the mycorrhizas present, at least in terms of production.

If case studies for multiple farms that accommodate identified risks and conditions can be designed for a range of cropping regimes, their benefits for different production systems and environments can be estimated and the magnitude of their influence on soil health can be inferred. In this way, evidence of the overall economic benefits of mycorrhizas in agricultural and horticultural ecosystems can be painstakingly constructed (Table 2.4). Because different crops have different responses to and aptitude for mycorrhizal colonisation, it is very important to ensure that the case studies cover a wide range of crops. As Smith and Read (1996, p. 454) have observed: 'Both cultivation and monoculture appear to change the species composition of the fungal populations and reduce their diversity, but the impact of these changes on crop production has not been adequately evaluated'. It is thus likely that mycorrhizas not only respond differently to different regimes but that their benefits might vary significantly between agricultural and natural ecosystems: they not only constitute an important element of biodiversity but they also respond to ecosystem biodiversity.

2.7 Conclusion

Although some of the contributions of mycorrhizas are well documented for reasons mentioned earlier, it is difficult to quantify their economic benefits in agricultural ecosystems. This is because there has been little work done, either to identify systematically all such benefits or to identify how variables that influence mycorrhizal function might interact with each other to influence overall benefits. To complicate matters further, it is possible that the nature and magnitude of such benefits might be site specific, so that all possible mycorrhizal impacts for specific rotations in specific paddocks during a particular season might need to be considered. Numerous studies have claimed explicit benefits for soil health and agricultural production from mycorrhizal colonisation. For example, Chaurasia (2004) viewed AM as having universal benefits for agriculture as well as for forests and other ecosystems, Smith and Read (1996, 2008) and Gazey et al. (2004) discussed their potential for improving crop productivity, while Delian et al. (2011) specifically referred to their role in increasing profitability. Albrechtova et al. (2012) mentioned their 'numerous benefits for sustainable crop production' as well as their possible role in the decontamination of soils polluted by residual organophosphates. Brito et al. (2012) also saw arbuscular mycorrhizas as having an important role in sustainable crop production, while other authors (e.g. Smith and Read 1996; Schulz 2001) explicitly mentioned economic benefits. It is important to note that all of the benefits mentioned above are, in fact, economic benefits. While most people tend to think of economics as particularly relating to commerce or finance, anything through which benefits accrue to humanity is deemed to be economic ('economics' means 'humanity's household', while 'ecology' means 'nature's household'). This

is one reason that the importance of nonmarket (intangible) values has been stressed here, especially as it relates to soil biodiversity. In its briefing paper, 'Valuing Nature', UNEP (2014, p. 1) observed that 'Part of the challenge is that the sheer range of benefits from ecosystems is often poorly understood. The term "ecosystem services"—the benefits derived from nature—is a useful concept for making the value of nature more explicit and relevant to human well being'. As mycorrhizas are part of soil biodiversity, and that they are part of an agricultural ecosystem, the 'sheer range' of benefits even from a relatively small-scale ecosystem is difficult to reflect accurately. While it is possible that unidentified elements and unknown benefits of mycorrhizas might be omitted from agricultural studies, thus reducing perceptions of their economic worth, it is also probable that their presence in agricultural ecosystems will have wider, undervalued, benefits to natural ecosystems, and vice versa.

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Chapter 3
Contribution of Dynamics of Root Colonisation by Arbuscular Mycorrhizal Communities to Ecosystem Function

Sutarman Gafur

3.1 Introduction

Arbuscular mycorrhizas (AM) form potentially symbiotic associations between species of Glomeromycota fungi and the roots of the majority of vascular plant species (Smith and Read 2008). Roots from natural ecosystems contain various mycorrhizal taxa (Brundrett and Abbott 1991, 1994; Clapp et al. 1995; Merryweather and Fitter 1998b; Moyersoen and Fitter 1999; Helgason et al. 1999). Most soils contain communities of AM fungi (Cuenca et al. 1998; Smilauer 2001). Investigation of root colonisation by communities of AM fungi has been hampered by the difficulty in distinguishing among fungi inside roots, but some morphological characteristics (Abbott 1982; Merryweather and Fitter 1998a) and molecular techniques (Turnau et al. 2001; Kohout et al. 2013) are overcoming these limitations.

The dynamics of root colonisation by communities of AM fungi is associated with their capacity to form propagules, their tolerance of environmental conditions and their competitive ability (Abbott and Gazey 1994). It is also influenced by the host plant through the availability of carbon substrates needed for fungal growth (Pearson et al. 1994) and root architecture (Hetrick 1991). The form and infectivity of propagules of AM fungi within soil are also important (Brundrett and Abbott 1991), and this in turn is associated with prior colonisation of roots, and relative susceptibility of plant species present to colonisation by different fungi present in the community.

Mycorrhizal communities are complex (Read 1991; Holland et al. 2014) and the fungi can interact with each other within these communities (Pearson et al. 1994). As a result of these interactions, the relative abundance of infective spores and

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hyphae in soil varies in association with different life cycle strategies of AM fungi. An understanding of the dynamics of colonisation of roots by communities of AM fungi is required for predicting the likely success of inoculation with introduced fungi (Dodd et al. 2002; Bell et al. 2003) and for selecting soil management procedures (Abbott and Gazey 1994) for maximising their benefits.

3.2 Colonisation of Roots by AM Fungi

The emergence of molecular tools for identifying AM fungi in roots has highlighted the complexity of root colonisation by these ubiquitous soil organisms (Holland et al. 2014) although not all are extremely diverse (Shi et al. 2012). As root colonisation by communities of AM fungi is dependent on the characteristics of the host plant, the soil as a medium for the plant to grow in and the characteristics of the fungi, the relative abundance of fungi present can change over time (Sanchez-Castro et al. 2012).

The infectivity of fungi can be associated both with differences in inoculum level and differences in their ability to colonise roots (Srivastava et al. 1996; Wilson and Tommerup 1992). Abbott et al. (1992) also explained that competitiveness among AM fungi depends on their relative infectivity but that the quantity of inoculum can interfere with this relationship. The types of propagules include hyphal fragments, living and dead/dried roots and spores. The relative abundance of forms of inocula can contribute to the infectivity of the fungi to different extents. Although dried roots of subterranean clover contained an effective form of inoculum for several AM fungi, they were ineffective as inocula for other AM fungi (Tommerup and Abbott 1981). Differences among AM fungi include physiological processes such as in bidirectional nutrient transport in fungal hyphae or in carbon metabolism as was indicated by the decline in infectivity following sporulation by *S. calospora* (Pearson and Schweiger 1994) and *A. laevis* (Jasper et al. 1993). In addition to the propagule characteristics of AM fungi, their capacity to colonise roots also depends upon how they react to chemical, physical and biological properties of soil. Soil organic matter, including plant root residues, influences soil structure, soil pH, nutrient and soil water-holding capacity, all of which, alone or in combination, can influence mycorrhizal colonisation (Gaur et al. 1998; Hamel et al. 1997; Nadian et al. 1998).

Mycorrhizal fungi have the potential to interact with a wide range of other soil organisms in the root, in the rhizosphere and in the bulk soil (Linderman 1992; Andrade et al. 1998). These interactions have a range of effects; some are competitive and others may be mutually beneficial. Effects can be seen at all stages of the AM fungal life cycle, from spore population dynamics through to root colonisation and external hyphal growth (Fitter and Garbaye 1994). Increased branching and orientation of the hyphae towards the root may enhance the subsequent process of colonisation (Tamasloukht et al. 2003), and AM fungi may produce substances that

are antagonistic to other rhizosphere organisms (Anderson 1992) which in turn could influence colonisation of roots.

Root anatomy can influence mycorrhizal colonisation, but AM fungi can also influence root structure. Plant species that have few root hairs are strongly mycorrhiza-dependent in phosphorus-deficient soils (Crush 1974; Schweiger et al. 1995). Secondary thickening of cell walls can affect colonisation, with invading fungi tending to penetrate and colonise cells that have little or no suberin deposition (Srivastava et al. 1996). AM fungal hyphae can grow easily on and inside young roots without secondary thickening; certain areas of a root may also be more susceptible to colonisation. Intercellular hyphae are formed more frequently as the fungi spread into the inner cortex and colonisation can be intense in the deeper layers of root cortex where fungi form complex intracellular arbuscules (Bonfante-Fasolo and Vian 1989). Differences in colonisation due to root diameter were highlighted by Fitter and Merryweather (1992) supporting evidence that plants with coarser roots and few root hairs were more typically mycorrhizal (Baylis 1970), but the extent of colonisation of fine-rooted plants varies with fungal isolate (Schenck and Smith 1982).

Root density, distribution and growth rate are each relevant to the formation of a primary entry point by AM fungi because the fungus must first intercept a root (Abbott et al. 1984). The intercepting hyphae may grow from fungal propagules in soil or from ramification of hyphae associated with mycorrhizal roots. Root density differs among plant species and over time and has the potential to influence colonisation by AM fungi. Therefore, consideration of the structure and distribution of roots (Hetrick 1991) are important factors to be taken into account in preparing a strategy to improve colonisation of roots by AM fungi.

Root exudates can influence root colonisation by AM fungi through influences on hyphal growth in soil and spore germination both positively and negatively (Gianinazzi-Pearson et al. 1990; Graham 1982). Elias and Safir (1987) observed that hyphal elongation of *G. fasciculatus* was enhanced by exudates from *Trifolium repens* but only when the plants were grown under phosphate deficiency. The function of the exudates as a promoter of fungal growth decreased when phosphate deficiency was overcome. In contrast, root exudates from non-mycorrhizal and mycorrhizal peas inhibited hyphal growth of *Gigaspora margarita* (Balaji et al. 1995). Mycorrhizal *Pisum sativum* and its non-mycorrhizal isogenic mutant were not different with respect to effects of their root exudates on *G. mosseae* (Giovanetti et al. 1993). The amount, type and complexity of the volatile compounds exuded from roots have the potential to influence fungal reactions before the fungus meets the root as well as after the hyphae contact the root. In addition, later stages of mycorrhiza development, such as the formation of appressoria and hyphal penetration of the root surface, may involve exudate molecules in recognition processes (Koske and Gemma 1992).

3.3 Relative Abundance of AM Fungi Within Roots

The relative abundance of AM fungi within roots is influenced by changes in environmental conditions and interactions with host plants (Sanchez-Castro et al. 2012). As this depends on interactions between fungi, environmental changes and presence of host plants, it is difficult to formulate a simple mechanism for the process of root colonisation by communities of AM fungi. Knowledge of phenomena related to both the quantity and types of propagules within the soil may not be sufficient for predicting the dynamics of root colonisation (Bowen 1987). Furthermore, one of the characteristics of communities of AM fungi is strong unevenness in the relative abundance of fungi (Allen et al. 1995; Brundrett and Abbott 1995). Thus, investigations of characteristics of individual AM fungi merely provide a starting point for understanding processes involved during simultaneous colonisation of roots by genetically diverse fungi.

Before microscopic observation of colonisation of AM fungi in roots, root sample is first cleared and stained using classic stains such as trypan blue (Phillips and Hayman 1970), acid fuchsin (Saito et al. 1993) and chlorazol black E (Brundrett et al. 1984). Other visual techniques involving staining include enzyme analysis (Rosendahl and Sen 1994; Tisserant et al. 1998) and fluorescent antibody techniques (Wilson et al. 1983). The emergence of molecular tools has greatly expanded the ability to detect communities of AM fungi in roots (Kohout et al. 2013), and they are occasionally used in conjunction with morphological methods (Shi et al. 2012). Morphological observations enable structural features of AM fungi in roots (Dickson 2004). There are discrepancies between quantifications of relative abundance of AM fungi in roots using both morphological (Abbott 1982; Merryweather and Fitter 1991) and molecular approaches (Robinson-Boyer et al. 2009) which are further highlighted when the same roots are assessed using different methodologies (Shi et al. 2012).

There have been many studies of the diversity of AM fungi in field soils based on spore type and abundance (e.g. Brundrett and Abbott 1994; Cuenca et al. 1998; Franke-Snyder et al. 2001). However, there is little relationship between the presence and abundance of spores of particular species of AM fungi in soil and the extent to which they are present within roots growing in the soil (Merryweather and Fitter 1998a; Scheltema et al. 1987). This may be partly due to preferential colonisation of roots (Helgason et al. 2002) and to host-dependent patterns of colonisation (Bever et al. 1996).

The abundance, distribution, effectiveness and aggressiveness of each AM fungus species within a community are determining factors of competitive success (Graham and Abbott 2000; Wilson and Tommerup 1992). High density of fungal propagules and localised distribution increased the rate of colonisation of roots compared to that of low density and dispersion of fungal propagules in soil (Wilson and Trinick 1983). Critical density levels for propagules may vary with species of fungus. In field soil, propagule densities can be highly variable even between adjacent soil cores (Brundrett and Abbott 1995). Furthermore, AM fungi differ in

their biological characteristics such as spore dormancy period and type of propagules (Abbott et al. 1992; Tommerup 1983; Tommerup and Abbott 1981) which combined with heterogeneity in distribution of infective propagules in soil will lead to difference in the proportion of each AM fungus within roots from time to time.

As AM fungi differ in their tolerance of conditions such as soil pH, nutrient content, water-holding capacity, soil organic matter, soil disturbance and other soil organisms, it is expected that these characteristics may result in the dominance of certain AM fungi in soil. Consequently, the dominance of fungi within the roots might change, but not necessarily in direct proportion to the abundance of spores (Merryweather and Fitter 1998a; Scheltema et al. 1987).

While most AM fungi can associate with a wide range of hosts, their performance relative to each other depends on the host characteristics. A comparison of inoculation with AM fungi on different host plants showed that *Polianthes* was highly colonised compared to *Capsicum* (Gaur et al. 1998). Studies of host dependence and species diversity of AM fungi in grassland (Bever et al. 1996) demonstrated that co-occurring plant species supported very different rates of sporulation by AM fungi. These differences were not affected by the time of sampling, suggesting that they reflect host-dependent differences in fungal growth rates, rather than host-dependent timing of sporulation. It was hypothesised that the host dependence of the relative growth rates of fungal populations may play an important role in the maintenance of AM fungal species diversity (Bever et al. 1996). Thus, the relationship between sporulation and the extent of colonisation by AM fungi is complex (Douds and Schenck 1990; Gazey et al. 1992) further compounding the dynamics of mycorrhiza formation by individuals within communities of AM fungi.

There is a high degree of variability in mycorrhizal dependency among host plants (Hoeksema et al. 2010). Plants range from highly dependent, whereby the plants are unable to survive without mycorrhizas in highly phosphate-deficient soils, to low dependency, where plants can survive under some conditions without mycorrhizas when phosphate is highly deficient (Janos 1980). This could be related to the fact that species of AM fungi differ in their ability to take up phosphorus and transfer it to the host plant (Solaiman and Abbott 2008) as well as to preferential colonisation of roots of some plants by some AM fungi (Helgason et al. 2002). Further investigation of the relevance of diversity within communities of AM fungi and mycorrhizal dependency is recommended.

Soil disturbance, including soil removal during mining operations (Jasper et al. 1989a, b, c, 1992), soil erosion (Powell 1980), mechanical disruption (Cuenca and Lovera 1991) and tillage (Kabir et al. 1997; Jansa et al. 2003) can reduce the abundance of AM fungi in roots. Species diversity in a community of AM fungi may also be reduced (Cuenca and Lovera 1991). Soil disturbance may have an indirect effect on the presence and abundance of AM fungi within roots by reducing the inoculum potential of members of the community of AM fungi present. However, the percentage of root length colonised by AM fungi in soil from an annual pasture was not decreased after disturbance, whereas colonisation of plants grown in disturbed soil from forest or heathland was only half that of the undisturbed soil

(Jasper et al. 1991). These differences were correlated with the number of infective propagules that survived the disturbance treatment. Another study on propagules of AM fungi in a disturbed habitat in the Kakadu region of tropical Australia revealed that propagules of AM and ectomycorrhizal (ECM) fungi occurred in all sites (Brundrett et al. 1996) and were sporadically distributed in highly disturbed areas. Both the relative abundance and frequency of occurrence of inoculum of AM and ECM fungi increased with vegetation cover in the older disturbed sites examined in this study.

Soil disturbance may change the effect that these communities of AM fungi have on host plants depending on how the disturbance influences each fungus and how it affects the way the fungi influence each other. In highly disturbed environments, low inoculum densities can lead to slow or low levels of colonisation of roots (Bellgard 1993; Jasper et al. 1989b). In contrast, seedlings in undisturbed vegetation may become rapidly colonised when their roots contact existing mycorrhizal hyphae in shallow layers of soil, reflecting a greater concentration of propagules in surface soils (Bellgard 1993).

Mining activities can reduce the abundance of propagules of AM fungi (Jasper et al. 1992) and destroy their infectivity in relocated topsoil (Jasper et al. 1989b). Severe soil disturbance has the pronounced effects of separating much of the external hyphae from the host root and of breaking up the soil hyphal network (-Jasper et al. 1989c; Miller and Jastrow 1990). Therefore, AM fungi which depend on intact hyphae as propagules may be less competitive in forming mycorrhizas than those which rely on more robust propagules such as spores when soil is disturbed.

3.4 Function of Communities of AM Fungi

As field soils contain communities of AM fungi, there can be no single mycorrhizal effect on plant growth. However, due to the difficulties in studying AM fungi as communities, most investigations of the function of AM fungi focus at the level of an individual species or isolates of AM fungus under controlled conditions. Nevertheless, the function of AM fungi varies depending on the environmental conditions, plant and AM fungus species, and observations of function under field conditions are the result of combined effects of dynamic communities.

The hyphae of AM fungi can extend up to several centimetres from the root, effectively extending the zone of nutrient depletion around roots to absorb immobile elements from the bulk soil (Jakobsen et al. 1992). A high diversity of AM fungi may be important for buffering an ecosystem against disturbance (Vogt et al. 1997). The number of AM fungal spores and abundance of mycorrhizal roots fluctuate with season (Brundrett and Kendrick 1988) associated with differences in life cycles and spore dormancy periods, but the effect of this instability could be minimised when the fungi are present in communities if they have complementary effects (Koide 2000). Consequently, the fluctuation in root

colonisation levels is expected to be less when roots are colonised by several species of fungi than if a single fungal species is present. The extent to which this influences mycorrhizal function is less clear.

3.5 Conclusion

The dynamics of root colonisation by communities of AM fungi occur in parallel with the changes in abundance of individual species of AM fungi. Characteristics of the fungi play a significant role in the fluctuation of root colonisation and demonstrate that it is not easy to predict root colonisation dynamics by communities of AM fungi based on the knowledge of the individual characteristics of AM fungi within the community. Differences in physiological characteristics of members of the AM fungal community are likely to influence the dynamics of root colonisation. Soil disturbance can also affect different AM fungi in different ways and this will alter the relative abundance of AM fungi in soil and in roots. This differential sensitivity and tolerance of particular fungi to soil characteristics and soil disturbance will also be dependent on the propagule potential in soil and emphasises that short-term studies overlook the possibility of identifying long-term contributions of communities of AM fungi in field soils.

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Chapter 4
Biofertilizers with Arbuscular Mycorrhizal Fungi in Agriculture

Olmar B. Weber

4.1 Introduction

An increase in crop production and land productivity in agriculture is necessary to meet the demand for food. If agricultural systems are to be sustainable in maintaining soil fertility and soil structure over a long period of time, they need management strategies that are economically viable, environmentally safe, and socially fair with soils managed to safeguard food security (Killham 2011). One strategy involves fertilizer (NPK) replacement by compounds that are less polluting, less persistent in soil, and less energy consuming (Lichtfouse et al. 2009). Considering that fluctuating crop and fertilizer prices occur and farmers rarely reduce expenditure on fertilizers, a knowledgeable farmer will use inputs to reach economical profitability, environmental safety, and social fairness farming systems. Another strategy involves applying ecological concepts and principles to the design, development, and management of sustainable agricultural systems (Lichtfouse et al. 2009).

Microbial inoculants, including arbuscular mycorrhizal (AM) fungi and plant growth-promoting rhizobacteria, are potential components of sustainable management systems (Adesemoye and Kloepper 2009). AM fungal inoculants are marketed as an important biological component to commercial horticulture and agriculture, but for successful application of AM fungi with economically profitable results, the soil environment must be suitable for the development of the AM fungal symbiosis (Baar 2008).

Companies have taken different market approaches for microbial inoculants, ranging from products with AM fungi alone to mixed products (Baar 2008). In order to exploit beneficial effects of AM fungi in sustainable agriculture,

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appropriate management practices have to be applied (Vosátka and Albrechtova 2009). By better understanding mycorrhizal symbioses for optimization of plant–soil systems, the need for biofertilizers that include inoculants of AM fungi in agricultural production will be clarified.

4.2 Fertilizer Use in Agriculture

The increase in food production and land productivity in agriculture has been due to the use of fertilizers, especially NPK, and significant increases in their use in the future are predicted (FAO 2010). As a result of extensive use of fertilizers (Vance 2001), the agrochemicals and agronomic practices commonly adopted in intensive production systems have generated environmental problems including deterioration of soil quality, surface water, and groundwater, and reduced biodiversity and function of ecosystems. An alternative way to increase soil fertility and supply nutrients based on the efficient use of mineral and manufactured fertilizers is to intensify the use of biofertilizers. The ability of the root systems to establish symbiotic relationships with soil microorganisms of the rhizosphere represents one strategy that land plants have developed to survive the abiotic and biotic stresses (Allen 1996). This approach is economically viable, environmentally safe, and socially fair (Lichtfouse et al. 2009). For instance, sustainable crop productivity depends largely on soil biological fertility, defined by Abbott and Murphy (2007) as the ability of soil organisms to contribute to the nutritional requirements of plants and foraging animals for productivity, reproduction, and quality while maintaining the biological processes that contribute positively to the physical and chemical states of the soil.

The use of biofertilizers as a source of plant nutrients for more sustainable agricultural practices (Gentili and Jumpponen 2006) involves a range of soil microorganisms including AM fungi which have potential to enhance productivity in combination with a reduction in application of mineral phosphate fertilizer (Marin 2006). AM fungi form symbiotic associations with the vast majority (>80 %) of families of land plants, but some members of the families *Brassicaceae*, *Chenopodiaceae*, *Caryophyllaceae*, *Cyperaceae*, *Juncaceae*, and *Amaranthaceae* do not form mycorrhizal associations (Cardoso and Kuyper 2006). Plant growth response to mycorrhizas can be positive, neutral, or negative (Smith and Smith 2011). In contrast to nitrogen-fixing bacteria, AM fungi do not add mineral nutrients to the soil and are therefore not strictly biofertilizers (Cardoso et al. 2010). There are various types of microbial cultures and inoculants available on the market today, and results are often difficult to extrapolate to field conditions.

4.3 Analysis of Biofertilizers with AM Fungal Used in Agriculture

AM fungal inoculants (Table 4.1) in commercial products (or biofertilizers) usually contain one or a few species of *Glomus*, particularly *G. intraradices* (Akhtar and Siddiqui 2008; Baar 2008; Öpik et al. 2008). Specially designed agricultural machines can be used to inoculate the seed or distribute AM fungal inocula with a relatively high speed over a large area (Baar 2008).

Murphy et al. (2007) reported that the performance of microbial inoculants in the field can be inconsistent. Measurement of mycorrhizal colonization of roots is usually used in bioassays with commercial products, but calibrations that take into account an understanding of the relationships between mycorrhizal colonization and soil conditions are necessary (Djuuna et al. 2009). Consistency of plant responsiveness to inoculation with selected strains of AM fungi should be a prerequisite to adoption of AM inoculation practices.

AM fungal inocula are commonly produced in association with host plants in a greenhouse or in growth chambers using "pot cultures" containing expanded clay and vermiculite (Gentili and Jumpponen 2006; Vosátka et al. 2008; IJdo et al. 2011). Other production techniques as well as substrate-free culture techniques (hydroponic and aeroponic) and in vitro cultivation methods have been attempted (IJdo et al. 2011), and higher quality of commercial products can be expected in the future.

The producers of AM fungal inoculants should specify the quantity and quality of the mycorrhizal fungi present in the marketed products. Accordingly to Wiseman et al. (2009), if manufacturers of commercial AM fungal inoculants desire large-scale acceptance of mycorrhizal technology, they must better demonstrate that their products are compatible with current retail distribution methods and can promote mycorrhizal colonization under the conditions of their intended use. Vosátka et al. (2008), when analyzing the market development for mycorrhizal technology, noted that while industry has developed inoculum using AM fungal strains, quite often they are not well characterized in terms of ecological requirements and stability. The success of an inoculum formulation depends on whether it is (1) economically viable to produce, (2) unaltered in viability and function, and (3) easy to carry and disperse during application.

A basic step is the AM fungi selection that depends on environmental conditions of the locality, soil, and host plant. AM fungi must be able to colonize roots rapidly after inoculation, absorb phosphate from the soil effectively and transfer it to the plant, persist in soil and reestablish mycorrhizal symbiosis during the following seasons, and form propagules that remain viable during and after inoculum production (Abbott et al. 1992; Tanu Prakash and Adholeya 2006). Selection of AM fungi may also consider tolerance to abiotic stress and resistance to soil pathogens. There are studies and comprehensive reviews exploring possibilities for AM fungi to protect endangered plants and habitats (Bothe et al. 2010), alleviation of salt stress (Evelin et al. 2009; Kaya et al. 2009; Mardukhi et al. 2011), bioprotection

Table 4.1 Examples of the use of biofertilizers containing AM fungal inocula

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
Commercial MicoFert® (Cuba) containing a mixture of AM fungi-colonized soil and AM fungi-colonized root fragments was used in high-input coffee plants grown in different coffee plantation soils	Study based on the results of 62 inoculation trials using soils from Cuban plains with a history of high-input agriculture and 68 trials conducted with pristine or seminatural soils, which were under low-input under-story coffee production	There was no significant relationship between plant response to AM fungal strains and soil properties in the high-input agriculture soil data set, may be due to variation induced by the use of different host plant species and to modification of soil properties by a history of intensive production. Findings indicate that AM strains must not only be highly effective, they must be able to function in the soil environment where they are introduced	Herrera-Peraza et al. (2011)
Commercial Mycorise (Canada) AM-1207 containing *G. intraradices* combined with one exotic *Glomus intraradices* (isolate AM-1004 from Canada) and mixed AM fungi (AM-1209, native from India) containing *Glomus*, *Gigaspora*, and *Scutellospora* spp.	Field trial with carrot (*Daucus carota* L.) to compare three AM inocula on the formation of infectious propagules in different fractions of inocula in a marginally sandy loam Alfisol amended with farmyard manure	The exotic isolate AM-1004 and mixed AM fungal (*Glomus*, *Gigaspora*, and *Scutellospora* spp.) inocula used either as roots, soil, or a mixture of both have a greater potential in producing more propagules in the shortest span of time. *G. intraradices* (AM-1004), root based, and the indigenous mixture (AM-1209), as crude inoculum, can be used as starter cultures for on-farm production of these AM fungal inocula	Sharma and Adholeya (2011)
Commercial Aegis Argilla (Italy) containing clays as granular carriers, leek root pieces, and *Glomus intraradices* spores	Greenhouse experiment with two cucumber (*Cucumis sativus* L.) genotypes (hybrid "Ekron" and variety "Marketmore") were inoculated with AM fungi grown in pots containing quartziferous sand mixed with slow-release fertilizer, pH values 6.0 and 8.1	The inoculated plants under alkaline conditions had higher total, marketable yield, and total biomass than noninoculated plant Mycorrhizal cucumber plants grown under alkaline conditions had a higher macronutrient concentration in leaf tissue compared to noninoculated plants	Rouphael et al. (2010)

(continued)

Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
Commercial TerraVital Hortimix (UK) containing *Glomus mosseae*, *G. intraradices*, *G. claroideum*, and *G. microaggregatum*, >50 infective units ml^{-1}) combined without and 135 mg P kg^{-1}	Greenhouse trial with cowpea (*Vigna unguiculata* L. Walp cv. IT18) on washed sand and rock phosphate treatments supplemented with nutrient solution and mycorrhizal inoculum	AM fungi are able to increase plant availability of RP when NO^{-3} is the major form of N in the soil and the substrate pH is in a neutral range Increased supply of NH^{-4} relative to NO^{-3} improved plant P availability from rock phosphate but also had a negative effect on the extent of AM fungal root colonization, irrespective of the plant P-nutritional status	Ngwene et al. (2010)
Commercial Endol® Biorize Sarl (France) combined with the inoculants of *Glomus mosseae* (isolates BEG 12, BEG 167), *G. intraradices* (BEG 141), and *G. etunicatum* (isolates BEG 168 and HB-Bd45)	Sweet potatoes (*Ipomoea batatas* L.) were inoculated with various combinations of AM fungi and grown under traditional condition in China	Inoculation with *G. intraradices* BEG 141 or *G. etunicatum* (HB-Bd45-Gsp4, BEG 167, and BEG 168) increased yields (10.2 and 14.0 %) AM fungi varied in their ability to establish after inoculation and in their effect on yield and quality of sweet potato tubers	Farmer et al. (2007)
Commercial products: TerraVital Hortimix (UK) containing *Glomus mosseae*, *G. intraradices*, *G. claroideum*, and *G. microaggregatum* (>50 infective units per ml inoculum); Endorize-Mix (France) with the *G. mosseae*, *G. intraradices*, and *Glomus* sp. (infective units not specified); and AMYkor (Germany) with *G. mosseae*, *G. intraradices*, and *G. etunicatum* (50 infective units per ml inoculum)	Pot experiment with horticultural pelargonium (*Pelargonium peltatum* L'Her.) on compost substrates and commercial AM inocula	The inoculation of three different commercial AM inocula resulted in colonization rates of up to 36 % of the total root length. Addition of compost in combination with mycorrhizal inoculation can improve nutrient status and flower development of plants grown on peat-based substrates	Perner et al. (2007)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
Two commercial biofertilizers: BioLife (USA), containing a combination of 13 bacterial strains, and Media Mix (USA), containing spores of four species mycorrhizal fungi as well as N_2-fixing and P-solubilizing bacteria combined with mineral fertilizers	Greenhouse trial with tomato plants (*Solanum lycopersicum* L. var. Belle) applying commercial inoculants and conventional fertilization (115 kg N ha^{-1}, 69 kg P ha^{-1}, 366 kg K ha^{-1} and 100 kg Mg ha^{-1})	The tomato yield can be increased by 32.0 % when using bacterial fertilizer BioLife and by 22.9 % when using mycorrhizal inoculum Media Mix, compared with conventional fertilization. The total energy input required in unheated greenhouses increases by 1.88 % with the application of BioLife and by 1.38 % with the application of Media Mix; however, they decrease by 22.31 % and 16.92 %, respectively, per ton of tomato production	Mihov and Tringovska (2010)
Commercial MYKE® PRO SG2 (Canada) containing a *G. intraradices*, covered by 1.3 kg of pasteurized soil, MYKE® PRO covered by unpasteurized soil, and control with pasteurized soil	Glasshouse experiment with maize (*Zea mays* L. hybrid IC 192) and AM fungal inoculant	The inoculant significantly improved the P content of the host in the presence of the resident AM fungal community. In contrast to inoculation, soil disturbance had a significant negative impact on species richness of AM fungi and influenced the AM fungal community composition as well as its functioning	Antunes et al. (2009)
Nine commercial AM fungal inoculants (seven granular and two root dipped) purchased through typical consumer channels	Greenhouse experiments using corn (*Zea mays*), sorghum (*Sorghum bicolor*), trident maple (*Acer buergerianum*), and sweet bay magnolia (*Magnolia virginiana*) as host plants and AM fungal inoculants	Commercial AM fungal inoculants had little effect on corn root mycorrhizal colonization when obtained anonymously through typical consumer channels and applied at the manufacturers' recommended rates In corn and sorghum, colonization rarely exceeded 5 % when plants were treated with commercial inoculants.	Wiseman et al. (2009)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
		Despite the near absence of colonization, commercial inoculants generally improved shoot growth and increased soil nutrient concentrations in a dose-dependent manner. Commercial inoculants tested had no effect on mycorrhizal colonization or shoot growth of trident maple or sweet bay magnolia liners	
Commercial Biorize Sarl (France) containing *Glomus mosseae* or *Glomus intraradices* was combined with a mixture of *Azotobacter chroococcum*, HKN-5, *Bacillus megaterium*, HKP-2, *Bacillus mucilaginosus*, HKK-2, organic fertilizer, and two levels (50 and 100 %) of NPK (300 mg N, 92.3 mg P, and 184.6 mg K per kilogram of dry weight of soil)	Greenhouse trial with maize (*Zea mays* L.) on peat moss-based bacterial inoculum and/or 15.0 g sand-based mycorrhizal inoculum	The use of biofertilizer (*G. mosseae* and three bacterial species) resulted in the highest biomass and seedling height Microbial inoculum increased the nutritional assimilation of plant (N, P, and K) and improved soil properties, such as organic matter content and total N in soil The presence of mycorrhizal fungi had different influence on the population of rhizobacteria. *G. intraradices* was able to stimulate the introduced beneficial bacterial growth in the rhizosphere soil. However, the high mycorrhizal infection with *G. mosseae* showed a strong inhibition of P and K solubilizers	Wu et al. (2005)
Commercial Mycogold (Malaysia) containing *Glomus* spp. (infective units not specified) combined with two native communities of AM fungi (Brazil):	Greenhouse experiment with dwarf cashew (*Anacardium occidentale* L. clone CCP76) without and with 87 mg de P l^{-1} soil and AM fungal inocula	The cashew seedlings presented a low response to the phosphorus treatment. The symbiotic plant association with *Glomus etunicatum*, *G. glomerulatum*,	Weber et al. (2004)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
(1) *Glomus etunicatum*, *G. glomerulatum*, *Scutellospora* sp., and *Acaulospora foveata* and (2) *G. etunicatum*, *Entrophospora* sp., and *Scutellospora* sp.		*Scutellospora* sp., and *Acaulospora foveata* (native community) and the commercial product allowed a better plant growth 4 months after cashew nut sown	
Seven commercially mycorrhizal inoculants obtained from different companies (USA)	Experiments with strawberry (*Fragaria* × *ananassa* Duch.) cultivars were evaluated side by side in organic strawberry production fields in central California	None of the seven commercially prepared mycorrhizal inoculants tested resulted in an increased marketable fruit yield in organic or non-fumigated fields. In one of six experiments, a commercial inoculant increased total yield over the nontreated control but did not influence marketable fruit yield	Bull et al. (2005)
Eight commercial AM fungal inoculants obtained anonymously (USA)	Greenhouse trial with maize (Z. mays L. var. Golden Cross) on sand/peat medium inoculated with AM fungi	Only three of the commercial inocula formed mycorrhizas when used at the recommended rate, and the extent of colonization ranged from 0.4 to 8 %. The failure of five of the eight commercial inocula to colonize roots when applied at the recommended rate suggests that preliminary trials should be made before commercial AM fungal inocula are used in important landings	Tarbell and Koske (2007)
Ten commercial mycorrhizal inoculants (six contains *G. intraradices*, three with one or more fungal species, and one endo-/ectomycorrhizal inoculum) were used in nursery conditions	Experiments with sweet corn (*Zea mays* L. hybrid Silver Queen) grown in a soil-based medium and in two different soilless substrates, a potting mix prepared with redwood bark, pine sawdust, calcined clay and sand, and the commercial Sunshine mix, mainly composed of sphagnum peat moss	Only the plants inoculated with the products that did not promote mycorrhizal colonization increased their growth relative to the noninoculated controls, suggesting the presence of other growth promoters in the inoculum products. Based on these results, nurseries should	Corkidi et al. (2004)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
		conduct preliminary tests to determine which inoculants will perform in their potting mixes to assure the best fit of inoculum with their particular conditions	
Different AM fungal species *Glomus mosseae*, *G. etunicatum*, and *G. intraradices* isolated from saline soils (Iran) were tested under saline conditions	Greenhouse and field trials with three wheat cultivars (Roshan, local; Kavir, genetically modified; and one mutated line, Tabasi) combined with and without AM fungal species	In both experiments, AM fungi significantly enhanced the concentrations of macro and micro-elements. The results indicated that the selected combination of AM species and wheat cultivar can result in the highest rate of nutrient uptake by wheat plants under saline conditions	Mardukhi et al. (2011)
Different AM fungal inoculants from soils of high-input continuous maize fields, low-input continuous maize fields, and undisturbed native vegetation were used	Greenhouse trials with maize (*Zea mays* L. cv. Landrace and hybrid H5), to assess the mutualistic functioning of AM fungi and the mycorrhizal responsiveness of maize genotypes	When maize was grown in field soil brought into the greenhouse, AM fungi and communities of other soil organisms did not benefit plant growth in high-fertility soil, but they did improve maize growth in low-fertility soil Landrace maize was more responsive to mycorrhizas than hybrid maize, and novel soil inoculum was more beneficial than inoculum from sites where the crop and organisms have long coexisted	Martinez and Johnson (2010)
Different AM fungal inoculants were used in greenhouse: *Glomus mosseae* (isolate IMA1 from the UK and isolate AZ225C from the USA) and *Glomus intraradices* (isolate IMA5 from Italy and isolate IMA6 from	Greenhouse and field trials to assess the effect of native and exotic selected AM fungal inocula on plant growth and nutrient uptake in a low-input, 2-year forage legume (*Trifolium alexandrinum*) cv. Tigri	The use of AM fungal inocula may be very effective in improving crop productivity and quality in low-input agricultural systems and their effects are persistent at least until 2 years after inoculation.	Pellegrino et al. (2011)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
France). In field experiment were used the four *Glomus* isolates single and mixed and a native AM fungal inoculum (mixed fungal population)	and maize (*Zea mays*) crop rotation	Differences in isolate performances indicate that the choice of the best AM fungal inoculum for field utilization is pivotal for the success of inoculation practices The use of native AM fungi, produced on farm with mycotrophic plants species, might represent a convenient alternative to commercial AM fungal inocula and offer economically and ecologically important advantages in sustainable or organic cropping systems	
Communities of fungi obtained from soils of conventional and low-input cropping systems. The conventional cropping system with a nonleguminous 6-year crop rotation (barley–barley–rye–oat–potato–oat) received different fertilizer rates	Pot experiment with leek (*Allium porrum* cv. Titan), barley (*Hordeum vulgare* cv. Arra), flax (*Linum usitatissimum* cv. Linetta), alfalfa (*Medicago sativa*, unknown cultivar), red clover (*Trifolium pratense* cv. Björn), white clover (*Trifolium repens* cv. Huia), and subclover (*Trifolium subterraneum*, unknown cultivar)	*Glomus claroideum* was the most commonly identified single species in the experimental area. A bioassay using roots as inoculum for isolation and culture of dominating AM fungi was successfully developed and yielded only *G. claroideum*. Such dominating AM fungi seem to compete successfully against other indigenous AM fungal species and would therefore ensure a predictable impact of AM fungal inoculation in field conditions	Vestberg et al. (2011)
Mycorrhizal inoculants with species *Glomus mosseae* and *Entrophospora colombiana* (=*Kuklospora colombiana*)	Plants of papaya (*Carica papaya* L cv. Maradol) inoculated with AM fungi under controlled conditions and transplanted into an experimental orchard fertilized with NPK (235–42–222 kg ha^{-1})	Inoculation with *G. mosseae* and *E. colombiana* increased papaya yield by improving setting and fruit weight *G. mosseae* had better influence in various aspects, possibly due to greater association with	Vázquez-Hernández et al. (2011)

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Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
		papaya plants and better adaptability to edaphic and environmental conditions	
Mycorrhizal inoculant with *Glomus mosseae* was combined with P levels (18.3, 48, 79.4, and 100 mg kg^{-1})	Pot experiment with papaya (*Carica papaya* L. cv. Sunrise), and pineapple [*Ananas comosus* L. Merr.] were inoculated with AM fungal and cultured for 5 (papaya) and 7 months (pineapple)	Mycorrhizal papaya plants exhibited higher biomass and macroelement contents in shoots than plants without mycorrhizas at any P level Mycorrhizal effects on pineapple at the lowest P level were significant in terms of plant development and P shoot contents. Differential benefits derived from mycorrhization seem to be correlated to each crop's internal P requirements	Rodriguez-Romero et al. (2011)
Mycorrhizal inoculant with *Glomus mosseae* combined without, 80, and 160 kg N ha^{-1} and tilled and no-tilled soils	Pot experiment with wheat (*Triticum aestivum* L.) plants combined with AM fungal inoculant and conventional tilled and no-tilled soils	In no-tillage, the plant colonization was greater than in conventional tillage, but it was reduced by the N fertilization. In conventional tillage, the inoculation with *G. mosseae* increased colonization. Both conventional tillage and N fertilization promoted wheat root growth. Inoculation did not affect root growth but enhanced N concentration in roots when fertilizer was not applied	Shalamuk et al. (2011)
Different AM fungal inoculants: *Acaulospora foveata* (HR0602), *A. appendicula* (HR0201), *A. denticulata* (RA2106), *Glomus dimorphicum* (WH0101), *G. tenerum*	Greenhouse experiment with chili (*Capsicum frutescens* L.) inoculated with AM fungi and cultivated on fumigated soil	*Acaulospora appendicula* HR0201, *A. denticulata* RA2106, and *G. clarum* RA0305 were found to be efficient chili growth promoters, with *G. clarum* RA0305 being the best. These findings suggest	Boonlue et al. (2012)

(continued)

Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
(WH0102), *G. clarum* (A0305), *A. denticulata* (HR0406), *G. globiferum* (PY0109), *G. globiferum* (PY0103), and *G. globiferum* (PY0107)		the potential of *G. clarum* RA0305 for use as an AM fungal inoculum for the production of organic chili in Thailand	
Twenty-three different AM fungal inoculants: *Acaulospora delicata*, *A. rugosa*, *Gigaspora candida*, *Glomus aggregatum*, *G. albidum*, *G. aurantium*, *G. claroideum*, *G. clarum*, *G. coronatum*, *G. etunicatum*, *G. fasciculatum*, *G. geosporum*, *G. glomerulatum*, *G. hoi*, *G. intraradices*, *G. macrocarpum*, *G. mosseae*, *G. occultum*, *G. versiforme*, *G. xanthium*, *Glomus* sp. 2, *Glomus* sp. 4, and *Glomus* sp. 5	Greenhouse experiment with long pepper (*Piper longum* L.) and indigenous AM fungi from India	Considering the shoot length, total biomass, nutrient content, chlorophyll content, and root infection, pre-inoculation with 6 AM fungal species (*Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp. 2, *G. mosseae*, and *G. etunicatum*) appeared to be promising AM fungi for inoculating this medicinal plant	Gogoi and Singh (2011)
Mycorrhizal inoculant with *Glomus mosseae* combined with N_2-fixing *Bradyrhizobium* sp. (strain BXYD3)	Field and greenhouse trials with soybean (*Glycine max* L. Merr.) genotypes HN89 (P-efficient) and HN112 (P-inefficient) on soil with low available N and P content	Co-inoculation with rhizobia and AM fungi increased soybean growth under low P and/or low N conditions as indicated by increased shoot dry weight, along with plant N and P content. A synergistic relationship dependent on N and P status exists between rhizobia and AM fungi on soybean growth. The deep root genotype, HN112, benefited more from co-inoculation than the shallow root genotype, HN89	Wang et al. (2011)

(continued)

Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
Seven AM fungal consortia isolated from coffee plantations with different agricultural inputs (low, intermediate, and high) were used	Greenhouse and field trials with coffee plants (*Coffea arabica* L.) inoculated with different AM fungal consortia under greenhouse and transplanted onto a coffee field in Mexico	The most effective AM fungal consortia on plant growth promotion and survival under field conditions were collected from intermediate-input agricultural plantations, which also had the greatest number of AM fungal species	Trejo et al. (2011)
Different AM fungal inoculants (mix of *Glomus clarum* and *Gigaspora margarita* and five isolates of *Glomus etunicatum*) were combined with P rates (0, 20, 40, 80, and 160 g P2O5 plant^{-1})	Field trial with coffee (*Coffea* sp.) inoculated under glasshouse conditions and transplanted onto a farm with Oxisol in Minas Gerais (Brazil)	Coffee bean yield measured for 5 consecutive years, showing consistent effects of P application. Based on the total yield of five harvests, maximal productivity was achieved with a mix of *Glomus clarum* and *Gigaspora margarita* at 20 g P2O5 plant^{-1} and with the same mixed inocula and *G. etunicatum* (isolate Var) at the highest P rate. Pre-colonization of coffee outplants with selected AM fungi and application of low to moderate P rates at planting is advantageous for coffee production in low-fertility soils	Siqueira et al. (1998)
Different inoculants of N-fixing bacteria (*Azospirillum brasilense*, *Azotobacter chroococcum*) combined with AM fungi (*Glomus fasciculatum* and *Glomus mosseae*)	Field conditions and nursery with pomegranate (*Punica granatum* L.) and microbial inoculant	In both conditions tested, the combined treatment of *Azotobacter chroococcum* and *Glomus mosseae* was found to be the most effective. A significant improvement in the plant height, plant canopy, pruned material, and fruit yield was evident in 5-year-old pomegranate plants in field conditions	Aseri et al. (2008)

(continued)

Table 4.1 (continued)

Biofertilizer or microbial inoculant	Experimental conditions	Main results	Selected references
Biofertilizer (mycorrhizal, nitrogen-fixing bacteria, phosphorous-solubilizing bacteria) combined with no fertilizer and chemical (135 kg ha^{-1} urea + 185 kg ha^{-1} triple superphosphate)	Field experiments with annual medic (*Medicago scutellata* L. cv. Robinson) under dry farming conditions in Iran	The biological fertilizers can modify the adverse effects of moisture stress conditions The highest pod yield was obtained after applying nitrogen-fixing bacteria + mycorrhiza	Shabani et al. (2011)

against plant pathogens (Akhtar and Siddiqui 2008; Saldajeno et al. 2008), and interactions with rhizobacteria (Adesemoye and Kloepper 2009) and other soil microorganisms (Das and Varma 2009; Javaid 2010; Reis et al. 2010; Miransari 2011).

In studies of the performance of AM fungal inoculants (Table 4.1), species of AM fungi are commonly obtained from work collections, where a small number of species are maintained (e.g., Stürmer and Saggin 2010). This may be one reason for using a limited number of AM fungal species in glasshouse or field experiments. In Brazil, for example, there are collections containing a variable number of isolates (3–50) and species of AM fungi (2–28) which are held in university laboratories and research organizations. Greater exploration of AM fungal diversity can be expected by assessing the substantial international collections of AM fungi including INVAM (http://invam.caf.wvu.edu), IBG (http://www.kent.ac.uk/bio/beg), and GINCO (http://emma.agro.ucl.ac.be/ginco-bel/ordering.php) which guarantee delivery of well-identified species and traceability.

The efficiency of AM fungi in promoting plant growth is commonly evaluated under controlled conditions, including micro-plots (Table 4.1). Such control allows study of certain effects of AM fungi on plants under specific environmental conditions. However, this does not mean that the same performance of plants inoculated with selected AM fungi will occur under field conditions. The overall contribution of AM to plant nutrition and crop productivity is determined by the interactions among the host plant, the AM fungal partner, soil properties, and other environmental factors (Kahiluoto et al. 2012). Environmental conditions (Baar 2008; Kahiluoto et al. 2012), production systems (Sieverding 1991), and agricultural practices (Brito et al. 2008) can all interfere in plant responsiveness to AM fungal inocula. Furthermore, Antunes et al. (2009) showed that soil disturbance may under certain conditions have greater consequences for AM fungal effects on

plant productivity and the structure of indigenous AM fungal communities than certain AM fungal introduction through commercial inoculation.

In agroecosystems where production is dependent on indigenous AM fungi and there is low potential inoculum of these fungi in the soil, a response in crop yield can be expected after application of selected isolates of AM fungal inocula (Sieverding 1991). Similarly, in areas where plant production has been limited by stresses, especially low Pi available in soil (Sieverding 1991; Cuenca et al. 2008; Osorio and Habte 2009), salt stress (Evelin et al. 2009; Kaya et al. 2009; Mardukhi et al. 2011), and stresses caused by soil bacteria (Miransari 2011) and plant pathogens (Akhtar and Siddiqui 2008; Saldajeno et al. 2008), responses to inoculation may be expected. Higher plant performance was observed after pre-inoculation of coffee plants (Siqueira et al. 1998), papaya (Vázquez-Hernández et al. 2011; Rodriguez-Romero et al. 2011), banana, and some vegetables (Cuenca et al. 2008) on soils with low and medium fertility, after application of suboptimal dosages of phosphate.

Kahiluoto et al. (2009) suggested that the low-input cropping system with recycled organic matter composted before incorporation favors AM contribution to crop performance in the long term compared with a conventional cropping system. Advantages have been observed for (1) co-inoculation of AM fungi and N_2-fixing bacteria (species not specified) under dry conditions for annual medic production (Shabani et al. 2011), (2) AM fungi and phosphate-solubilizing bacteria (species not specified) with reduction of phosphate fertilization under dry conditions for corn production (Zarabi et al. 2011), (3) AM fungi and rhizobia under low P and/or low N conditions for soybean growth (Wang et al. 2011), (4) AM fungi (*Glomus fasciculatum* and *G. mosseae*) combined with the N_2-fixing bacteria *Azospirillum brasilense* and *Azotobacter chroococcum* (Aseri et al. 2008), (5) - co-inoculation of AM fungi and phosphate-solubilizing bacteria under drought stress condition for corn grain production (Zarabi et al. 2011), and (6) inclusion of AM fungi in biofertilizer in organic systems (Tanu Prakash and Adholeya 2006).

In cropping systems, on-farm production of AM fungal inocula may offer an alternative to a commercial AM fungal inocula (Douds et al. 2010; Sharma and Adholeya 2011; Pellegrino et al. 2011; Vestberg et al. 2011). Of importance for exploiting mycorrhizal technology in agriculture is the function of AM fungi in the soil and cropping systems where they are managed (Vosátka and Albrechtova 2009; Antunes et al. 2009; Barea et al. 2011; Herrera-Peraza et al. 2011).

4.4 Principles of AM Fungal Effectiveness in Cropping Systems

The majority of crop plants naturally form arbuscular mycorrhizas. AM fungi live in two environments, the root from which they receive C and to which they deliver nutrients and the soil from which they absorb those nutrients. Factors related to both

the soil conditions and internal plant environment are involved in the function of AM symbiosis. Soil management and agronomic practices controlling the effectiveness of AM fungal strains need to be understood for a reliable use of AM inoculation in agriculture.

Smith and Smith (2011) identified factors that may influence mycorrhizal growth responses of plants to colonization by AM fungi as hyphae, interfaces, and the root and soil environments (Herrera-Peraza et al. 2011). Evaluated the performance of coffee in different cropping systems after inoculation with AM fungi and observed that under seminatural soils, typically from mountainous areas of Cuba, their performance was not the same in all soils. The diversity in fungal efficiency can be related to ecological factors in plant–soil systems (Solaiman and Abbott 2004; Yang et al. 2010; Barea et al. 2011). The plant genotype, phosphate and its equilibrium with other nutrients, and the need for alleviation of abiotic and biotic stresses all need to be considered in AM fungi selection programs (Abbott et al. 1992).

AM fungal effectiveness and plant responsiveness involve the interaction of independent plant and fungus genomes (Janos 2007). The mycelia (extra and intraradical) produced by different AM fungi have quite varied characteristics in terms of hyphal diameter, the extent of growth away from the root, and the ability to absorb nutrients from soil away from the root (Jansa et al. 2003). These authors observed acquisition and transport of substantial amounts of phosphorus and zinc located 15 cm away from the roots by *Glomus intraradices* in symbiosis with maize. Thonar et al. (2011) observed for mycorrhizal *Medicago truncatula* that *G. intraradices*, *G. claroideum*, and *Gigaspora margarita* were able to take up and deliver Pi to plants from distances of 10, 6, and 1 cm from the roots, respectively. The differences among *Glomus* species related to C requirements. *G. margarita* provided low P benefits to plants that formed dense mycelium networks close to the roots where P was probably transiently immobilized. Tracer studies also provide insights into the role of the fungal symbionts in determining diversity in plant responsiveness (Grace et al. 2009).

Diversity in responsiveness to AM fungi may reflect the diversity in function of the symbiosis. Phosphate uptake by mycorrhizal plants involved two pathways: (1) the soil–root system which involves uptake from the rhizosphere by root epidermis and root hairs and (2) the AM fungal pathway which involves uptake by extraradical mycelium, rapid translocation over many centimeters, delivery to the symbiotic interfaces, and transfer to the plants (Javot et al. 2007; Smith and Smith 2011). These pathways involve different cell types and nutrient transporters, providing capacity for independent and coordinated regulation (Smith and Smith 2011). In nonresponsive associations, the mycorrhizal pathway can also be functional (Grace et al. 2009) and may reflect an estimation of mycorrhizal effectiveness when P is measured. Similarly, the pathway for N uptake has been demonstrated in soil-grown plants using $^{15}NH_4$ and $^{15}NO_3$, but it is not known what proportion of total plant N requirement is delivered via this route (Smith and Smith 2011).

The effectiveness of mycorrhizal inoculation can involve a mixture of native AM fungi or exotic AM fungi and compatible hosts. Antunes et al. (2011) compared the growth responses of bluegrass (*Poa pratensis*) and Bermuda grass (*Cynodon*

dactylon) inoculated with AM fungi assemblages originating from distant areas with contrasting climates and observed that AM fungal isolates originating from contrasting climates consistently and differentially altered plant growth, suggesting that AM fungi from contrasting climates have altered symbiotic function. AM fungi may adapt to different climatic conditions but of importance is how they compete with indigenous AM fungal communities in cropping systems. Nevertheless, the use of native AM fungi produced on-farm with mycorrhizal plants species (Douds et al. 2010; Sharma and Adholeya 2011; Vestberg et al. 2011) may represent a convenient alternative to commercial inoculum production. Pellegrino et al. (2011), evaluating native and exotic AM fungal inocula on plant growth and nutrient uptake in a low-input *Trifolium alexandrinum* and *Zea mays* in crop rotation, observed that the native fungal inoculum was as effective as highly efficient single exotic fungi.

Soil disturbance and cropping systems can affect the abundance and diversity of AM fungi and the function of the symbiosis. Soil disturbance associated with tillage systems can disrupt extraradical mycelium (Kabir et al. 1997). However, extraradical mycelia can survive the summer dry conditions in Mediterranean climate, allowing the next crop to benefit from the mycelium developed by the previous crop in the rotation (Brito et al. 2011). Duan et al. (2011) tested effects of soil disturbance and residue retention on the function of the symbiosis between medic and two AM fungi (*Glomus intraradices* and *Gigaspora margarita*) in an experiment simulating a crop rotation of wheat followed by medic and observed that the AM fungi responded differently to disturbance. *G. intraradices*, which was insensitive to disturbance, compensated for lack of contribution by the sensitive *G. margarita* when they were inoculated together. With respect to intercropping systems, Bainard et al. (2011) showed that intercropping systems supported a more abundant and diverse AM fungal community compared to conventionally managed systems. A positive influence on diversity of AM fungi can be related to more compatible host species, and the common mycorrhizal networks allow different plants to communicate with each other (Song et al. 2010).

In the field, the AM fungal interactions with other microorganisms (Das and Varma 2009; Javaid 2010; Reis et al. 2010) may affect mycorrhizal function. Wang et al. (2011) evaluated the co-inoculation of *G. mosseae* and *Bradyrhizobium* sp. on soybean genotypes HN89 (P-efficient) and HN112 (P-inefficient) and observed a synergistic relationship on plant growth, especially under low P and low N conditions for HN112, but there were no effects of inoculation under adequate N and P conditions. The N and P status influenced the effectiveness of inoculation, but AM fungal colonization reduced total root length, root surface area, and root volume in soybean. Furthermore, the genotype root architecture may affect the symbiosis. Yao et al. (2009) inoculated trifoliate orange (*Poncirus trifoliata*) seedlings with *G. intraradices*, *G. caledonium*, *G. margarita*, and *G. versiforme* and observed that AM colonization affected the distribution of root classes, increasing the proportion of fine roots (0–0.4 mm) and decreasing the proportion of coarse roots (0.6–1.2 mm), and reducing the total root length and root volume.

4.5 Conclusions

Agricultural practices and soil nutrient management practices can affect the mycorrhizal symbiosis and plant performance. As AM fungi inoculation becomes more popular, inoculation techniques need to be improved, always ensuring that inoculants are applied as close to seeds and roots as possible. Training programs for farmers may be necessary for successful use of mycorrhizal technology to avoid ineffective use of costly biofertilizers that contain AM fungi. Studies with AM fungal inoculants have demonstrated that some products can improve plant uptake of nutrients and thereby increase the use efficiency of applied artificial fertilizers.

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Chapter 5
Mycorrhizal Inoculum Production

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5.1 Introduction

The reliance on chemical fertilizers and pesticides in current agriculture and horticulture can cause pollution of natural environments as crop production rises rapidly. Therefore, development and management of sustainable agriculture is a primary issue in the agriculture sector worldwide. To enhance this practice, one feasible way is to generalize the recyclable organic agricultural cultivation methods such as utilizing naturally occurring plant growth-promoting microbes (PGPM).

Arbuscular mycorrhizal (AM) fungi can form symbiosis with virtually 80 % of all cultivated plants. They infect the roots and colonize invasively inside root cells. AM fungi are mainly characterized by arbuscules which are formed by fine, bifurcate branching hyphae in cortical cells. Hyphae of AM fungi extend outwards from the root surface, expanding the accessibility of the root system for nutrient uptake. AM fungi can therefore contribute as a "biofertilizer" by facilitating access to nutrients (Sylvia 1990; Leyval et al. 2002; Srivastava and Sharma 2011; Turnau and Haselwandter 2002).

Commercial application of AM fungal inoculum is increasing. In 2001, Sylvia listed 21 companies in North America, eight in Europe, two in South America, and two in Asia with involvement in production of inocula of AM fungi, but there are many more established companies which aim to produce and use inocula in various

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sectors of plant production (Gianinazzi and Vosátka 2004). Reasons for development of this agricultural biotechnology industry include the fact that AM fungi are increasingly being considered as a "natural plant health insurance" (Gianinazzi and Gianinazzi-Pearson 1988). Their positive impacts on plant development and health, land reclamation, and phyto-remediation are well recognized (Leyval et al. 2002; Turnau and Haselwandter 2002), and there is higher awareness of biodiversity issues, including those concerning soil microbial communities, and acceptance of these natural technologies as alternatives to agrochemicals (Barea 2000; Gryndler 2000). Furthermore, society is demanding more sustainable means of production, with a consequent feedback to farmers and land conservationists.

Producing microbial inoculum is a complex procedure that involves the biotechnological expertise and the ability to respond to associated legal, ethical, educational, and commercial requirements. This is particularly the case for obligate endosymbiotic microorganisms such as AM fungi because satisfying the aforementioned requirements is closely associated with the particular method of inoculum production.

5.2 Techniques Employed to Cultivate AM Fungi Propagules

"Pot culture" (Wood 1985) is the main technique employed for the large-scale production of inocula of AM fungi. It is a traditional and widely practiced method which employs trap plants (Chellappan et al. 2002). Potty (1985) reported that *Glomus mosseae* multiplied on cassava (*Manihot esculenta*) tuber peel yielding 3–4/cm^2 and suggested that this peel could be used for mass multiplication of AM fungi. Subsequently, Ganesan and Mahadevan (1998) claimed that hyphae, arbuscules, and vesicles of *G. aggregatum* developed on the surface of cassava tuber could be used as inoculum. Selvaraj and Kim (2004) used sucrose-agar globule with root exudates (SAGE) as a source of inoculum to increase the production of spores of AM fungi. The exudates led to higher percentage of root colonization (by about 10 % more) and increases in the number of spores (by about 26 %) and dry matter content (by more than 13 %) for the inoculum of AM fungal spores compared with their soil inoculum. A range of techniques such as the nutrient film technique (Mathew and Johri 1988), aeroponic culture systems (Hung and Sylvia 1987, 1988), and root-organ cultures (Declerk et al. 1996) have been employed for the production of AM fungal inocula in near-sterile environment (Raja and Mahadevan 1991). For all methods, including pot culture, only a few spores are usually used to initiate the production of the inocula.

The sources of AM fungal inocula are defined by the biology of the fungi. All infective structures of the fungi including fungal spores and/or the mycelium produced inside or outside the host root can be used as inoculum. However, arbuscules and the auxiliary cells formed by some fungi are not known to be a

source of inoculum. Infected roots and substrates (carriers which contain infected root and/or mycelium and spores) are commonly used. Spores are an important source for the establishment of clean cultures of AM fungi on host plants in a previously sterilized substrate. This is because (1) a small number of spores can be isolated relatively easily from a soil substrate, (2) spores can be morphologically distinguished for the identification of the fungi, and (3) spore surfaces can be satisfactorily disinfected (with the object of producing inoculum free from contaminants).

It is well known that a single spore can initiate the mycorrhizal symbiosis (Sieverding 1991) and that spores are suitable sources of inoculum for experiments (Nopamornbodi et al. 1987) and other special cases of plant growth (e.g., in nurseries or in conditions where aseptic inoculum is required). Spores of AM fungi can be produced on artificial media at low cost or established as by-products in the process of manufacturing other forms of AM inoculum such as those embedded in expanded clay as the carrier. Although it is technically possible to inoculate crops with spores, the use of spore inoculum is questionable for other reasons. Some spores require several days or longer to germinate and spore dormancy has been reported (Tommerup 1987). Due to the slow initial development and slow spread in the root system, it cannot be expected that a spore inoculum can compete with indigenous AM fungal propagules in the presence of other soil microorganisms. A rapid and high level of colonization of roots by the inoculated fungus is a prerequisite for the desired inoculant fungus to be beneficial to the host plant (Douds et al. 2005).

Widespread application of AM fungal inocula has been limited by difficulties in obtaining large quantities of pure inoculum. Mass production of AM fungal inocula became technically feasible with the introduction of the pot culture technique (Wood 1985) by culturing with plant hosts on substrates such as sand, peat, expanded clay, perlite, vermiculite, soilrite (Mallesha et al. 1992), rock wool (Heinzemann and Weritz 1990), and glass beads (Redecker et al. 1995).

Soil inoculum contains all AM fungal structures and can be highly infective as an inoculum for some fungi. A soil inoculum is generally chopped up before being applied in quantities depending on the inoculation technique. Many plants including maize, sorghum, Bahia grass, and Sudan grass have been shown to be suitable hosts for inoculum production. Soils and climates vary regionally, and locally available materials for inoculum production need to be tested. An important consideration for the selection of the host is that it should not have pathogens in common with those crops which are to be inoculated.

The time required for cultivation of AM fungal inocula in soil-based culture systems is relatively long, and the product quality can be inconsistent due to the possibility of introducing contaminants. Spores produced in pot cultures are generally more conducive to identification than those collected from the field which usually consist of mixtures of species. Cultures may be produced by various methods, including soil trap cultures, pot substrate cultures, plant trap cultures, and soil culture (Brundrett et al. 1999). Soil trap cultures involve growing plants in field soil for up to 6 months and then separating spores of the different AM fungi

into separate pot cultures. These cultures can be used as base for further purification. For pot substrate culturing, a small quantity of substrate from an existing pot culture can be mixed thoroughly with disinfected substrate, added to a mycorrhiza-free plant or placed under seeds in a sterilized soil. These cultures usually establish quickly, and spores were normally produced within a month or two of the subculturing attempt depending on the fungal species. Plant trap cultures involve removing plants from an area of interest, washing the roots thoroughly to remove all traces of soil and external mycelium, and planting them in a suitable sterile substrate. Mixed culture produced by this method can be used for subsequent further purification. Soil culture involves adding a layer of pot culture or other inoculum soil in sterilized media over several cycles to enhance mycorrhiza formation by the fungi present.

5.3 On-Farm Production of Arbuscular Mycorrhizal Fungus Inoculum

On-farm production of AM fungus inocula is an alternative to commercially produced inocula as the quantities of inoculum necessary for large-scale agriculture may be costly. Producing the inoculum on-site saves processing and shipping costs. These factors are the primary reason why most on-farm methods have been utilized in developing nations. Another benefit of on-farm production of inoculum is that locally adapted isolates can be used which may be more effective than introduced ones in certain situations (Sreenivasa 1992) and a taxonomically diverse inoculum can be produced.

Significant advances in on-farm production of AM fungal inocula have been made in developing countries in the tropics. For example, Sieverding (1987, 1991) produced inoculum of an effective strain of the AM fungus *Glomus manihotis* in Columbia using 25-m^2 fumigated field plots. Dodd et al. (1990) used a similar method in Columbia to produce an inoculum containing three AM fungi. Gaur (1997) and Douds et al. (2000) used raised beds of fumigated soil to produce inocula. Douds et al. (2005) also developed a modified raised bed method for on-farm production of AM fungus inoculum in temperate climates. The raised bed enclosures, 0.75 m × 3.25 m × 0.3 m, were constructed with silt fence walls, weed barrier cloth floors, and plastic sheeting dividing walls between 0.75-m square sections. The enclosures were 20-cm deep with mixtures of compost and vermiculite. In all cases, the choice of fumigants needs to comply with government regulations.

The compost utilization trial at the Rodale Institute Experimental Farm, Kutztown (Reider et al. 2000), used three treatments for comparing bulked AM fungal inocula which were (1) a commercially available AM fungus inoculum, (2) an on-farm inoculum, and (3) a control treatment. The commercially available AM fungus inoculum (MYKE® Garden, Premier Tech Biotechnologies, Rivièredu-

Loup, Quebec) contained 30 propagules of the AM fungus *Glomus intraradices*, as determined by the manufacturer, in a peat vermiculite mixture. The on-farm inoculum (compost–vermiculite = 1:9 vol/vol) contained 3,225 propagules of *G. mosseae*, *G. etunicatum*, *G. claroideum*, and AM fungi indigenous to the small amount of soil mixed into the compost. The two soil fertility management regimes in this experiment were a conventional chemical fertilizer and dairy cow manure + leaf compost. Final yields of tubers showed that the mycorrhizal treatments outproduced the control by 33-45 % and that the on-farm inoculum performed as well as the commercial mix (Douds et al. 2005).

Colonized roots contain internal fungal mycelium as well as external mycelium (and sometimes AM fungal spores) and can be used as an inoculum. Before use, roots are often chopped into smaller pieces. The infectiveness of colonized roots can be higher than that of spores, with new root colonization occurring within 1–2 days of inoculation with infected roots (Sieverding 1991). In addition to using colonized roots in greenhouse experiments, this form of inoculum has been used to inoculate plants in nursery experiments (Janos 1980). The quantities of roots used in inocula vary from up to 20 g fresh weight per plant, but even 1 g per plant was sufficient to obtain growth responses in nurseries and greenhouse experiments (Howeler 1985) in sterilized soil. When the colonized roots contain AM fungal spores, they can be dried and stored at ambient temperature or in a cold room for up to a month without any loss of their infectiveness, but when stored at 4 °C in water or moist vermiculite, infectivity was maintained for 2 months (Hung and Sylvia 1987).

5.4 Aeroponic and Hydroponic Inoculum Sources

Aeroponic culture of AM fungi is a complete culture system that starts with relatively few spores of the selected fungus to inoculate culture plants (Singh et al. 2012). These plants were then transferred into the aeroponic environment for more extensive root growth, colonization, and sporulation of the fungus. The resulting colonized root and spores may be used in a variety of ways (research, horticulture, floriculture, vegetable crops, forestry, scrublands, landscaping). Large-scale production of colonized roots with single AM fungi is practicable in aeroponic culture (Hung and Sylvia 1987; Sylvia and Hubbel 1986). When horticulture crops including tomato, sweet potato, and strawberry were produced with this system, their roots were by-products which can be used as inocula. Hence, the cost of this inoculum source may be fairly low in regions where these systems are used. Aeroponic culture of AM fungi is a biotechnology that allows both efficient production of AM fungal inoculum and soil-free investigation of mycorrhizas.

Aeroponic culture was first explored for legume rhizobia interaction by Zobel et al. (1976) and then for AM fungi by Sylvia and Hubbel (1986). It is a more aerated system than hydroponics and has proven to be an efficient system for growing AM fungal inoculum without a physical substrate (Hung and Sylvia

1988) as used in a nutrient flow system (Mosse and Thompson 1984). Inoculum production of AM fungi in aeroponic culture allows easy extraction of spores, hyphae, and roots. In addition, the roots may be sheared to produce inocula with a high propagule density (Sylvia and Jarstfer 1992a, b). In aeroponic culture, plants are grown in a closed or semi-closed environment by spraying the roots with a nutrient-rich solution where the environment is kept free from pests and diseases so that the plants may grow healthier and quicker than plants grown in a medium. However, if aeroponic environments are not completely sealed, then pests may pose a problem.

Problems are encountered during inoculum production at an industrial scale, including maintaining non-contaminated conditions. Martin-Laurent et al. (1997) demonstrated that soilless culture method such as aeroponic culture was a promising way to produce pure inoculum. Saplings inoculated with AM fungi in pots and subsequently grown in aeroponic conditions showed significantly higher rates of mycorrhizal colonization and P content than saplings grown in soil (Martin-Laurent et al. 1997). The potential of AM inoculum production in aeroponic culture for industrial applications was similarly demonstrated on *Paspalum notatum* and *Ipomoea batatas*. These plants were inoculated with *Glomus etunicatum* using a water-soluble polymer as the sticking agent for AM inoculum (Hung et al. 1991). However, the conventional spray nozzle and ultrasonic fog to produce fine mist of nutrient solution result in rapid loss of nutrient solution through evaporation and diminishing the possibility of rapid absorption of nutrients by the aeroponically cultured roots (Carruthers 1992). Another disadvantage of spraying roots with a nutrient solution with larger droplets is stifled root growth (Carruthers 1992). These problems limit rapid growth and AM fungal colonization of roots in the system. To overcome these limitations, piezo-ceramic element technology was used by Carruthers (1992), employing high-frequency sound that blasted the nutrient solution and nebulized it into microdroplets size of 1 mm in diameter.

It has been demonstrated that colonized roots sporulate rapidly in aeroponic culture (Martin-Laurent et al. 1997; Sylvia and Jarstfer 1992a, b). It was also demonstrated that both colonized roots and spores produced in aeroponic chambers can serve as infective AM fungal inocula which can be mixed directly and thoroughly with growing media if plants are to be immediately planted or transplanted. However, inoculum viability declines with storage time (Sylvia and Jarstfer 1992a).

Hydroponic culture of mycorrhizal plants provides a controlled nutrient environment and allows the harvest of mycorrhizal plants free from soil. This type of culture is infrequently used for growing and studying mycorrhizal plants. Hydroponic culture of mycorrhizal fungi was reported first by Peuss (1958) for *Glomus mosseae* with *Nicotiana tabacum*. Mycorrhizal plants were also grown in nutrient solution culture by Cress et al. (1979, 1986) and Karunaratne et al. (1986), and Dugassa et al. (1995) reported a culture of *Glomus intraradices* with *Linum usitatissimum*. To avoid microbial contaminants, frequent refreshment of the nutrient liquid is needed. The soaked state of the host plants and AMF therein caused by aquatic environment used in this method is not the natural growth condition for AM fungi and may limit sporulation.

Nutrient film culture is a technique developed for commercial production that entails continuous recycling of a large volume of nutrient liquid in a film which flows over the roots of the plant. MacDonald (1981) used a compact autoclave hydroponic culture system for the production of axenic mycorrhizas between *Trifolium parviflorum* and *Glomus caledonium* and others (Elmes et al. 1984; Elmes and Mosse 1984; Howeler et al. 1982; Mathew and Johri 1988; Mosse and Thompson 1984).

Besides the variation of techniques used in aseptic inoculation of AM fungi to the host plant, the major concern in the nutrient film technique system is the concentration of nutrients. The preferred values for the various nutrient elements vary from one particular mycorrhizal system to another depending particularly on the size and other features of the plant (Sharma et al. 2000). Another important factor is the compromise between plant growth and mycorrhizal colonization as waterlogged conditions affect mycorrhizal growth adversely (Tarafdar 1995).

As AM fungi are integrated components of most terrestrial plants, the nutrient exchange and other benefits due to AM fungi are sufficient for research conditions without contaminations (Diop 2003). The development of the root-organ culture technology system has opened new avenues for studying the symbiosis (Elsen et al. 2001).

5.5 Root-Organ Cultures

The root-organ culture technique was developed by White (1943) and others (Mosse 1962; Butcher and Street 1964; Butcher 1980) using excised roots on synthetic mineral media supplemented with vitamins and carbohydrates. The formation of lower-order roots is essential for rapid increase in root biomass and the establishment of continuous cultures. Both axenic and monoxenic approaches using different sources of propagules of AM fungi aimed to acquire root-organ cultures of AM fungi (Bécard and Piché 1992; Chabot et al. 1992; Diop 1990, 1995; Declerk et al. 1996).

Initiation of roots requires pre-germination of seeds after surface sterilized with classical disinfectants (sodium hypochlorite, hydrogen peroxide) and then thoroughly washed in sterile distilled water. Following germination of seeds on water agar or moistened filter papers, the tips (2 cm) of emerged roots can be transferred to a nutrient-rich media such as modified White medium (Bécard and Fortin 1988) or Strullu and Romand medium (Strullu and Romand 1987). With pH of the medium adjusted to 5.5, fast-growing roots can be cloned by repeated subcultures. This method of artificial culture is a valuable tool for the study and inoculum production of arbuscular mycorrhizal fungi as it avoids the interaction of other inhabitants of the rhizosphere.

The vegetative development of AM fungi in monoxenic cultures has been followed using either transformed or non-transformed roots (Bécard and Piché 1992; Fortin et al. 2002). The long-term behavior of *G. margarita* on Ri-TDNA-

transformed roots of the carrot showed 80 % of the fungal infection units were produced during the period of root aging (Bécard an Fortin 1988; Diop et al. 1992). The use of Sunbags in in vitro system is another alternative to obtain large-scale AM fungal inoculum without contaminations. The axenic AM fungal propagules can be conserved at 4 °C in the dark for several months or used for fundamental or inoculation practices. The possibility of continuous culture and cryopreservation has resulted in an international collection of in vitro AMF (websites: http://www.mbla.uclac.be/ginco-beland; http://res2.agr.ca/ecorc/gino.can/).

5.6 Qualitative and Quantitative Production of Mycorrhizal Inocula

The industrial activity of inoculum producers has been developed using different AM fungi, which are quite often not well characterized in terms of ecological requirements and stability. Along with this, the lack of quality control for several marketed inocula is among the main reasons for the low acceptance of mycorrhizal technology in horticultural and agricultural practices (Gianinazzi and Vosátka 2004). This situation has led to the need for mycorrhizal inoculum production industry to develop, in its own interest, criteria that will satisfy minimum requirements of quality. Whatever the mode of inoculum production chosen and the formulation procedure adopted by the companies, the marketed product has to meet the expected requirements of end users. Although these objectives may vary according to the companies, they should all aim at the use of AM fungi as a natural plant health insurance (Gianinazzi and Gianinazzi-Pearson 1988). In this context, the following criteria should be fulfilled by the companies: (1) plants to be inoculated must be able to form mycorrhizas; (2) the AM fungal inoculum must be free of agents that could negatively affect normal plant growth and development; and (3) the shelf life of the inoculum should be sufficient to suit end-user markets. The introduction of such criteria by the inoculum producers could contribute to the definition of conditions for the registration of products at national or international levels (Von Alten et al. 2002). Furthermore, in the product description, inclusion of the following recommendations for quality standards may be considered: pH, nutrient carriers, and additives. Additives could be included if their primary aim is to support mycorrhizal development, but additives which are general fertilizers should not be included unless this is stated clearly.

For better quality and production, the relevant number of AM fungal propagules should be determined. Therefore, there is a need for an independent testing service that can be used by producers to check that batches of inocula meet baseline standards that have been established and agreed to by individual companies on the basis of a voluntary code of best practices (Gianinazzi and Vosátka 2004). The inoculum formulation procedure usually consists of placing fungal propagules (root fragments colonized with AM fungi, fragments of fungal mycelium, and spores) in

a desired carrier (perlite, peat, inorganic clay, zeolite, vermiculite, sand, etc.) for a given application. Some companies producing AM fungal inocula have adopted the approach of one type of formulation (i.e., single fungal species) for all markets, while others produce a range of products for their target buyers.

The outcome of the AM symbiosis depends on environmental factors, AM fungal characteristics, and plant variables. However, present knowledge makes it difficult to predict the effectiveness of inocula. For example, the procedure called direct inoculum production process could help to improve predictability of AM fungal inoculum effectiveness (Feldmann and Grotkass 2002). Quality control of commercial inoculum must be dealt with, and a reference system for information concerning inoculum effectiveness based on the results of standard tests should be established for the buyers as well as a list of examples where the relevant inoculum had already been successfully used.

5.7 Ethical and Legal View of Using Inoculum

Suitable legislation based on quality control adapted to AM fungal inocula is essential for the development of mycorrhizal technology. At present, registration procedures for AM fungal inocula vary between countries, with some having very strict regulations (e.g., France and Canada) and others being less demanding or even without regulations. No regulation or lack of adherence to strict regulation will encourage the marketing of ineffective products. Overregulation could also destroy the market by preventing the development of small and medium enterprises and inoculum producers and distributors (Gianinazzi and Vosátka 2004). In France, beneficial microbes such as *Rhizobium* are considered as biofertilizers, and their registration requires a complex and expensive procedure that implies detailed description of the biological properties of the relevant microbes (identification, dissemination, toxicity, etc.). Furthermore, demonstration of the beneficial effects of the microbe via several controlled field trials (three to five per year) was performed, and finally, tests of the lack of toxicity or allergenicity of the formulated products on humans, animals, and plants were done. At the EU level, there is no registration for biofertilizers. However, the directive 91/414/EEC regulates the use of microbial products for plant protection. The data requirements for approval of plant protection products focus on possible unacceptable impacts on plants or the environment, harmful effects on human or animal health, and contamination of groundwater. The cost of such a process would handicap attempts to introduce mycorrhizal technology into plant production systems. Because of attempts to apply this directive to AM fungi, the European network on AM fungi, Cost Action 8.38 (2001), initiated discussions within the EU on the need for a registration procedure for AM fungal inocula. Because AM fungal inocula do not produce toxins, they should be regarded as a natural part of the plant, and the guidelines for approval of microbial plant protection products are not directly applicable to them, and the "risk assessment" criteria are also inappropriate (http://www.dijon.inra.fr/

cost838/index.html). However, unexpected consequences of transport of inocula should be considered (Schwartz et al. 2006).

5.8 Conclusion

Intensive agricultural practices are currently being reevaluated and are coming under increased scrutiny as the awareness of the consequences of excessive use of fertilizers and chemical pesticide usage improves. The concept of biofertilizers is to domesticate some of these microorganisms in agricultural production systems, so that additional natural reservoirs of nutrients in the atmosphere, hydrosphere, and pedosphere can be tapped to meet the requirements of sustainable agriculture. This approach also augments yield and monetary returns to the farmers, particularly to the small landholders, for which the incremental input cost is low.

The conventional difficulty of keeping low cost compatible with high quality of final products is always a point of concern of cultivating AM fungi using root-organ culture. The cheaper and lower technical methods of the abovementioned potted and hydroponic ones lead to difficulties in product control and product quality which discourages adoption. Soilless culture methods such as aeroponics have been demonstrated to be a promising way of inoculum production with potential for intensive production systems such as in horticulture. On-farm production of inocula combined with strategic management practices may be more practical for a large-scale agricultural production.

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Chapter 6
Use of Arbuscular Mycorrhizal Fungal Inocula for Horticultural Crop Production

Keitaro Tawaraya

6.1 Introduction

Rock phosphate is the raw material of phosphate fertilizer and the global reserves are limited. The expected global peak of phosphate production has been predicted to occur around 2030 (Cordell et al. 2009) but this is a complex issue. The sudden emergence of the concept of peak phosphorus within the debate on global phosphorus scarcity in the international arena may have raised more questions than it has resolved (Cordell and White 2011). Excessive application of phosphate fertilizer is common practice on horticultural crops (Mishima et al. 2010; Reijneveld et al. 2010); this means that phosphorus use efficiency is usually low. If phosphorus is applied in excess of plant requirement, it can accelerate phosphorus enrichment of water leading to eutrophication of rivers, lakes, and marshes (Maguire et al. 2005). It is necessary to respond to the problem of depletion of the resource of rock phosphate by (1) reducing application of phosphate fertilizer to agricultural crops to a level related to that which is required for plant requirement within one cropping cycle, (2) selecting crop plants that are more efficient at acquiring and using soil phosphorus, and (3) recycling organic phosphorus for agricultural use. Inoculation with arbuscular mycorrhizal (AM) fungi is a promising technique in the horticultural industry, especially for plants exposed to diverse abiotic stresses. Zhang et al. (2014) suggested that inoculation with AM fungi increases the tolerance of loquat seedlings to drought stress, and that improved nutrient uptake by AM fungi greatly contribute to this tolerance. AM fungi can promote the growth of many plants by enhancing increasing the efficiency of use of phosphate and zinc fertilizers (Watts-Williams et al. 2014). However, growth responses of horticultural crops following inoculation with AM fungi have most commonly been investigated under pot culture conditions and because mycorrhizal dependency varies among

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plant species and cultivars, plants are not equally responsive. Ortas et al. (2013) observed in an inoculation experiment in tomato, with many AM fungi species, that plant response differed with AM fungi, and that there was no single inoculant species which showed superiority compared with the others examined. They also showed inoculation at seeding stage has higher mycorrhizal dependency than inoculation at seedling stage. But the effects of inoculation with AM fungi under field conditions are not widely demonstrated.

6.2 Application of Chemical Fertilizers to Horticultural Crops

Horticultural crops include vegetable crops, flower and ornamental plants, and fruit trees. Inoculant AM fungi have been mainly applied to vegetable crops and fruit trees. Vegetables crops are classified as leaf vegetables, fruit vegetables, and root vegetables. Leaf vegetables include onion (*Allium cepa*), garlic (*Allium sativum*), Welsh onion (*Allium fistulosum*), leek (*Allium porrum*), and lettuce (*Lactuca sativa*) all of which are mycorrhizal. Non-mycorrhizal leaf vegetables include cabbage (*Brassica oleracea*), rape (*Brassica campestris*), and spinach (*Spinacia oleracea*). Mycorrhizal fruit vegetables include tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), pumpkin (*Cucurbita maxima*), and cucumber (*Cucumis sativus*) and mycorrhizal root vegetables include carrot (*Daucus carota*). Non-mycorrhizal root vegetables include radish (*Raphanus sativus*), turnip (*Brassica campestris*), and beet (*Beta vulgaris*). *Allium* plants including Welsh onion (*Allium fistulosum*), onion (*Allium cepa*), Chinese chive (*Allium tuberosum*), and garlic (*Allium sativum*) have coarse root systems and their capacity for phosphate uptake can be lower than for species with fibrous root systems (Greenwood et al. 1982). Therefore, mycorrhizas can be particularly beneficial in increasing the efficiency of phosphate uptake mostly of *Allium* species in phosphate deficient soil.

6.3 Inoculation of Horticultural Crops with Arbuscular Mycorrhizal Fungi

6.3.1 Mycorrhizal Dependency of Horticultural Crops

The degree of plant growth response associated with colonization of roots by AM fungi is expressed as mycorrhizal dependency and differs among plant species (Habte and Manjunath 1991; Howeler and Sieverding 1983; Planchette et al. 1983; Singh et al. 2012; Tawaraya 2003). Mycorrhizal dependency is generally high in the genus *Allium* because crop plants in Alliaceae such as Welsh onion,

Chinese chive, garlic, and leek have less well-developed root systems than do many other species (Greenwood et al. 1982). Mycorrhizal dependency can also differ among cultivars. For example, in a study of 16 Japanese cultivars of Welsh onion (*Allium fistulosum*) colonized with AM fungi *Glomus fasciculatum* (Tawaraya et al. 1999), 12 cultivars showed a positive response to AM colonization but one cultivar showed negative response. Furthermore, under glasshouse conditions, peanut grain production showed mycorrhizal dependency when inoculated with *Glomus rosea* in the presence of a low supply of P but for *G. clarum*, mycorrhizal dependency was only observed in the absence of applied P (Hippler and Moreira 2013).

It is necessary to check the mycorrhizal dependency of local cultivars and to select appropriate cultivars when AM fungi are inoculated in field conditions, or where the aim is to capitalize on contributions of indigenous AM fungi. The potential to increase the benefits from AM fungi will depend on the tradition of soil and agronomic management practices as well as plant cultivar used. For example, different degrees of dependency on the activity of AM fungi between native maize landraces and hybrids have been reported (Sangabriel-Conde et al. 2014), although the moderate level of fertilization used did not appear to have affected the mycorrhizal symbiosis, as all the maize types used this in experiment demonstrated mycorrhizal dependency. Cultivars of horticultural species bred in Japan are unlikely to be highly mycotrophic because of a history of heavy application of P fertilizer (Tawaraya et al. 2001). Breeding programs for plants used in intensive horticulture do not usually consider the AM fungi and their symbiotic associations with crop plants. Therefore, highly mycotrophic cultivars could be bred for use in horticulture in order to use phosphate resources more sustainably.

6.3.2 Inoculation Under Field Condition

Advanced scientific understanding of arbuscular mycorrhizal symbioses has recently demonstrated the potential for implementation of mycorrhizal biotechnology in horticultural plant production (Vosátka et al. 2012). Effects of AM fungal inoculation on nutrient uptake and growth of horticultural crops have been demonstrated in the field but most field experiments have been carried out at only one P level (Table 6.1). For example, inoculation with indigenous AM fungi increased the weight of marketable lettuce (*Lactuca sativa*) at one soil P level, 45 days after planting (Cimen et al. 2010b). In a different field experiment, inoculation with *Glomus intraradices* increased the bulb yield of onion (*Allium cepa*) at one soil P level 115 days after inoculation (Cimen et al. 2010a). Field inoculation with a selected inoculant of *Glomus intraradices* increased shoot dry weight of grapevine (*Vitis berlandieri* × *Vitis rupestris*) at 15 mg P kg^{-1} soil after 5 months (Camprubi et al. 2008).

Table 6.1 Growth response of horticultural crops with inoculation of AM fungi under field conditions

Crop species	AM fungi	Growth period	P levels	Growth responses	References
Allium fistulosum	*Glomus* R-10	109	4	+M > −M	Tawaraya et al. (2012)
Allium sativum	*Glomus fasciculatum*	145	4	+M > −M	Al-Karaki (2002)
Allium sativum	*Glomus mosseae*	–	4	No difference	Sari et al. (2002)
Colocasia esculenta	*Glomus*, *Gigaspora*	60	1	+M > −M	Li et al. (2005)
Lycopersicon esculentum	Indigenous	70	1	+M > −M	Cavagnaro et al. (2006)
Lycopersicon esculentum	*Glomus intraradices*	88	1	+M > −M, no difference	Subramanian et al. (2006)
Solanum tuberosum	*Glomus intraradices*	109	1	No difference	Douds et al. (2007)
Allium porrum	*Glomus 3 species*	36	1	+M.−M	Sorensen et al. (2008)

AM fungi can affect aspects of the quality of plant production as well as their yield. For example, inoculation with *Glomus mosseae* or *G. versiforme* increased survival rate and growth of tissue culture of taro plants (Li et al. 2005). In this study, the contents of nitrogen, phosphorus, potassium, calcium, copper, and zinc, the formation of corms, number of second and third branch corms and corm yield, and contents of protein, starch, and amino acids in the corms were also enhanced in response to mycorrhizas (Li et al. 2005). However, such responses are not always consisted. It was demonstrated that yields of potato tubers were increased by AM fungal inoculum (Douds et al. 2007) whereas inoculation with AM fungi did not affect shoot biomass of strawberry (*Fragaria* × *ananassa*) at 498 mg P kg^{-1} soil 14 weeks from transplanting (Stewart et al. 2005). In another example, the pre-inoculation of peach (*Prunus persica*) seedlings with AM fungi did not increase shoot growth at 85.3–95.8 mg P 100 g^{-1} soil after 2 years of transplanting (Rutto and Mizutani 2006).

Fruit yield of mycorrhizal tomato grown under drought stressed conditions was higher than that of non-mycorrhizal tomato at 0.10 g kg^{-1} soil 60 days after transplanting (Subramanian et al. 2006). On the other hand, when inoculation of garlic (*Allium sativum*) was investigated at four soil P levels, the AM fungal-inoculated garlic had higher mean bulb weight than did uninoculated plants at low P application levels (0 and 20 kg P ha^{-1}) under field conditions, 145 days after planting (Al-Karaki 2002). In another study, preinoculation with AM fungi increased shoot dry weight of leek (*Allium porrum*) 36 days at three soil P levels (20, 32, and 44 mg P kg^{-1}) after transplanting (Sorensen et al. 2008).

Growth improvement following inoculation of AM fungi has not been as clearly shown under field conditions as under pot culture conditions. For example, in spite

of well-known mycorrhizal dependency of *Allium* spp., mycorrhizal inoculation did not increase yield and bulb weight of garlic (*Allium sativum* cv. Urfa local) at four P application levels (0, 40, 80, and 120 kg P_2O_5 ha^{-1}) under field conditions (Sari et al. 2002). Furthermore, shoot and fruit biomass of a wild-type tomato were not different from that of a mutant tomato although the wild type had higher AM colonization and higher concentrations of both P and Zn in shoot and fruits than did the mutant when grown in the field (Cavagnaro et al. 2006). Many factors such as temperature, moisture conditions, solar radiation, and pests can affect growth of plants under field conditions. It is difficult to claim that soil P is the most growth-limiting factor, especially in many horticultural field conditions; however, there are situations where P responses to mycorrhizal inoculation can be demonstrated even where P appears to be adequate for plant growth. Recently, we examined the effects of inoculating AM fungi on the growth, P uptake, and yield of Welsh onion (*Allium fistulosum* L.) under non-sterile field conditions (Tawaraya et al. 2012). Yield of inoculated plants grown in soil containing 300 mg P_2O_5 kg^{-1} soil was similar to that of non-inoculated plants grown in soil containing 1,000 mg P_2O_5 kg^{-1} soil. In this case, the cost of inoculation was US$2,285 ha^{-1} which was lower than the cost of phosphate fertilizer (US$5,659 ha^{-1}) added to soil containing 1,000 mg P_2O_5 kg^{-1} soil for non-inoculated plants. Thus, AM fungal inoculation can achieve marketable yield of *A. fistulosum* under field conditions with reduced application of P fertilizer.

6.4 Factors Affecting Contributions of AM Fungi to Horticultural Crops Under Field Conditions

There are many inconsistencies in studies of inoculation of horticulture plants with AM fungi in terms of the soil used, nutrient availability compared with plant requirement, plant species, and environmental conditions, so generalizations cannot be made about predicted responses in any one situation without local knowledge. Site-specific investigations are necessary to identify situations where there is potential for increasing benefits from AM fungi. These investigations should be incorporated into best management practice for individual horticulture crops at particular locations.

AM fungi should only be introduced if the population of indigenous AM fungi is low and/or the effectiveness of the indigenous AM fungi is low. If there is a high indigenous population of AM fungi with high ability to affect plant growth and nutrient uptake, heavy application of P fertilizer and fungicide should be avoided in order to maintain the population of indigenous AM fungi. However, it is difficult for farmers to determine the indigenous population of indigenous AM fungi and its capability in their fields. Therefore it is necessary to establish protocols and training workshops for farmers for evaluation of indigenous AM fungi.

Growth improvement following inoculation of AM fungi is not expected in soil with high concentrations of available P because plant root itself can take up sufficient amount of P from soil. Differences in plant growth between inoculated and non-inoculated plant are usually negligible in soil with high concentrations of available P. In some situations, AM fungi can be inoculated during the nursery stage for transplanting crops. Nursery soil may need to be sterilized either to remove indigenous AM fungi prior to inoculation with selected AM fungi, or to eliminate pathogens. Commercial soil media which contain high concentrations of available P need to be adjusted to reduce the P concentration if it is at a level that inhibits AM colonization during the nursery stage.

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Chapter 7
Management of the Arbuscular Mycorrhizal Symbiosis in Sustainable Crop Production

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7.1 Introduction

We live at a unique time in planet Earth's history where humans significantly impact the geochemical cycles that sustain life. Among the negative impacts of humans on the Earth's chemical equilibrium, loss of nitrogen (N) and phosphorus (P) and nitrous oxide (N_2O) emissions from cultivated fields affect air and water quality in addition to wasting non-renewable P and fossil fuel resources. This is not sustainable.

The challenges we face include the need to reduce the negative impacts of crop production on the environment, while further increasing land areas under cultivation to produce renewable fuels, food and industrial crops for a rapidly growing and developing world population. Clearly, sustainability will only be achieved by improving the efficiency of nutrient cycling in human-managed systems. Nutrients exported from production fields end up as organic wastes from urban and industrial sources. These nutrients should be recycled in crop production systems, and the contribution of biological N_2 fixation to crop production should be increased. Nutrient-efficient cropping systems should also be designed.

The improvement of nutrient-use efficiency in crops and concurrent reduction in the levels of labile N and P in cultivated soils are necessary to minimize undesirable losses of N and P to the environment. The natural processes of soil nutrient mobilization and cycling are largely driven by microorganisms about which very little is known. This makes managing soil bioresources a difficult task. Nevertheless, some 50 years of research has built an important body of knowledge on a group of microorganisms that are central to nutrient cycling in agroecosystems: the arbuscular mycorrhizal (AM) fungi. In the first part of this chapter, we show how the AM symbiosis improves the efficiency of nutrient use by crops. We review the agronomic practices influencing the AM symbiosis in the second part of the chapter and present ways to enhance the contribution of the symbiosis to cropping systems' efficiency. The agronomic practices discussed relate to soil tillage, fertilization, pesticide use, AM inoculation of crops and grazing management. We also discuss how bioactive molecules and crop genotypes creating effective AM symbioses may contribute to the sustainability of agroecosystems.

7.2 Arbuscular Mycorrhizas: A Component of Sustainable Crop Production

Nutrient-use efficiency in crop production is a function of two main factors, which are both influenced by the AM symbiosis. The first factor relates to the efficiency of the mechanisms involved in nutrient transformation in soils. The quality of crop plants as a sink for nutrients is another very important factor.

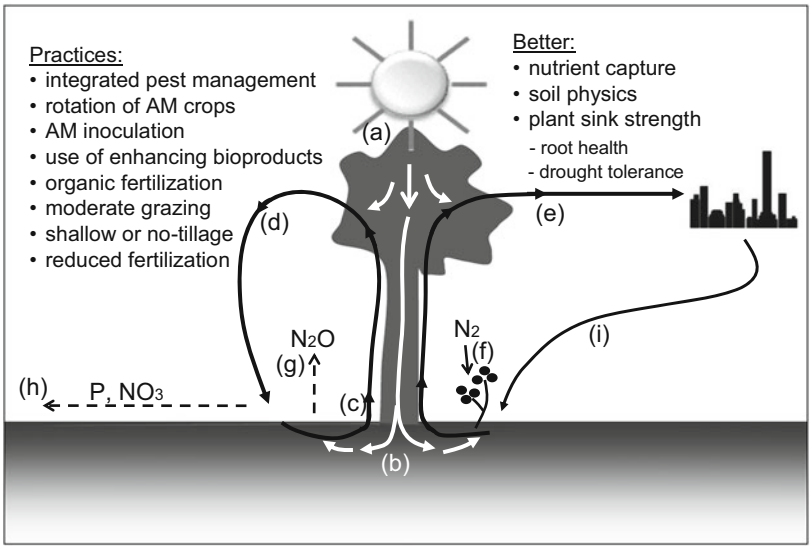

Fig. 7.1 Representation of the central role of the AM symbiosis in nutrient cycling in sustainable agroecosystem. Carbon and energy captured by the crop canopy (**a**) is transferred below ground and partly used to support the activity of AM fungi and associated microorganisms (**b**). Microbial activity mobilizes soil nutrients and the AM fungi facilitate their uptake by the crop (**c**). Part of the crop biomass is recycled in soil (**d**) whereas crop yield is exported to consumers' markets (**e**). The AM symbiosis favours the close cycling of N and P within the soil and plant components of the system, enhancing N_2 fixation in legumes (**f**) and reducing N and P losses through denitrification (**g**), leaching and run-off (**h**). The system is sustainable if the nutrients exported in yield are returned to the field (**i**). The agronomic practices listed in the *top left corner* leads to the AM-related benefits listed in the *top right corner* of the figure

7.2.1 Efficiency of Soil Nutrient Dynamics

Arbuscular mycorrhizas are the interface between the soil and those plants that can form this symbiosis. They connect roots to photosynthetic aerial plant parts and extensive networks of coenocytic hyphae that absorb water and nutrients, exude carbon (C)-rich compounds in the soil and interact with soil processes (Hamel and Strullu 2006). An AM symbiosis can be seen as a solar-powered soil management system where photosynthesis may increase in response to belowground demand (Fig. 7.1). The AM symbiosis, supported by plant photosynthesis, facilitates soil–plant processes that benefit crop production.

AM hyphae absorb and transport nutrients (Liu et al. 2007), which are translocated to plants through a symbiotic interface (Takeda et al. 2009). The symbiosis has been shown to improve plant nutrition by increasing plant uptake of nutrients present in growth-limiting amounts in soil, so the role of AM hyphae in plant P nutrition is particularly important to sustainability. The hyphae upload phosphate ions against a concentration gradient, concentrating phosphate in polyphosphate molecules (Takanishi et al. 2009; Tani et al. 2009) which are

transported to the symbiotic interfaces located in root cortical cells, where P is downloaded into the plants tapping into the AM hyphal network (Karandashov and Bucher 2005; Bucher 2007).

The role of the AM symbiosis in efficient plant N nutrition may also be important. AM hyphae can translocate N to host plants (Govindarajulu et al. 2005; Rains and Bledsoe 2007; Cappellazzo et al. 2008; Whiteside et al. 2009), and the symbiosis can be important for N uptake from fertilizers (Azcón et al. 2008). However, the contribution of the this symbiosis to crop N nutrition is often thought to be small, as nitrate (NO_3^-), the main source of N for crops, is freely mobile in soil and, thus, transport of N in AM hyphae brings little advantages to crop N nutrition in conventional crop production systems. Nevertheless, in sustainable production, mineral N levels should be kept low as NO_3^- can be reduced to N_2O, a potent greenhouse gas, or lost from the soil–plant system through leaching (Herzog et al. 2008; van der Heijden 2010) and negatively impacting surface and ground water quality. Ammonium (NH_4^+), the other major soil mineral N form, can be lost from dry soils through volatilization or oxidized to NO_3^-. The large amount of soil N held in organic form and organic soil amendments must be mineralized by soil microorganisms before the N they contain can be used by plants. AM hyphal networks can stimulate the organisms involved in mineralization. The presence of AM fungi in the plant–soil system can enhance mineralization of N from organic residues in soil, and the N released can be better used by plants tapping AM networks located in the vicinity of mineralizing residues (Hodge et al. 2001). The amount of N from decomposing plant residues that is acquired by plants through AM networks may be important and was demonstrated to reach 25 % of residue N in 24 weeks and overcome Russian wild rye growth limitation (Atul-Nayyar et al. 2009). Furthermore, it accounted for about 20 % of *Plantago lanceolata* N content (Leigh et al. 2009). Plants and soil microorganisms compete for soil-available N, and large AM hyphal networks potentially offer the crop a pool of available N inaccessible to competing microorganisms (Whiteside et al. 2009).

The AM symbiosis is an asset particularly for mycorrhizal crops relying on organic N sources. An effective AM symbiosis should maximize the benefit of legume crops in rotations and the utilization of compost and other organic sources of nutrients, the use of which is necessary in a sustainable world. The gap in nutrient cycling created by the unidirectional movement of nutrients from production field to cities and industries must be removed and N fertilizer use must be reduced. Nitrous oxide is the most important greenhouse gas emitted from agriculture, with levels increasing with the abundance of plant-available soil N, and fertilizer manufacturing and transport is the other main source of greenhouse gas from agriculture (Snyder et al. 2009).

Phosphorus is required in large amount by plants, but phosphate deposits exploitable with current technologies are finite and rapidly disappearing (Gilbert 2009). The AM symbiosis allows efficient plant P uptake from soils with low levels of available P, reducing the risk of wasteful P loss, thus preserving the quality of water and aquatic ecosystems (van der Heijden 2010).

AM fungi can play an important role in forage systems, especially in mixtures of legume and grass species, as they improve biological N_2 fixation in legumes (Hayman 1986; Xavier and Germida 2002; Chalk et al. 2006). Their extraradical hyphal networks also facilitate the transfer of N from N_2-fixing plants to companion grasses (Sierra and Nygren 2006; Jalonen et al. 2009) and improve the efficiency of nutrient cycling, reducing nutrient losses from the soil–plant system (van der Heijden 2010). AM fungi can decrease the productivity of components of a plant mixture while promoting that of another (Gange et al. 1993; van der Heijden et al. 1998), giving a competitive advantage to AM-dependent species (Grime et al. 1987). AM fungi favoured the proliferation of perennial forbs (Gange et al. 1993) and of most legumes in plant mixtures, an effect attributed to the high P demand of these plants (Karanika et al. 2008). Increasing the proportion of legumes in pasture or mixed hay fields would improve stand productivity and reduce dependence on N fertilizers.

More efficient use of the AM symbiosis in crop production would improve the sustainability of crop production. The AM symbiosis can reduce nutrient loss from ecosystems in three main ways: (1) by improving crop nutrient extraction capacity (van der Heijden et al. 2008) allowing the production of good yield at lower levels of soil fertility; (2) by increasing soil aggregation via physical particle enmeshment and cementing with "sticky" exudates, which results in better soil nutrient storage and retention (Rillig and Mummey 2006) and in reduced erosion (Tisdall 1991; Wright and Upadhyaya 1996) and leaching losses (Querejeta et al. 2009); and (3) by promoting growth of host crops, thus increasing the size of this desirable nutrient sink (van der Heijden 2010).

7.2.2 Reduced Biotic and Abiotic Limitations Increase Nutrient Capture by AM Crops

Although the effect of AM fungi in field settings is complicated by plant-to-plant interactions (Koide and Dickie 2002), AM fungi generally have the potential to enhance nutrient supply and the productivity of plant stands (van der Heijden and Horton 2009). However, the effect of AM fungi on plant growth promotion goes beyond nutrition (Newsham et al. 1995; Finlay 2008). Plant disease and insufficient water are major plant growth-limiting factors which are also influenced by the AM symbiosis.

7.2.2.1 Arbuscular Mycorrhiza and Plant Bioprotection

The extraradical mycelium of AM fungi is a large structure often representing over 20 % of soil microbial biomass (Leake et al. 2004). This fungal structure is important in improving plant nutrition, but it also has a profound influence on the

soil ecosystems. The extraradical AM mycelium derives its C and energy from plants and constitutes a sink similar in size to that of fine roots (Johnson 2010). Plants contribute considerable amounts of C and energy into soil systems, and this influences soil biodiversity and function (Finlay 2008). The AM symbiosis also influences soil microbial community structure through AM hyphal exudates, which have different effects on different organisms (Filion et al. 1999; Lioussanne et al. 2010), and through modification of root exudation (Sood 2003; Lioussanne et al. 2008). These processes influence the way that AM fungi interact with other soil microorganisms and can lead to protection against soilborne pathogens (St-Arnaud and Vujanovic 2007).

Several mechanisms have been proposed to explain how the AM symbiosis enhances plant tolerance to pests (see reviews by Azcón-Aguilar and Barea 1997, Harrier and Watson 2004, and St-Arnaud and Vujanovic 2007). They include the improvement of plant nutrition (especially P nutrition which modifies root exudation), increased cell wall lignification, competition with pathogens for C source and space in roots, antagonistic effects on pest especially in association with some bacteria (Bharadwaj et al. 2008; Siasou et al. 2009) and stimulation of the plant defence system (Pozo and Azcón-Aguilar 2007). The bioprotection conferred by the AM symbiosis to a plant probably results from the collective effects of more than one mechanism acting simultaneously (Pozo and Azcón-Aguilar 2007). Plant protection and other beneficial effects of AM fungi on host plants, such as tolerance to environmental stresses and improved soil physical properties, make AM fungi an important multifunctional component of sustainable agroecosystems.

7.2.2.2 Arbuscular Mycorrhiza and Plant Drought Stress

Limited water availability in agroecosystems is often a main cause of yield loss. The frequency of drought period is expected to increase in several regions of the world, with climate change. Severe drought may cause the xylem and rhizosphere to become air-filled and disrupt water flow, whereas milder water shortage leads to a state of C starvation exacerbated by photoinhibition (McDowell et al. 2008). The AM symbiosis can improve both plant and soil water relations in addition to increasing plant water use efficiency by mobilizing nutrients in dry soils (Augé 2004; Sheng et al. 2008). Mycorrhizal plants often contain more water and leaf chlorophyll than non-mycorrhizal plants (Colla et al. 2007; Al-Karaki and Clark 1999; Subramanian and Charest 1997, 1999; Srivastava et al. 2002) and have better gas exchange (Ruiz-Lozano and Azcón 1995; Aroca et al. 2009; Benabdellah et al. 2009) under drought.

The effect of the AM fungi on plant drought tolerance depends on the host–fungus combination (Davies et al. 2002; Pande and Tarafdar 2002). The better growth and water status of AM plant symbiosis is usually attributed to effective water extraction by an extraradical AM mycelium giving access to tightly held soil water and increasing soil–root hydraulic conductance (Gonzalez-Dugo 2010) and better osmotic adjustment and stomatal regulation (Augé 2001, 2004). Osmotic

adjustment is an important drought-tolerance mechanism in plants (Martinez et al. 2004) that is influenced by the AM symbiosis (Wu et al. 2007). The symbiosis has also been shown to enhance antioxidant defence (Garg and Manchanda 2009; Hajiboland et al. 2010) protecting photosystem II against the reactive oxygen species (ROS) created by photoinhibition.

The AM symbiosis may also improve plant performance through drought periods by increasing the capacity of the soil to store water (Augé 2004). The amount of water a soil can hold depends on its structure, especially its porosity. The spaces between sand particles are large and water tends to drain through easily leaving a water film on sand grain surfaces and air-filled interstices. By contrast, gaps between silt or clay size particles are small, which restricts water flow (Brady and Weil 2001). Mycorrhizal roots promote the aggregation of soil particles improving soil porosity (Oades 1993; Rillig and Mummey 2006; Lambers et al. 2007), water infiltration (Kabir and Koide 2000, 2002) and storage (Augé 2004) in fine-textured soils. They stimulate the package of fine particles into microaggregates containing medium-sized pores with good water-holding capacity and macroaggregates of size suitable for the creation of interstitial macropores conducive to soil drainage and aeration.

The importance of the different mechanisms of protection operating in AM plants to provide protection against drought probably varies with the plant–fungus associations and conditions. However, Augé (2004) showed that soil hyphae abundance explained lethal leaf and soil water potential better than did root colonization, root density, soil aggregation and leaf phosphorus or osmotic potential (Augé 2004), indicating the importance of the extraradical AM mycelium in explaining the AM effect.

7.3 Managing the AM Symbiosis in Cropping Systems

The AM symbiosis can provide important benefits to the plant, the soil and the environment, and this makes the management of the symbiosis desirable in sustainable crop production. The management of the AM symbiosis is achievable through a variety of agronomic practices, in particular: (1) tillage, (2) crop nutrition, (3) grazing and (4) integrated pest management (IPM), as well as by (5) the selection of crop genotypes and crop rotation sequences, (6) the use of AM inoculants and (7) the use of biotechnologies that enhance the AM symbiosis of crop plants.

7.3.1 Understanding Tillage Effect to Optimize AM Symbiosis-Related Benefits

No-till or conservation tillage is practiced to reduce surface run-off and loss of sediments, nutrients and pesticides from topsoil to surface water. No-till (i.e., direct seeding into the standing stubbles of a previous crop) is an excellent way of conserving soil water resources in semiarid areas. Under moist climate, by contrast, soil inversion by mouldboard ploughing in fall and harrowing in spring is practiced to accelerate soil warming and remove excess moisture. Ploughing deeply disturbs soil and disrupts AM hyphal networks which are mostly located in the top 25 cm of the soil (Kabir et al. 1998). Tillage may also increase soil bulk density restricting root growth (Lampurlanés and Cantero-Martínez 2003; Yau et al. 2010) and AM colonization (Mulligan et al. 1985).

The terminology used to refer to different tillage managements is a source of confusion leading to the widespread belief that AM fungi contribute little to crop nutrition in developed countries. "Conservation tillage" refers to diverse practices including ridge tillage, reduced tillage, shallow tillage and strip tillage, which result in different patterns and extents of disturbance. "Conventional tillage" also refers to a range of different practices and has regionally defined meanings. Whereas inversion of the top 20–25 cm of the soil by fall ploughing and soil levelling by a few harrowing operations in spring is the conventional practice in temperate humid areas, it is conventional not to use any fall tillage and to harrow only once the top 7.0–7.5 cm on the soil before seeding, in dryer areas. Thus, conventional tillage under dryer climates corresponds to conservation tillage in wetter areas. It is important to consider the depth and intensity of soil disturbance resulting from a tillage system before concluding on its likely impact on AM fungi.

Tillage systems imposing disturbance only to the few top centimetres of the soil preserves AM hyphae networks and AM fungi functionality (Kabir 2005). However, the effects may depend on the farming system and/or on the inoculum level in the soil. Plants connected to fragmented AM extraradical hyphae networks can have reduced access to soil nutrients (O'Halloran et al. 1986) even if AM root colonization levels are not reduced (Evans and Miller 1990).

Low or no soil disturbance favours an abundance of active AM hyphae (Borie et al. 2006; Md González-Chávez et al. 2010; Roldán et al. 2007), and although the effect of tillage on AM fungi proliferation disappears with time, the restoration of the AM fungal biomass also takes time. A negative impact of severe soil disturbance on active AM fungal biomass may persist, even after 1 year (Wortmann et al. 2008), and can persist for 5 years after only one tillage event (Drijber 2002). Reduced AM fungal biomass in soil was associated with reduced soil mycorrhizal inoculum potential and reduced colonization of crop roots (Garcia et al. 2007; Lekberg et al. 2008; van Groenigen et al. 2010). Thus, low or no soil disturbance favours the abundance of AM fungi propagules (spores and hyphae) in soil (Evans and Miller 1990; Borie et al. 2006; Cornejo et al. 2009), which is particularly important for short-season annual crops.

Tillage not only affects AM fungal development and symbiosis functionality but can also change the structure of the AM fungal community. Richer AM fungal communities were found in no-till than in tilled soil and tillage was found to select for AM species of the genus *Glomus* (Alguacil et al. 2008; Boddington and Dodd 2000). The dominance of AM fungi species is also altered by tillage (Douds et al. 1995; Jansa et al. 2003), and the disappearance of some species, namely, *Glomus ambisporum* and *Glomus etunicatum*, following a disking-fallow treatment was reported (Rasmann et al. 2009). The selection of AM genotypes by tillage may be beneficial or detrimental to crops, depending on the species involved. Nevertheless, it is logical to expect that the loss of AM fungal diversity will at least result in reduced ability of the soil system to adapt to changes.

It may be advantageous to markedly disturb the soil despite the disruptive effect this has on extraradical AM hyphae. Crop residues remaining at the surface of soils under no-till or under reduced tillage systems are mixed in the few uppermost centimetres of the soil. The soil organic matter consequently accumulates at the soil surface resulting in nutrient stratification and enrichment of the top layer. Crop residues are relatively rich in nutrients, particularly in N and P. Concentrations of N and P in soil surface under conservation tillage may increase to inhibitory levels, as high fertility inhibits AM symbiotic development (Garcia et al. 2007; White and Weil 2010). Punctual ploughing operations may also reduce the risk of P loss from soils enriched by decades of P fertilization on which a layer of organic matter accumulates. Phosphate ions are very reactive in soil where they bind to surfaces. Organic molecules compete with phosphate ions for fixation sites in soil, and soluble P may not only be released through mineralization of crop residues, but P ions can also be displaced from fixation sites into the soil solution by organic molecules (Ouyang et al. 1999), raising available P to unhealthy levels. Mixing the organic layer accumulating on the surface of soils under conservation tillage with mineral soil increases the amount of binding sites available to fix both P ions and organic molecules, reducing the availability of these P ions (Guertal et al. 1991; Simard and Beauchenmin 2002). A host crop with abundant root production and low AM dependency such as wheat should follow a deep tillage operation that disrupts AM hyphae networks, in order to re-establish these networks without yield penalty.

7.3.2 Crop Nutrition Management in Sustainable Agroecosystems

Plants and AM fungi require a certain level of nutrients to growth and function, and the nutrients exported from the soil system through harvest and sale of products must be replaced somehow for the production system to be sustainable. In poor fertility soils, fertilization stimulates root colonization (Ramirez et al. 2009) and plant response to AM inoculation (Covacevich and Echeverría 2009). In contrast,

cultivated soils are often nutrient rich as fertilization is a common component of crop management strategies. Although mineral N, P and K fertilizers and manures are sometimes abundantly used, they are not always necessary for higher crop production (McKenzie et al. 2003); fertilizers can be seen as cheap production insurance. A problem of using fertilization rate exceeding that exported in crop yield is P build-up in soil (Fixen 2006). The accumulation of residual P fertilizer in the soil creates unfavourable conditions for AM symbiotic development and can select for less mutualistic AM fungal strains (Johnson 1993).

Soil fertility management, particularly P fertility, is certainly a key element needing improvement in crop production. The soil P-testing methods usually used to determine fertilizer rates are quite coarse and inefficient tools. Plant-available P is estimated using different extraction protocols designed to dissolve easily soluble forms of P from soil calcium, aluminium and iron phosphates (Olsen and Sommers 1982). The different soil P-testing methods, such as Bray and Kurtz (1945) or (Olsen et al. 1954), are selected based on dominant regional soil type. They provide rudimentary indices of soil P fertility—"low", "medium" or "high"—that were calibrated to indicate the P requirement of each crop, often on varieties and in cropping systems that have long been replaced. Soil K testing is conducted in a similar way, and soil N may not be tested at all as current test results are largely unrelated to plant N nutrition (Ziadi et al. 1999).

Notwithstanding that nutrient calibration could be redone using new varieties and cropping practices, soil testing has important intrinsic limitations. It gives no indication on N and P availability from organic sources, yet these are major nutrient sources in soil. Most of the N and more than two-thirds of the P can occur in organic forms, and while soil microbial P is a small pool, it cycles rapidly and contributes importantly to plant nutrition (Stevenson and Cole 1999). Soil testing also gives no information on the contribution of AM fungi to crop nutrient uptake in the soils being tested.

Despite these important limitations, soil testing has been the tool available to manage crop nutrition since the mid-1900s, but as the environmental consequences of the poor nutrient-use efficiency of current agriculture are being unveiled, new tools are being proposed to better manage soil fertility. Models based on direct measurements of soil nutrient supply capacity and plant development models can be used to forecast the amount of nutrient needed to achieve a desirable crop yield (Greer et al. 2003). The precision of such models for P forecasting would be greatly improved by the inclusion of information on the AM hyphae network contributing to uptake. The information needed, i.e., the effective AM hyphae surface area for uptake, could be estimated using quantitative PCR (polymerase chain reaction) when reliable DNA markers are identified. Another approach to assessing the contribution of AM fungi to crop nutrition is to model the distribution of AM fungi in the landscape. Recent advances provide useful information towards the development of systems to monitor AM fungi and the function of the AM symbiosis in the field (Zimmerman et al. 2009; Johnson 2010; Tian et al. 2010).

Soil nutrient levels (Liu et al. 2000a), particularly N and P balance (Liu et al. 2000b), have a large influence on AM symbiosis development and function. Plants control AM symbiosis development and this influences their growth. As described by the functional equilibrium model developed by Johnson (2010), plants partition more C below ground under conditions of P limitation to develop capacity for soil P extraction. However, when the C cost to the plant exceeds the P uptake benefit derived from the AM symbiosis and becomes growth limiting, C export below ground is reduced. Plant growth may become limited by P availability when photosynthesis and N fertilizer rates are high. At this point, C supply to the AM symbiosis is increased to relieve P limitation, but high soil N levels increase the risk of harmful N losses to the environment (Snyder et al. 2009). Abundant P fertilization of AM crops reduces AM development, along with AM-related benefits to soils and crops, and increases the risk of nutrient loss from agroecosystems (van der Heijden 2010). Among the AM-derived benefits, the ability of AM fungi to link N mineralization from organic residues to plant demand is particularly relevant to nutrient cycling efficiency and sustainability of crop production. The presence of AM hyphae in patches of organic residues was shown to enhance mineralization (Hodge et al. 2001) with positive impact on host plant N nutrition (Atul-Nayyar et al. 2009; Leigh et al. 2009), as illustrated in Fig. 7.2.

The AM symbiosis enhances organic matter mineralization improving organic fertilizer availability to plants (Hodge et al. 2001; Amaya-Carpio et al. 2009; Atul-Nayyar et al. 2009). Organic fertilizers slowly release phosphorus; thus, they are less likely to raise soil P fertility to AM-inhibiting levels, as do readily available P sources. Organic fertilization can improve AM symbiosis development (Perner et al. 2006) even when mineral fertilization reduces it (Gryndler et al. 2006), but this depends on the amounts added. Compost may have a positive effect on the AM symbiosis (Valarini et al. 2009) and, used in combination with the AM symbiosis, can be as effective as mineral fertilizers (Perner et al. 2007; Salami 2007). The efficiency of sewage sludge can be increased by AM fungi (Arriagada et al. 2009), but these materials can be rich in P, explaining the negative impacts they have had on AM fungi (Tanu et al. 2004). Providing crops with a balanced nutrition will involve nutrient inputs from various sources, including N_2 fixation.

Rotation including N_2-fixing legumes reduces the dependence of cropping systems on N fertilizers and enhances AM symbiosis development and function (Bagayoko et al. 2000; Houngnandan et al. 2000; Alvey et al. 2001). Intercropping cereals and legumes may also increase mycorrhiza formation, with positive impacts on nodulation, N and P acquisition and use (Li et al. 2009), and is another agronomic practice enhancing efficient nutrient cycling in agroecosystems. The ability of AM fungi to improve plant N nutrition through better access to organic N source is advantageous in crops receiving N in organic form (He et al. 2003).

The AM symbiosis associated with different crops is influenced differently by soil fertility level (Johnson 2010). Similarly, some AM fungal strains stimulate plant growth best at relatively high soil fertility level, whereas others function best at lower level of fertility (Herrera-Peraza et al. 2011). The inclusion of detailed information in models will be needed to develop the most appropriate fertilization

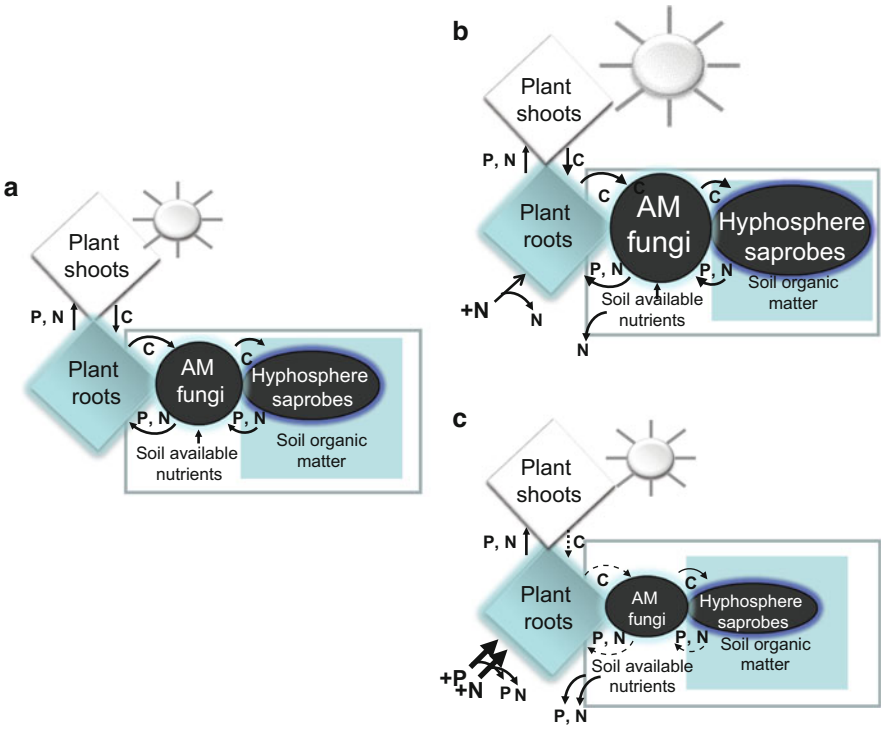

Fig. 7.2 Models representing dynamics of crop plant–AM fungi–saprobe relationships involving the distribution of C, P and N resources above and below ground, modified from Johnson (2010). (**a**) Mutualistic relationships in the distribution of fixed C and soil nutrients from inorganic and organic pools between crop plants, AM fungi and associated hyphosphere saprobes. (**b**) Increased photosynthesis or N fertilization stimulates plant growth, increasing plant P demand and AM symbiosis development and stimulating microbial mobilization of soil nutrients. Increased soil N availability increases the risk of N loss to the environment. (**c**) Fertilizer N and P applications beyond levels required to fulfil plant–AM fungi needs, by contrast, reduce C allocation to the AM symbiotic system. Fertilization increases the risk of N and P loss to the environment and may increase soil P saturation, with negative impact on the AM symbiosis in subsequent years

plan for each crop situation. Overall, slow release fertilizers generally favour AM symbiosis development and function when used in reasonable amounts, which varies with the requirements of different crops. The use of organic fertilizers tends to enhance the AM symbiosis, but care should be taken as their nutrient content may be unbalanced and it may be necessary to combine different fertilizing materials, organic or minerals. The use of crop rotations including N_2-fixing legumes is an excellent strategy both to enhance AM symbiosis-related benefits and reduce the footprint of crop production on the environment.

7.3.3 *Pest Management: A Challenge and Opportunity in AM Resource Management*

Pests and pathogens are responsible for about 45 % of all crop production losses worldwide (Agrios 2005). As the demand for food and bioproducts rises, pest management in agricultural systems is critical, and the use of agrochemicals, traditionally applied to control pests, is raising concerns. In addition to concern for human health (Lynch et al. 2009; Rull et al. 2009; Gilden et al. 2010), they can trigger the development of resistance in target organism populations (Sukhoruchenko and Dolzhenko 2008; Miyamoto et al. 2009; Hollomon and Brent 2009) and negatively impact beneficial organisms (Van Zwieten et al. 2004; Kinney et al. 2005). Although some cases of neutral and even positive effects of pesticides on the AM symbiosis have been reported (Reis et al. 2009), fungicides (Campagnac et al. 2008; Campagnac et al. 2009; Assaf et al. 2009; Zocco et al. 2008), herbicides (Vieira et al. 2007) and several insecticides (Veeraswamy et al. 1993) often have negative impacts on the performance and function of AM fungi. Pest control should be harmless to AM fungi because they are involved in many important ecological processes related to ecosystem function (Finlay 2008). Furthermore, AM fungi can enhance plant resistance to pests (Harrier and Watson 2004; Elsen et al. 2009), but pesticide application negatively impacting on AM fungi may increase crop vulnerability to pests.

Preserving the performance of the AM symbiosis with crops is aligned with the strategies and goals of IPM which is the road map of pest management in sustainable agroecosystems (Cuperus et al. 2004). IPM is defined as a comprehensive strategy for the control of pest populations in an environmental context to keep them below an economic threshold (FAO 1967). Sanitation, crop rotation, mechanical pest removal, use of chemical and biological control agents and host resistances are simultaneously used in IPM to control pests and minimize risks of environmental pollution and development of resistance in pests and to preserve the services provided by nontarget organisms such as AM fungi.

Improving AM fungi performance in agroecosystems could be a goal of IPM programmes, as AM fungi have important biocontrol activity in addition to improving plant nutrition and stress tolerance (St-Arnaud and Vujanovic 2007). The presence of AM hyphae in soil can have a sanitizing effect and reduce the abundance of pathogenic propagules (St-Arnaud et al. 1997; Fortin et al. 2002). The abundant extraradical AM hyphae leading to rapid and extensive AM root colonization could also reduce the incidence of diseases caused by parasitic nematodes (Pandey et al. 2009) which compete with AM fungi for occupation of plant roots and as food resource (Hol and Cook 2005). AM fungi are particularly important in the protection of plants against root diseases (Nogales et al. 2009; Azcón-Aguilar and Barea 1997), which may be symptomless and undetected in the field but decrease crop productivity and nutrient cycling efficiency.

Pesticides that are safe for the AM symbiosis (Wan and Rahe 1998; Reis et al. 2009) should be preferred (Wilson and Williamson 2008; Habte 1997;

Pimienta-Barrios et al. 2003), and alternative control measures should also be safe or beneficial to maximize the services of AM fungi in an IPM context. Well-planned crop rotations should always be practiced in order to break disease cycles. Interestingly, crop rotation can enhance AM symbiosis function, as mutualism in AM fungi appeared to be favoured by crop rotation (Johnson and Pfleger 1992). Host specificity level is low in the AM symbiosis (Smith and Read 1997), but often high in plant–pathogen interactions (van der Plank 1975) explaining how crop rotation with AM crop species can simultaneously reduce pathogenic infection and enhance AM symbiosis development and function.

The AM fungi are obligate biotrophs, making the presence of a host plant a mandatory condition for the symbiosis to exist. While weeds pose a real and serious risk of yield reduction, some weed species may benefit the agroecosystem by increasing biodiversity and abundance of beneficial AM fungi (Jordan et al. 2000). The removal of weed species hosting AM fungi from agroecosystems can alter the diversity, abundance and functioning of these important fungi, with an impact on crop growth (Feldmann and Boyle 1999; Kabir and Koide 2000). Weeds have been almost completely eliminated from cultivated fields by extremely effective technologies, in particular by glyphosate and glyphosate-resistant crops (Dewar 2009) although glyphosate-resistant weed genotypes are emerging (Duke and Powles 2009).

Sustainable crop production and IPM involve more planning and record keeping than conventional production. Easy-to-use agrochemicals simplify crop production but may reduce ecosystem functional efficiency with impact on the environment. Improving the sustainability of crop production is a realistic goal. The complexity of agroecosystems could be better monitored and managed through further application of knowledge in biology, meteorology, remote sensing and environmental modelling. A range of tools that support agronomic decisions could be further developed, but to reach a postmodern sustainable agriculture, silver bullet strategies must be put aside.

7.3.4 Manipulating AM Fungi Through Plant Selection

The host plant appears as a key element for the management of the AM symbiosis as it controls the development of the symbiosis in interaction with environmental conditions (An et al. 2010). Although most plants may host AM fungi, mycorrhizal development and plant response to AM colonization vary with the crop species (Saif 1987; Sudová 2009) and even with the genotypes of a same species (Krishna et al. 1985; Kaeppler et al. 2000; Eason et al. 2001; Karliński et al. 2010). Different plants are also associated with different levels of extraradical hyphae proliferation (Bingham and Biondini 2009) and different AM fungal community composition (Johnson et al. 1992; Mummey and Rillig 2006).

Variation in AM symbiosis formation has implications in cropping systems. Altering plant species in crop rotations can influence the AM potential of soils

(Johnson et al. 1991; Karasawa et al. 2002; Higo et al. 2009), an effect that is more marked in no-till systems (Gavito and Miller 1998). An AM population-mediated feedback effect of a previous crop on a following crop may occur as crop plants may modify AM fungal communities and as different host plants respond differently to different AM fungi (Saif 1987; Oliveira et al. 2006; Sudová 2009). A good crop rotation sequence should maximize the contribution of AM fungi to cropping systems in addition to minimizing pest population build-up, as mentioned in the previous section.

Plant response to AM fungal colonization varies interspecifically but also intraspecifically, revealing the possibility to breed plants which benefit from a more effective AM symbiosis either directly or indirectly. Kaeppler et al. (2000) identified one quantitative trait locus (QTL) for AM responsiveness in maize inbred lines, indicating the possibility to efficiently select maize varieties for AM symbiotic traits using genetic markers.

Different strategies could be used in the selection of plant genotypes for improved AM symbiosis. As plant response to AM colonization depends strongly on the plant–fungus combination involved (Facelli et al. 2009; Javaid et al. 2009), genotypes could be selected for effective association with highly effective AM (HE) strains. Such crop genotypes could be used with inocula containing these HE strains. Crop genotypes selected for their compatibility with HE strains would be an interesting tool for AM management in rotations involving non-host crop species or in biologically degraded soils. The advantage and disadvantage of this strategy would be the simplification of the systems, as it would make it easier to manage, but possibly less biodiverse. Whereas the negative impact of some desirable cropping practices (such as the production of desirable non-host crops) would be reduced by the possibility of rapidly restoring AM symbiosis-related beneficial effects on the agroecosystem, the performance of inoculated crops may vary geographically. Specific AM fungal strains appear to function well only within a range of environmental conditions beyond which their effect on plant productivity is reduced or even become negative (Herrera-Peraza et al. 2011). Thus, crop genotypes and HE strain-based inoculants should be developed specifically for different geographical locations. Alternatively, crop genotypes could be selected for their performance in association with AM fungi naturally occurring in cultivated soils. It would be important in this case to evaluate crop genotypes in different regions, as the dominant AM fungal genotypes naturally present in cultivated soils were shown to vary in a predictable manner with environmental conditions (Dai et al. 2012). This would be compatible with current practices in plant breeding. The evaluation of crop genotypes is normally done in multiple locations to assess yield stability (Tester and Langridge 2010). Thus, selecting plant genotypes for performance with local AM strains appears to be a feasible and ecologically sound option.

7.3.5 *Inoculation with Highly Effective AM Strains*

Tools for the management of the AM symbiosis in crop production are required (Facelli et al. 2009). Cultivars forming efficient AM symbioses are certainly important tools, but effective AM inoculants are also potentially useful where the ultimate goal of producing food and bioproducts sustainably sometimes conflicts with the maintenance of healthy AM fungal communities in cultivated soils. Despite their negative effect on AM fungi, disrupting tillage operations or the production of non-host crops may be desirable at times, and inoculation of AM-dependent crop species with selected AM fungal strains may restore ecosystem function where indigenous AM communities were negatively impacted.

Arbuscular mycorrhizal inoculants could also be used to stimulate plant growth beyond the capacity of an indigenous AM community. Inoculation with HE strains may have positive effects on cropping systems with little ecological consequence on indigenous AM communities, as shown by Antunes et al. (2009). A strain of *Glomus intraradices* inoculated onto maize positively interacted with the indigenous AM community in promoting plant growth, but did not influence the structure of the indigenous AM fungal community.

Host plant growth can be increased or decreased by AM inoculation (Johnson et al. 1997; Gosling et al. 2006). Different AM fungi have different abilities to build mutualistic relationships. They also have different abilities to compete for host plant roots, and competitive strains may be less effective mutualists (Bennett and Bever 2009). Highly effective strains are expected to enhance nutrient uptake and crop yield under field conditions. They only need to be competitive enough to produce a stable effect, and they should not be invasive. As the cost of production is an important consideration, HE strains with high propagule yield should be sought for the formulation of commercial inoculants. Species producing infective extraradical hyphae, such as *G. intraradices*, *G. etunicatum* and *Acaulospora spinosa*, will be preferred over species of *Gigaspora* and *Scutellospora* whose only propagules are spores (Klironomos and Hart 2002).

Inoculation of field crops has been shown to be effective (Sharma and Adholeya 2004; Stewart et al. 2005; Rivera et al. 2007; Higo et al. 2009; Mehraban et al. 2009). The effectiveness of an AM inoculation depends on the plant (Johnson et al. 1992; Gosling et al. 2006; An et al. 2010) but also on soil type (Herrera-Peraza et al. 2011). Different AM fungi are adapted to different soil types (Johnson et al. 1992; Herrera-Peraza et al. 2011; Dai et al. 2012). Highly effective strains often produce good effects on a range of crops, and in Cuba, crops are inoculated with HE strains selected based on the soil type where they will be introduced rather than on the crop species (Rivera et al. 2007).

7.3.6 Managing AM Fungi in Sustainable Crop Production Using Bioactive Molecules

Compatibility between crop plants and AM fungi can be enhanced through manipulation of signal molecules. The flavonoid "formononetin" was commercialized to enhance AM symbiosis development in plants (Koide et al. 1999) and to improve the yield of AM inoculum (de Novais and Siqueira 2009) by the biotech industry. Plant symbioses, including the AM symbiosis, are regulated by a crosstalk between plants and their microsymbionts and influence the formation and function of the AM symbiosis (Brachmann 2006).

Several plant compounds can influence the AM symbiosis (Brachmann 2006; Steinkellner et al. 2007). Strigolactones, a group of sesquiterpene lactones, can increase hyphae branching, mitochondrial density and respiration of AM fungi (Akiyama et al. 2005; Besserer et al. 2006a, b). Some plant flavonoids (Nair et al. 1991; Siqueira et al. 1991; Gianinazzi-Pearson et al. 1989; Tsai and Phillips 1991; Ishii et al. 1997; Fries et al. 1998; Aikawa et al. 2000; Cruz et al. 2004), ethylene (Ishii et al. 1996; Geil and Guinel 2002) and polyamines (El Ghachtouli et al. 1996) can stimulate AM fungi growth. Alginate oligosaccharide (Ishii et al. 2000) and nucleoside derivative (Kuwada et al. 2006) from a brown alga, *Laminaria japonica*, also stimulated AM hyphae growth, in vitro. The dipeptide Trp–Trp isolated from water-stressed bahia grass roots promoted growth and attracted the germ tube of germinating AM spores (Horii et al. 2009). The tryptophan dimer and Leu–Pro remarkably stimulated AM spore formation in *Glomus clarum*, *G. etunicatum* and *Gigaspora albida* grown in the absence of host roots or root exudates (Ishii and Horii 2009). Such signal molecules stimulating AM fungi growth and sporulation may facilitate the production of high-quality inoculants in bioreactors, with positive impact on the production costs of AM inoculants.

In natural ecosystems, plants manage their interactions with soil microorganisms using biochemical signals that selectively trigger responses in symbiotic partners (García-Garrido et al. 2009), but selectivity in signalling may not be perfect. Care should be taken as the application of signal molecules to crops or their inclusion in inoculant formulations may result in the attraction of undesirable organisms (Steinkellner et al. 2007) and reduce the ability of the crop to manage its rhizosphere. A good understanding of plant–microbe interaction is required to manipulate the rhizosphere exogenously. Crop genotypes with enhanced but regulated signalling systems selected in targeted plant-breeding programmes, appear as a safer avenue to AM symbiosis management in cropping systems. Rapid and early symbiosis development in crops would increase AM-derived benefits in short-season crops. Growing green manure plants with signalling properties highly promoting AM fungal development immediately following agronomic interventions unfavourable to the AM symbiosis, such as tillage or rotation with non-host crop species, would help counteract the negative impact of these cropping practices.

Plant signals may become useful bioproducts for sustainable production, but conversely, fungal signals may also lead to the development of useful

biotechnologies. Plants recognize microbe-derived compounds and adjust their responses according to the type of microorganisms encountered (Mabood et al. 2006). AM fungal signals stimulating root receptivity to indigenous or introduced HE strains could be a useful symbiosis management tool. Such signal molecules could be formulated and applied to crops or introduced into AM inoculant formulations to improve symbiosis formation and function (Gianinazzi and Vosátkatka 2004).

7.3.7 AM Fungi in Pastures

Growing forage crops or pastures instead of annual crops is a sustainable alternative for the use of marginal lands. Perennial forage stands offer a permanent soil cover, are usually drought tolerant (Acuña et al. 2010), are a main source of feed for the livestock industry and maintain soil quality through their contribution to soil C (Shrestha and Lal 2010). Forage stands are typically low-disturbance and low-input systems, and thus they are good habitats for AM fungi.

Grazing management is the main way to manipulate and maintain pasture ecosystem health. Grazing increases the diversity of grassland plant species (Watkinson and Ormerod 2001) and that of AM fungi, although it decreases their proliferation (Murray et al. 2010). Defoliation reduces plant C export below ground (Ilmarinen et al. 2008), and reduced AM root colonization was attributed to grazing (Jirout et al. 2009). Grazing increases system heterogeneity, which is important to preserve biodiversity in grasslands (Klimek et al. 2008), but it may reduce AM function in intensive systems. Overgrazing, in turn, leads to reduced AM fungal diversity (Su and Guo 2007) and ecosystem degradation (Watkinson and Ormerod 2001).

The loss of AM diversity may negatively impact ecosystem function. Concurrent seasonal changes in AM fungi and plant community composition in native Canadian grasslands (Yang et al. 2010) suggested that seasonal shifts in above- and belowground diversity are necessary to maintain the function of ecosystems as the environmental conditions vary. The diversity of AM fungi creates a patchy soil environment (Mummey and Rillig 2008) conducive to the coexistence of different plant species. Different AM fungi influence different plants differently (Aldrich-Wolfe 2007), and variation in AM hyphae density and ribotype distribution in soil, with spatial structure at less than 30 cm (Mummey and Rillig 2008), indicates that seedling establishment occurs over a mosaic of AM fungal influence.

Marginal land conversion to native grasslands could be a sustainable alternative in dry areas at this time of climate change. Much of the brown soil zone of the Canadian Prairie, for example, is forecasted to become dryer and only marginally suitable for cereal production by mid-twenty-first century (Nyirfa and Harron 2002), a conclusion supported by trends in climate change (Cutforth 2000). In contrast, the productivity of native Canadian grasslands should be sustained under the conditions forecasted by climate models (Thorpe et al. 2008). The diversity and

adaptation of native ecosystems gives them a resilience that tame forage species may not have. Ecovar seeds of several native plant species are commercially available and seeded native ecosystems could protect soils in marginal areas while supporting the cattle industry. It remains to be seen if the AM fungal diversity will also need to be restored in these soils.

7.4 Conclusion

Agrochemicals have increased the productivity of cultivated lands and simplified crop production. However, the application of agricultural technologies may reduce the efficiency of agroecosystems in the long term. Knowledge should be applied and technology developed that enhances ecosystem efficiency through the management of their complexity. In this context, it is particularly important to improve the precision and sustainability of crop nutrition management in a manner that protects and maximizes benefits from AM fungal resources. Technologies that mitigate negative effects of some necessary cropping practices on the functionality of the AM symbiosis in agriculture are also required.

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Chapter 8
Application of Arbuscular Mycorrhizal Fungi in Production of Annual Oilseed Crops

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8.1 Introduction

Oilseed crops are the second most in importance after cereals and significantly contribute to the Indian economy. Oilseeds cover about 13 % of the total arable land and generate nearly 10 % of the total value of the agricultural products in India (Singh et al. 2006). The country grows nine dominant oilseed crops, with groundnut (*Arachis hypogaea* L.), soybean (*Glycine max* L. Merrill) and rapeseed-mustard (*Brassica juncea* L.) accounting for 87 % and 75 % of total oilseed production and acreage, respectively (Agricultural Statistics at a Glance 2004). In India, soybean is the premier oilseed crop and growing parallel with groundnut followed by rapeseed-mustard. When compared to other countries, the productivity of these oilseeds per unit area is very low in India and their productivity is declining due to the recurrence of drought, low nutrient use efficiency of crop, nutrient deficiency in soil and other biotic and abiotic stresses.

Microbial interactions with plant roots may involve either endophyte or free living microorganisms and can be symbiotic, associative or casual in nature.

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Beneficial symbionts include N_2-fixing bacteria (e.g. rhizobia) in association with legumes and interaction of roots with AM fungi, with the latter being particularly important in relation to plant P uptake (Richardson et al. 2009). Legume crops are generally cultivated in nutrient poor environments in India and have a high P requirement for nodule formation, nitrogen fixation and optimum growth. The mycorrhizal condition in legume crops increases vegetative growth and seed yield in addition to improving nodulation (Mathur and Vyas 2000).

During the past 50 years, the widespread use of chemical fertilisers to supply N and P has had a substantial impact on food production and has become a major input in crop production around the world (Tilman et al. 2002). However, further increases in N and P application are unlikely to be as effective at increasing yields (Wang et al. 2011) as only 30–50 % of applied N fertiliser and 10–45 % of P fertiliser are taken up by crops (Adesemoye and Kloepper 2009; Garnett et al. 2009). In addition, the abundant use of chemical fertilisers in agriculture has had some deleterious environmental consequences and is a global concern (Bohlool et al. 1992; Tilman et al. 2002).

The scientific community must look for alternate technologies which can play a major role in sustaining and increasing the productivity of oilseed crops. One approach could be the use of combinations of plant growth-promoting microorganisms (PGPMs) that can fix atmospheric nitrogen and solubilise or mobilise phosphorus, zinc and other soil nutrients to stimulate plant growth and improve soil health (Babalola 2010; Sharma et al. 2010).

The rhizosphere is the dynamic environment where much interaction takes place and AM fungi are important biotrophic plant associates. These fungi colonise the root cortex and develop an extrametrical mycelium which is a bridge connecting the roots with the surrounding soil microhabitats (Barea et al. 2005). They are obligate symbionts and require a host plant to complete their life cycle (Wardle et al. 2004). AM fungi form a symbiotic association with most agricultural crops and are able to increase plant nutrition and plant health (Jansa et al. 2009). In addition, AM fungi establishment in the root causes changes in the microbial community of the rhizosphere (Meyer and Linderman 1986; Marschner et al. 2001) and increases plant tolerance to a wide range of biotic and abiotic stresses (Auge et al. 2004; Whipps 2004; Jansa et al. 2009). Many studies have demonstrated on field crops, including oilseeds, the benefits of AM inoculation on plant nutrition (Cardoso and Kuyper 2006; Hamel and Strullu 2006), nodulation (Meghvansia et al. 2008; Aryal et al. 2006), N-fixation (Peoples and Craswell 1992) and plant protection (Whipps 2004; Doley and Jite 2013a, b) under ideal conditions.

Certain cooperative microbial activities involving plant growth-promoting microorganisms can be exploited as a low-input biotechnology and form a basis for a strategy to help sustainable, environment-friendly practices fundamental to the stability and productivity of agricultural ecosystems (Kennedy and Smith 1995). The purpose of this review is to discuss (i) the current status of major oilseed crops in India; (ii) the application of AM fungi (single and dual inoculation) in the plant growth, nutrition and control of soil-borne diseases associated with major oil seeds; (iii) the strategies of manipulating soil and agricultural practices to manage

indigenous AM fungi and quality performance and (iv) commercialisation possibilities of AM fungi.

8.2 Oilseed Crops of Global Importance

About one-third of the land area of the world comprises arid and semiarid climates. The increasing economic and agricultural utilisation of arid lands has emerged as a critical element in maintaining and improving the world's food supply (Zahran 1999). India plays a major role in global oilseeds and vegetable oil economy contributing about 15 % of the world's oil crops area of nine oilseeds (groundnut, soybean, rapeseed, mustard, sesame, sunflower, linseed, safflower and castor), 7 % of the world's oilseeds production and 6.7 % of vegetable oils production. However, the productivity in India is only 1,005 kg/ha as compared to the world average of 1,957 kg/ha (FAOSTAT). India has the largest area in groundnut, sesame, safflower and castor and ranks first in production of safflower, castor and sesame and ranked second in groundnut, third in rapeseed, fourth in linseed, fifth in soybean and tenth in sunflower (Table 8.1). In the domestic agricultural sector, oilseeds occupy a distinct position after cereals sharing 14 % of the country's gross cropped area and accounting for nearly 1.5 % of the gross national product and 7 % of the value of all agricultural products. India encompasses diverse agro-ecological conditions ideally suited for growing nine annual oilseed crops including groundnut, rapeseed-mustard, sunflower, sesame, soybean, safflower, castor, linseed and niger and two perennial oilseed crops (coconut and oil palm) and secondary oil crops such as maize and cotton. In addition to the above, more than 100 tree species of forest origin that have the potential to yield about one million tonnes of vegetable oil are grown in the country.

8.3 AM Fungi in the Production of Oilseed Crops

8.3.1 AM Fungi Inoculation Responses for Enhanced Growth and Nutrient Uptake

AM fungi are the most common type of association involved in agricultural systems. AM fungi are associated with improved growth of many plant species due to increased nutrient uptake, production of growth-promoting substances, induced tolerance to drought, salinity and transplant shock and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilisers (Sreenivasa and Bagyaraj 1989). Symbiotic associations of plant roots with AM fungi can result in enhanced growth because of increased acquisition of P and nutrients with low mobility in soil. Effective nutrient acquisition by AM fungi is generally attributed to the extensive hyphal growth beyond the nutrient depletion

Table 8.1 Area, production and yield of oilseeds: global and Indian scenario (AICRPS 2009; Damodaram and Hegde 2010)

Oilseeds	Area ('000 ha)		Yield (kg/ha)		Production ('000 MT)		India's position in the world		Percent area to total oilseed area in India	Percent production to total oilseeds production in India
	World	India	World	India	World	India	Area	Production		
Groundnut	24,590	6,850	1,554	1,071	38,201	7,338	First	Second	22.37	25.86
Soybean	96,870	9,600	2,384	942	23,095	9,045	Fifth	Fifth	34.51	35.74
Safflower	691.44	350	890	643	615.21	225	First	First	1.07	0.68
Castor	1,524.7	880	1,037	1,276	1,580.6	1,123	First	First	3.14	4.22
Sunflower	25,024	2,050	1,424	542	35,643	1,112	Fourth	Tenth	6.58	4.18
Rapeseed and mustard	30,987	5,750	1,883	1,014	58,364	5,833	Third	Third	22.85	25.98
Linseed	2,437	550	903	296	2,200	163	Second	Fourth	1.48	0.61
Sesame	7,534	1,750	478	381	3,603	666	First	First	6.56	2.31

zone surrounding the root (Tisdale et al. 1995). In this way, AM fungi enable their host plants to gather mineral nutrients from a much larger volume of soil than the roots could reach on their own (Jansa et al. 2009).

8.3.1.1 AM Responses Under Glass House/Nursery and Field Conditions

AM fungi responses vary with AM fungal species used, soil pH, experimental conditions (Clark and Zeto 1996), root-geometry/architecture of the host plant which influences the nutrient uptake particularly soil supply of P and soil temperature (Raju et al. 1990). For example, in soybean, manganese (Mn) and iron (Fe), protection was more efficient when the plants were inoculated with *Glomus macrocarpum* than with *Glomus etunicatum*, whereas *Gigaspora margarita* was not effective with the inocula used (Cardoso and Kuyper 2006). Jalaluddin et al. (2008) found that the AM fungus *Scutellospora auriglobosa* increased the uptake of P in sunflower resulting in increased yield and reduced incidence of *Macrophomina phaseolina* which causes charcoal root rot in sunflower var. Helico-250 cultivated in Sindh region of Pakistan. Wang et al. (2011) while examining the tripartite symbiotic associations with rhizobia and AM fungi and correlating their relationships to root architecture as well as N and P availability of two soybean genotypes contrasting in root architecture grown in a field showed variable responses to AM fungi. The deep root soybean genotype had greater AM fungi colonisation at low P, but better nodulation with high P supply than the shallow root genotype. Co-inoculation with rhizobia and AM fungi significantly increased soybean growth under low P and/or low N conditions as indicated by increased shoot dry weight, along with plant N and P content. Moreover, the effects of co-inoculation were related to root architecture. The deep root genotype (HN112) benefited more from co-inoculation than the shallow root genotype (HN89).

AM fungal inoculation has been shown to reduce Mn and Fe toxicity in plants, and the concentration of Mn in shoots and roots of mycorrhizal plants can be lower than in non-mycorrhizal plants (Kothari et al. 1991; Nogueira et al. 2004). Mycorrhizal soybeans grew better and had lower shoot concentrations of Fe and Mn than did non-mycorrhizal soybeans under greenhouse conditions. In roots, the results were the same for Mn and the reverse for Fe. The decrease of Mn in shoots was attributed to reduced availability, while the decrease of Fe in the shoots was attributed to its retention in the roots. In excess, both Mn and Fe can be toxic to plants; thus, mycorrhizas may protect the plants from their toxicity (Nogueira et al. 2004).

Under field conditions, AM fungal inoculation enhanced biomass, nutrient uptake and yield of sesame applied with conventional P fertiliser (superphosphate) and slow release P source (rock phosphate) (Anil-Prakash and Tandon 2002). The influence of AM fungus on P and Fe uptake of mycorrhizal groundnut (*Arachis hypogaea* L.) and sorghum (*Sorghum bicolor* L.) plants was studied by Caris et al. (1998) using radiolabelled elements (^{32}P, ^{59}Fe). Plants possessing different strategies for the acquisition of Fe (Marschner 1995) were selected for this experiment. Groundnut is dicotyledonous and is a strategy I plant (Fe-deficiency

response: enhanced net excretion of protons from the roots, increased Fe-reducing capacity), while sorghum is monocotyledonous (graminaceous) and is a strategy II plant (Fe-deficiency response: enhanced release of phytosiderophores from the roots). In both plant species, P uptake from the labelled soil increased more in shoots of mycorrhizal plants than in non-mycorrhizal plants. Mycorrhizal inoculation had no significant influence on the concentration of labelled Fe in shoots of peanut plants. In contrast, ^{59}Fe increased in shoots of mycorrhizal sorghum plants. The uptake of Fe from labelled soil by sorghum was particularly high under conditions producing a low Fe nutritional status of the plants providing evidence that hyphae of an AM fungus can mobilise and/or take up Fe from soil and translocate it to the plant.

Meghvansia et al. (2008) reported variations in efficacy of different treatments (involving AM fungal species and cultivar-specific bradyrhizobia) with different soybean cultivars indicating the specificity of the inoculation response. This provides a basis for selection of an appropriate combination of specific AM fungi and *Bradyrhizobium* which could further be utilised for verifying the symbiotic effectiveness and competitive ability of microsymbionts under particular agro-climatic conditions. Inoculation response of single or mixed species of AM fungi to soybean has shown enhanced growth, mineral nutrition and nutrient uptake (Sharma et al. 2012a, b; Ilbas and Sahin 2005; Meghvansia et al. 2008; Waceke 2003; Sanginga et al. 1999). The role of mycorrhiza-mediated *Rhizobium* symbiosis on soybean showed enhanced production of soybean under field conditions (Antunes et al. 2006). Synergistic effects of AM fungi and *B. japonicum* have a high potential to improve the nutrient supply of soybean including P and soil quality (Meghvansia et al. 2008). However, a much larger genetic variability of bradyrhizobia and AM fungi strains exist in different cultivar regions than was assumed previously (Taiwo and Adegbite 2001). Soybean can form tripartite symbiotic associations with nodule-inducing rhizobia and AM fungi, which may benefit both P and N efficiency (Lisette et al. 2003). Co-inoculation of soybean roots with *B. japonicum* 61-A-101 considerably enhanced colonisation by the AM fungus *Glomus mosseae* and increased N and P uptake (Xie et al. 1995). El-Azouni et al. (2008) studied the associative effect of AM fungi with *Bradyrhizobium* as biofertilisers on growth and nutrient uptake of *Arachis hypogaea*. The biomass and grain yield were significantly improved by using the dual bio-preparations of AM fungi and *Bradyrhizobium*. The bacterial mycorrhizal-legume symbiosis increased nodule number, nitrogenase activity, total pigments and carbohydrate, protein and lipid content. The N, P and K uptake was significantly increased due to the single or dual inoculation. Moreover, inoculation with AM fungi and *Rhizobium* enhanced nodulation and yield of groundnut applied with inorganic P fertiliser (Mandhare et al. 1995; Lekberg and Koide 2005) and organic amendments (Iyer et al. 2003).

Mostafavian et al. (2008) showed that besides *Rhizobium*, inoculation of AM fungi with *Thiobacillus* increased soybean yield. Jackson and Mason (1984) found positive relationships among P availability, mycorrhizal colonisation and pod yield in groundnut (*Arachis hypogaea* L.). Mirzakhani et al. (2009) indicated that seed yield and yield components of safflower were influenced by inoculation with *Azotobacter* and AM fungi. They showed that inoculation of seeds with

Table 8.2 Examples of AM fungi responses (applied singly or combined) to enhance growth and mineral nutrition of major oilseed crops

AM fungi species	Interaction/significant treatments	Crop	References
G. fasciculatum	Phosphorus levels	Soybean	Ilbas and Sahin (2005)
Indigenous *Glomus* sp.	Crop rotation and *Rhizobium*	Soybean	Sanginga et al. (1999)
Mixed AM fungi	Conventional, GM soybean and *Bradyrhizobium* sp.	Soybean	Powell et al. (2007)
G. mosseae	Root architecture and *Bradyrhizobium* sp.	Soybean	Wang et al. (2011)
Glomus intraradices, *Acaulospora tuberculata*	*Bradyrhizobium japonicum*	Soybean	Meghvansia et al. (2008)
Gigaspora gigantea			
Glomus fasciculatum	*Pseudomonas striata*, P sources	Soybean	Mahanta and Rai (2008)
Glomus etunicatum	Salt stress	Soybean	Sharifia et al. (2007)
Glomus intraradices	Glyphosate, *Bradyrhizobium japonicum*	Soybean	Powell et al. (2009)
G. fasciculatum	*Pseudomonas striata*, Rock phosphate	Soybean	Mahanta and Rai (2008)
Glomus mosseae, *Glomus etunicatum*, *Gigaspora rosea*	Phosphatic fertilisers	Soybean	Bethlenflavay et al. (1997)
G. mosseae	Heavy metals, phosphatic fertilisers	Soybean	Dev et al. (1997)
G. mosseae	*Bradyrhizobium japonicum*	Soybean	Shalaby and Hanna (2000)
G. intraradices	Phosphorus application	Groundnut	Lekberg and Koide (2005)
Glomus caledonium	Salt stress	Groundnut	Gupta and Krishnamurthy (1996)
G. fasciculatum	*Rhizobium* and phosphatic fertilisers	Groundnut	Mandhare et al. (1995)
G. fasciculatum	Phosphatic fertilisers	Groundnut	Singh and Chaudhari (1996)
Glomus sp.	*Bradyrhizobium*	Groundnut	Elsheikh and Mohamedzein (1998)
G. intraradices	*Azotobacter chroococcum*	Safflower	Mirzakhani et al. (2009)
G. intraradices	*Azotobacter chroococcum*	Sunflower	Mirzakhani et al. (2009)
Glomus mosseae, *Glomus intraradices*	Heavy metals	Sunflower	Adewole et al. (2010)

(continued)

Table 8.2 (continued)

AM fungi species	Interaction/significant treatments	Crop	References
G. fasciculatum	Phosphorus levels	Sunflower	Chandrashekara et al. (1995)
G. fasciculatum	–	Linseed, niger	Srinivasulu and Lakshman (2002)
AM fungi	Rock phosphate	Sesame	Anil-Prakash and Vandana (2002)
G. fasciculatum	–	Castor	Sulochana and Manoharachary (1989)
G. constrictum			
Gigaspora sp.			

Azotobacter and AM fungi (*G. intraradices*) at the time of planting increased the grain yield of safflower to about 38 % over control plants. Groundnut is an important food legume of Egypt, and to enhance the production of groundnut, new reclaimed soils were brought under cultivation. The lack of indigenous soil populations of AM fungi and rhizobia has restricted potential yields of groundnut cultivated in this area. A summary of AM fungi inoculation responses for enhanced growth and nutrient uptake is stated (Table 8.2).

8.3.2 AM Fungi Responses in the Stressed Environments (Drought, Heavy Metals and Salinity)

AM fungal responses have also been encouraging in stressed environments like acid/salt (Gupta and Krishnamurthy 1996; Sharifia et al. 2007), drought (Ruiz-Lozano 2003; Auge et al. 2004; Manoharan et al. 2010; Liu et al. 2007), heavy metals (Göhre and Paszkowski 2006; Nogueira et al. 2004) and modified micro-environmental conditions such as genetically modified soybean (Powell et al. 2009). AM fungi have also been observed to play a role in metal tolerance (Del Val et al. 1999) and accumulation (Zhu et al. 2001; Jamal et al. 2002). For example, groundnut is a major cash crop in the semiarid tropics where it is mainly grown under rainfed conditions. Poor soil fertility, drought and diseases are important factors causing low yields. Groundnut forms symbiotic associations with two types of soil microorganisms, one with *Bradyrhizobium* and another with AM fungi. The positive effect of AM fungi on plant growth and development make mycorrhiza a potentially very useful biological resource of assuring high plant productivity, with minimum application of chemical fertilisers or pesticides. Quilambo (2002) studied the effects of two AM inoculants on root colonisation, leaf growth and dry matter accumulation and distribution in two groundnut cultivars: Local and Falcon. The indigenous Soil Mozambique inoculants significantly increased root colonisation, leaf growth and dry matter in both cultivars under drought stress conditions. The commercial Hannover inoculant increased growth

only under well-watered conditions. Drought stress effects could be alleviated by inoculation with Soil Mozambique inoculants. Therefore, peanut productivity, particularly under drought stress, may be improved by an adequate management of the AM symbiosis.

Most studies conducted on sunflower indicate that besides growth promotion, mycorrhizal colonisation of sunflower enhanced the ability to store more heavy metals in the roots. Adewole et al. (2010) found that AM inoculation to sunflower increased pollution tolerance to cadmium (Cd) and lead (Pb) and consequently increased the yield of sunflower. External mycelium of AM fungi provides a wider exploration zone (Khan et al. 2000; Malcova et al. 2003), thus providing access to greater volume of heavy metals present in the rhizosphere. However, the effectiveness of AM fungal isolates in improving plant growth also depends on the level of heavy metals in soil (Awotoye et al. 2009). Del Val et al. (1999) reported six AM fungal ecotypes showing consistent differences with regard to their tolerance to the presence of metals in soil. AM fungi may play a role in the protection of roots from heavy metal toxicity by mediating interactions between metals and plant roots (Leyval et al. 1997). Contaminated soils, which are often nutrient poor with low water-holding capacities, may provide an advantage to plants colonised by AM fungi by enabling them to act as pioneering species (Khan et al. 2000). Wu et al. (2004) used an intercropping system to examine the interactions of mycorrhizosphere and rhizosphere on metal uptake by growing mycorrhizal non-hyperaccumulator *Zea mays* and non-mycorrhizal hyperaccumulator *Brassica juncea* in a split-pot experiment. The intercropping system achieved higher phytoremediation efficiency in metal-contaminated soil, especially with dual inoculation of beneficial rhizobacteria and AM fungi. Similar studies were conducted by Zhang et al. (2004) who grew groundnut (leguminous crop) and maize (nonleguminous crop) and found that the iron-deficient maize released phytosiderophores which improved iron nutrition of groundnut through influencing its rhizosphere processes.

Among the biological approaches to enhance plant growth in saline conditions, the role of AM fungi is well established. Most native plants and crops of arid and semiarid areas are mycorrhizal, and it has been suggested that AM fungal colonisation might enhance salt tolerance of some plants (Tain et al. 2004). Under salt (base and acid) stress conditions, AM fungi response in terms of yield on groundnut was almost tripled in mycorrhizal plants compared with non-mycorrhizal control plants. Furthermore, they showed that AM inoculation promoted the establishment of groundnut plants under acid stress conditions (Gupta and Krishnamurthy 1996). Therefore, the additional beneficial effects of AM fungi in reducing salinity stress imposed on them (*Arachis hypogaea* var. *hypogaea* cv. Florunner) were studied by Al-Khaliel (2010) to understand the growth and physiological changes of groundnut plants under induced saline conditions. These investigations indicated that the AM fungi (*Glomus mosseae*) could improve growth of groundnut under salinity through enhanced nutrient absorption and photosynthesis. Chlorophyll content and leaf water content increased significantly under salinity stress by the inoculation with mycorrhizal fungi.

8.3.3 AM Fungi Inoculation Responses on the Control of Soil-Borne Diseases and Other Plant Pathogens

8.3.3.1 Influence of AM Fungi on Soil-Borne Diseases

The potential for AM fungi to suppress root diseases caused by soil-borne pathogens (Dehne 1982; Linderman 1994) has been intensively studied. *Sclerotium rolfsii* is an important soil-borne pathogen and causes disease in numerous crops including groundnut. The loss of yield caused by pathogen infection generally is 25 %, but it can be as high as 80–90 % (Grichar and Bosweel 1987). AM fungi have been shown to influence fungal diseases caused by root pathogens (Karagiannidis et al. 2002). Most studies concluded that disease severity could be reduced by root colonisation of AM fungi through several mechanisms including increasing the mineral absorption and plant growth (Smith and Read 1997), phenolic compounds (Devi and Reddy 2002) and pathogenesis-related proteins (Pozo et al. 1999). Ozgonen et al. (2010) studied the effects of AM fungi against stem rot caused by *Sclerotium rolfsii* Sacc. in groundnut. In field trials, the effect on disease locus of AM fungi ranged between 30 and 47 % with AM fungi differing in their benefit.

Disease and poor soils are considered to be the main causes of loss in the groundnut production. Rosette virus disease (RVD) and *Cercospora* leaf spots (CLS) are the major worldwide diseases that infect groundnut plants. In Cameroon, up to 53 % loss has been estimated (Fontem et al. 1996). CLS are caused by *Cercospora arachidicola* Hori (early leaf spot) and *Cercosporidium personatum* (Berk. and Curt.) Deighton (late leaf spot). Depending on the moment of contamination during the growing season, groundnut plants infected by RVD do not produce pods and, consequently, do not give any harvest (Savary 1991). Management against phytoviruses is very difficult because viral infection can be transmitted through seeds and also through some insect vectors (*Aphis* sp.). Strullu et al. (1991) showed that the symbiosis between mycorrhizas and roots of many crops has a positive influence on the plant's nutrition and in protection against some diseases. Zachee et al. (2008) determined the effect of mycorrhizal inoculation on the development of diseases (RVD and CLS) and on the physiology of groundnut plants (variety A-26) infected by RVD. A urea treatment and an absolute control were also used. It was observed that root colonisation rate was very low in control and urea plots compared to mycorrhiza-inoculated plots. Mycorrhizal applications reduced disease infection by almost 40 % and 54 %, respectively, for RVD and CLS. It was evident that mycorrhizal symbiosis with groundnut roots increased the resistance of plants to RVD and CLS and positively influenced the physiology of groundnut plants infected by RVD.

Fungal root pathogens can be reduced in crops by AM inoculation (Caron et al. 1986), including *Phytophthora* species (Davis and Menge 1980; Cordier et al. 1996), *Rhizoctonia solani* (Yao et al. 2002) and *Pythium ultimum* (Calvet et al. 1993). Bacterial diseases may also be reduced by mycorrhiza establishment on roots (Dehne 1982). Evidence of the suppression of nematode penetration and

development following AM fungi inoculation has been reported by many workers (Elsen et al. 2001; Diedhiou et al. 2003). Harrier and Watson (2004) illustrated the role of AM fungi in organic and/or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant diseases. However, the mechanisms by which AM fungi colonisation confer the protective effect are not well understood. Bio-protection within AM fungal-colonised plants is the outcome of complex interactions between plants, pathogens and AM fungi. These interactions have been shown to result in reductions in disease incidence (Matsubara et al. 2001), pathogen development (Cordier et al. 1996) and disease severity (Matsubara et al. 2001). The extent of AM fungi-induced protection of host plants against pathogens suppression ranges from complete protection (Torres-Barragan et al. 1996) to partial protection (Matsubara et al. 2001). The extent of partial protection is influenced by the AM fungal species and cultivar used (Yao et al. 2002). Information related to oilseed crops is summarised in Table 8.3. Effects may relate to direct interaction between mutualists and pathogens (Abdalla and Abdel-Fattah 2000), competition for infection sites (Abdel-Fattah and Shabanam 2002) and improved nutrition of AM fungi plants which offset the damage caused by the pathogen involved (masking effect). Inoculation with soil-based mixture of AM fungi (*Glomus fasciculatum*) decreased incidence of disease caused by *Macrophomina phaseolina* (Tassi) in groundnut and increased growth and

Table 8.3 Examples of AM fungi application providing protection to oilseed crops against soil-borne diseases and other plant pathogens

AM fungi	Pathogen	Plant	References
G. mosseae	*Rhizoctonia solani*	Groundnut	Abdalla and Abdel-Fattah (2000)
Glomus sp.	Rosette virus disease (RVD), *Cercospora* leaf spot (CLS)	Groundnut	Zachee et al. (2008)
Gigaspora sp.			
G. intraradices	*Fusarium oxysporum* f. sp. *lini*	Linseed	Dugassa et al. (1996)
G. mosseae	*Fusarium solani*	Groundnut	Abdalla and Abdel-Fattah (2000)
G. mosseae	*Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*	Soybean	Zambolim and Schenck (1983)
G. fasciculatum	*Sclerotium rolfsii*	Groundnut	Krishna and Bagyaraj (1982)
AM fungi	*Meloidogyne arenaria*		Carling et al. (1995)
AM fungi	*Meloidogyne incognita*	Soybean	Kellam and Schenck (1980)
Glomus sp., *Gigaspora* sp.	*Heterodera glycines*	Soybean	Tylka et al. (1991)
G. intraradices	*H. glycines*	Soybean	Price et al. (1995)
G. mosseae	*H. glycines*	Soybean	Todd et al. (2001)

production of defence-related enzymes (Doley and Jite 2013a, b). The various defence-related biochemical parameters such as protein, proline, total phenol, total chlorophyll content, acid and alkaline phosphatase activity, peroxidase and polyphenol activity showed marked increase in their content or activity in mycorrhizal healthy or diseased plants in comparison to non-mycorrhizal diseased or control ones (Doley and Jite 2013a, b). Zambolim and Schenck (1983) reported that *Glomus mosseae* reduced the influence of *Macrophomina phaseolina* (Tassi.), *Rhizoctonia solani* (Kuhn.) and *Fusarium solani* (Mart.) in soybean. The suppression of endoparasitic nematodes by AM fungi has been recently reported by many workers (Habte et al. 1999). Several mechanisms have been proposed to explain the nematode suppression by AM fungi (Pinochet et al. 1996). Carling et al. (1995) observed the individual and combined effects of two AM fungal species, *Meloidogyne arenaria* and P fertilisation on groundnut plant growth and pod yield. They found that the groundnut growth and yield were generally stimulated by AM fungi, which increased groundnut plant tolerance to the nematode and offset the growth reductions caused by *M. arenaria* at the two lower P levels. Price et al. (1995) investigated the effects of the AM fungi, *Glomus intraradices*, on the soybean cyst nematode (SCN), *Heterodera glycines*, on two soybean cultivars, cv. "Bragg" (nematode intolerant) and cv. "Wright" (moderately nematode tolerant) grown in the greenhouse in soils with low (35 μg/g) and high (70 μg/g) P. They found variable AM responses to cultivar. The cultivar "Wright" was more responsive than "Bragg" and exhibited greater nematode tolerance. Dugassa et al. (1996) demonstrated the effects of AM fungi on the health of *Linum usitatissimum* infected with wilt (*Fusarium oxysporum* f. sp. *lini*) and AM fungi showed increased resistance against the wilt pathogen; the level of these effects depended on the plant cultivars which all showed the same level of root colonisation by AM fungi.

8.3.3.2 Interaction Between AM Fungi and Other Plant Growth-Promoting Rhizobacteria (PGPR) Leading to Inhibition of Fungal Pathogens

Rhizosphere microorganisms can affect presymbiotic phases of mycorrhiza development (Barea et al. 1998). The bacteria have been found adhering to the AM fungi hyphae (Bianciotto et al. 1996) and as well as embedded within the spore walls (Walley and Germida 1996). Bacteria adhering to AM fungal mycelium may utilise hyphal exudates or use mycelium as vehicle for colonisation of rhizosphere (Bianciotto et al. 1996). Bacteria from genus *Paenibacillus*, which are antagonistic to a broad range of root pathogens and are able to stimulate mycorrhizal colonisation, were found frequently to be associated with *Glomus intraradices* mycelium (Mansfeld-Giese et al. 2002). Therefore, it should be mandatory to detect the cohesiveness of both AM fungi and PGPR participating in a particular rhizosphere while maintaining the healthy rhizosphere. The key step is to ascertain whether an antifungal biocontrol agent will negatively affect the AM fungi populations. Several studies have demonstrated that microbial antagonists of fungal pathogens,

either fungi or PGPR, do not exert antimicrobial effect against AM fungi (Barea et al. 1998). There is a need to exploit the possibilities of dual (AM fungi and PGPR) inoculation to provide plant defence against root pathogens (Barea et al. 2005). Barea et al. (1998) conducted a series of experiments to evaluate the effect of *Pseudomonas* strains producing 2, 4-diacetylphloroglucinol (DAPG) on AM fungi formation and functioning. Three *Pseudomonas* strains producing DAPG were tested under in vitro and in situ for their effects on AM fungi; it was found that there was no negative impact on AM spore germination. Rather, there was stimulation of hyphal growth of *G. mosseae*. Under field conditions, none of the *Pseudomonas* strains affected the diversity of native AM fungi in the rhizosphere soil, root colonisation and AM functional symbiosis and rather improved plant growth and nutrient (N and P) acquisition by AM-mediated plants (Barea et al. 1998). Sanchez et al. (2004) showed that a fluorescent pseudomonad and *G. mosseae* had similar impacts on plant gene induction, supporting the hypothesis that some plant cell programmes may be shared during root colonisation by these beneficial microorganisms. Gram-positive and gamma-proteobacteria are more frequently associated with AM fungi than are gram-negative bacteria (Table 8.4), but their synergistic interaction is yet to be confirmed (Artursson et al. 2005).

Table 8.4 Examples of synergistic interactions between AM fungi and bacteria or PGPR leading to inhibition of fungal pathogens

Bacterial species	AM fungi species	Interaction effect	Inhibition of fungal pathogen	References
Bacillus pabuli	*Glomus clarum*	+	ND	Xavier and Germida (2003)
B. subtilis	*G. intraradices*	+	ND	Toro et al. (1997)
Paenibacillus validus	*G. intraradices*	+	ND	Hildebrandt et al. (2002)
Paenibacillus sp.	*G. mosseae*	+	+	Budi et al. (1999)
Paenibacillus sp.	*G. intraradices*	+	ND	Mansfeld-Giese et al. (2002)
Pseudomonas sp.	*G. versiformis*	+	ND	Mayo et al. (1986)
Pseudomonas sp.	*G. mosseae*	+	+	Barea et al. (1998)
Pseudomonas putida	Indigenous mixed AM fungi	+	ND	Meyer and Linderman (1986)
P. fluorescens	*G. mosseae*	+	+	Edwards et al. (1998)

Modified from Artursson et al. (2006)
+ positive, *ND* not determined

8.3.4 *Soil and Agricultural Management Practices Influencing AM Fungi Response*

To benefit from mycorrhizal associations (or more generally beneficial biological processes in the rhizosphere), emphasis has to be on agricultural practices that promote the occurrence and functioning of soil organisms, including AM fungi. The low host specificity of AM fungi may allow mycelial networks of a particular fungus in the soil to be connected directly to roots of plants of different species, forming hyphal links between their mycorrhizal roots. It has been shown that in fragile tropical agroecosystems, conventional agriculture, relying on tillage and external inputs (mineral fertilisers, biocides) for increase of productivity, may result in large ecological disturbances and may not be sustainable in the long term. Most of the cultivated plant species are able to form the mycorrhizas. However, the plant families *Brassicaceae* and *Chenopodiaceae* include species that do not usually form mycorrhizal symbiosis; among them, sugar beet and rape (Tester et al. 1987) are important. Growing these crops subsequently does not lead to multiplication of AM fungi, unless there are weeds that can act as hosts (Abbott and Robson 1991; Jansa et al. 2002).

8.3.4.1 Fertilisers, Manures, Fungicides and Tillage Practices

Application of farmyard manure can increase densities of AM fungal spores, although this depends on the soil types (Harinikumar and Bagyaraj 1989). Several studies indicated that cumulative P fertilisation decreases the spore density under Northern European field conditions (Martensson and Carlegren 1994; Kahiluoto et al. 2001). Another study showed that AM fungal colonisation was not affected by P addition when plants were deficient in N, but, when N was sufficient, P addition suppressed root colonisation (Sylvia and Neal 1990). Thus, there are agronomic soil management practices available for the farmer to regulate the AM fungi at the field site. An important measure, apart from the choice of cropping systems in conventional agriculture, is the use of fungicides particularly systemic fungicides applied in the field has shown to reduce the functioning of the AM fungi (Menge et al. 1978; Kling and Jakobsen 1997). AM fungi can be sensitive to certain but not all fungicides. Mancozeb, thiram and ziram are all dithiocarbamates and, as a group, appear to be deleterious to mycorrhizal fungi, at least when tested in groundnut (Sugavanam et al. 1994). Emisan (a mercuric treatment) and carbendazim (a benzimidazole) were both negative for AM fungi when tested in groundnut. Copper, however, appeared to provide a stimulus to mycorrhizae in groundnut. Application of fungicide to soil reduced sporulation and the root length colonised by AM fungi, although interaction of AM fungi and fungicide was observed to be highly variable depending on fungus-fungicide combination and on environmental conditions (Turk et al. 2006).

Fungicide seed treatments alter the microbial population dynamics in the rhizosphere by reducing root pathogen infection but may also affect nontarget organisms (Rodriguez-Kabana and Curl 1980; Trappe et al. 1984). Soil applications of metalaxyl have been reported to favour AM colonisation in corn and soybeans (Groth and Martinson 1983). Seed-applied captan had no effect on AM colonisation in studies conducted by Kucey and Bonetti (1988), and it reduced symptoms of *Fusarium solani* when applied along with AM inoculum in *Phaseolus vulgaris* plants (Gonçalves et al. 1991). Other fungicides such as benomyl, captan, pentachloronitrobenzene and emisan have been reported to also have negative effects on AM colonisation when applied as soil drenches (Kjoller and Rosendahl 2000; Schreiner and Bethlenfalvay 1997; Sugavanam et al. 1994). Murillo-Williams and Pedersen (2008) showed that under natural pathogen inoculum (non-fumigated soil), seed-applied fungicides with fludioxonil seemed to favour AM colonisation due to a reduced competition with aggressive pathogens like *Rhizoctonia* spp., an organism that is targeted by this fungicide.

Function of AM fungi and species composition may also be affected by farming systems. This is evidenced from a long-term field trial established in Switzerland designed to compare long-term effects of "conventional" vs. "organic" farming systems (Mäder et al. 2002). In this trial, about 40 % more roots were colonised by AM fungi in the organic systems than in the conventional system (Mäder et al. 2000). They suggested that AM fungal species differ in functional characteristics such as spore production and plant growth promotion (Van der Heijden et al. 1998). Moreover, less efficient AM fungal species might be selected by high-input farming (Scullion et al. 1998). Tillage affects the mycorrhizal hyphal network (Cardoso and Kuyper 2006). Mulligan et al. (1985) observed that excessive secondary tillage reduced AM colonisation of *Phaseolus vulgaris* L. Mycorrhizal root colonisation of corn growing in NT (no-tilled) and ridge till plots was greater than that in CT (conventional-tilled) plots (McGonigle and Miller 1993). AM hyphae and spores were more abundant in the top 0- to 15-cm layer of the soil profile and decreased dramatically below this depth (Kabir 2005). Similar results were reported for AM spores by An et al. (1990) in Kentucky, USA, under soybean. This suggests that tilling the soil to a depth of 15 cm would affect most of the AM fungi and that ploughing below this depth would dilute the AM propagules in the zone of seedling establishment (Kabir 2005). The role of glomalin in soil aggregation (Rillig 2004) was correlated with stabilisation of soil aggregates after a 3-year transition of a maize cropping system from conventional tillage to no tillage (Wright et al. 1999), and there are indications that some crop rotations favour glomalin production and aggregate stabilisation more than others (Wright and Anderson 2000). Thus, management of cropping systems to enhance soil stability and reduce erosion may benefit from consideration of the factors controlling production and maintenance of extraradical hyphae and glomalin (Cardoso and Kuyper 2006).

8.3.4.2 Crop Rotation and Sequences

AM fungi show only a limited degree of specificity; different plant species stimulate the amount and occurrence of different species of AM fungi; thus, through the management of plants, it is possible to modify mycorrhizal populations in the soil (Colozzi and Cardoso 2000; Hart et al. 2001). Mycorrhizal inoculum density declines when soils are kept fallow for extensive periods of time (Thompson 1987). The quantity of AM fungi in soils also differs between host species (Vivekanandan and Fixen 1991). Even the preceding crop in a crop rotation system affects the AM fungal spore densities in the field and thereby the yield of the following crop (Karasawa et al. 2001). Oehl et al. (2003) found that increased land use intensity was correlated with a decrease in AM fungal species richness and with a preferential selection of species that colonised roots slowly but formed spores rapidly. Soils used for agricultural production have a low diversity of AM fungi compared with natural ecosystems (Menendez et al. 2001) and are often dominated by *Glomus* species (Daniell et al. 2001; Oehl et al. 2003; Troeh and Loynachan 2003). One reason for this is the low diversity of hosts, which reaches an extreme in crop monoculture (Oehl et al. 2003). Monoculture may select for AM fungal species that provide limited benefits to the host plant. Johnson et al. (1992) found that maize yielded higher and had higher nutrient uptake on soils that had grown soybean continuously for the previous 5 years than on soil that had grown maize continuously for the previous 5 years. Conversely, soybean yielded higher and had higher nutrient uptake on soil which had grown 5 years of maize than 5 years of soybean. The most abundant AM fungal species in the continuous maize soil was negatively correlated with maize yield but positively correlated with soybean yield; there was a similar effect with soybean soil. They hypothesised that monocropping selects AM fungal species which grow and sporulate most rapidly and that these species will offer the least benefit to the plant because they divert more resources to their own growth and reproduction. The result can be reduced benefits of AM colonisation to the host plant while monocropping continues. Crop rotation effects on mycorrhizal functioning have repeatedly also been observed by other workers. Harinikumar and Bagyaraj (1988) observed a 13 % reduction in mycorrhizal colonisation after 1-year cropping with a non-mycorrhizal crop and a 40 % reduction after fallowing. Lack of inoculum or inoculum insufficiency after a long bare fallow (especially in climates with an extended, dry, vegetation less season) may result in low uptake of P and Zn and in plants with nutrient deficiency symptoms that have been described as long-fallow disorder. The use of mycorrhizal cover crops can overcome this disorder (Thompson 1996). Sanginga et al. (1999) found evidence for increased mycorrhizal colonisation of soybean if the preceding crop was maize and increased colonisation of maize if the preceding crop was *Bradyrhizobium*-inoculated soybean in the savanna of Nigeria. Similarly, Bagayoko et al. (2000) reported higher AM colonisation in cereals (sorghum, pearl millet when grown in rotation with legumes (cowpea, groundnut) than in

continuous cropping. Osunde et al. (2003) reported that AM colonisation in maize benefited from previously grown soybean plants.

In a long-term experiment involving three tillage systems and four soybean-based crop rotations after six cropping seasons, rotation produced significantly higher grain yield and supported higher inoculum potential of AM fungi in the rhizosphere soil (Sharma et al. 2012a). On the other hand, irrespective of crop rotations, the tillage system did not all have the same effect. Moreover, the inoculum potential of resident AM fungi in soybean rotation involving maize in conservation tillage was highly correlated with grain yield of soybean implicating the resident AM fungi in enhancing the soybean yield.

8.3.5 Inoculation vs. Field Management of Indigenous AM Fungi

Selection of the appropriate AM fungi is among one of the critical issues for the application of AM technology in agriculture (Estaun et al. 2002). Ecologically sound selected strains of AM fungi inoculum are not presently available in large quantities at a low price. Alternatively, inoculum can be produced on site (on farm) under local agronomic conditions (Sieverding 1991). The successful introduction of a foreign microorganism into the soil depends on how well it adapts, develops and competes for nutrients. AM fungal consortia isolated from organic farms were more effective in plant growth promotion under conditions of low nutrient availability than were consortia from conventional farms (Scullion et al. 1998). Therefore, it is likely that on-farm selected strains (site specific) are better due to their adaptability to edaphic conditions than selected strains produced in vitro or in vivo under controlled conditions. Given limitations of bulk inocula requirements or instances where inoculation may not be feasible, the management of native and resident AM fungi through crop sequences and soil management practices (e.g. minimum tillage) could be a better option.

8.4 Production and Commercialisation of AM Fungi

8.4.1 Conventional Methods

The obligate biotrophic nature of AM fungi has complicated the development of cost-efficient large-scale production technologies to obtain high-quality AM fungal inoculum. This is one of the bottlenecks to commercial exploitation (IJdo et al. 2011). There are various techniques currently used to culture AM fungi on hosts such as on-farm production (Douds et al. 2005, 2006; Sharma and Sharma, 2006; Sharma and Sharma 2008; Sharma and Adholeya 2011), pot culture

techniques using traps (Gaur and Adholeya 2000), nutrient film technique (Mosse and Thompson 1984) and aeroponics (Jarstfer and Sylvia 1995). The most frequently used technique for increasing propagule number has been the propagation of AM fungi on a suitable host in disinfested soil using pot cultures. Other factors for creating a favourable environment for culturing of AM fungi are a balance of light intensity, adequate moisture and moderate temperature without detrimental addition of fertilisers or pesticides (Jarstfer and Sylvia 1992; Al-Karaki et al. 1998). Cultures reaching high propagule density (e.g. 10 spores per gram) after a number of multiplication cycles can be stored using suitable methods after air-drying (Kuszala et al. 2001).

AM fungi have been cultured with plant hosts in different substrates such as sand, peat, expanded clay, perlite, vermiculite, soilrite (Mallesha et al. 1992), rockwool (Heinzemann and Weritz 1990) and glass beads (Redecker et al. 1995). They can also be produced aeroponically (Sylvia and Hubbell 1986). The aeroponic system was adopted for mycorrhiza production by the utilisation of seedlings with roots pre-colonised by an AM fungus and the use of modified Hoagland's nutrition with a very low P level (Hoagland and Arnon 1938). *Entrophospora kentinensis* was successfully propagated with bahia grass and sweet potato in an aeroponic system by Wu et al. (1995).

The nutrient film technique (NFT) was adapted for AM fungi inoculum production by Mosse and Thompson (1984). Further, Lee and George (2005) proposed a modified nutrient film technique for large-scale production of AM fungal biomass with the help of improved aeration by intermittent nutrient supply, optimum P supply and the use of glass beads as support materials.

8.4.2 In Vitro/Root Organ Culture (ROC) Method

In vitro culture of AM fungi was achieved for the first time in the early 1960s (Mosse 1962). Since then, various pioneering steps were aimed at axenic culturing of AM fungi. Continuous cultures of vigorous ROCs (Ri T-DNA-transformed) have been obtained through transformation of roots by the soil bacterium *A. rhizogenes* (Tepfer 1989) that provided the new way to obtain mass production of roots in a very short span of time. In most cases, purified and surface sterilised spores (Becard and Piche 1992) isolated from the field or from traps have been successful for establishing dual cultures under in vitro conditions. The root organ culture (ROC) is an attractive mass multiplication method for providing a pure, viable, rapid and contamination-free inoculum using less space and has an advantage over the pot culture multiplication/conventional system (Fortin et al. 2002; Cranenbrouck et al. 2005; Dalpe et al. 2005). Different production systems have been derived from the basic ROC in Petri plates. For example, root organs and AM fungi were cultured in small containers, by which large-scale production was obtained (Adholeya et al. 2005). Douds (2002) reported monoxenic culture of *G. intraradices* with Ri T-DNA transformed roots in two-compartment Petri dishes

as a very useful technique for physiological studies and the production of clean fungal tissues. Various inocula based on inert or sterilised substrata, such as peat, expanded or calcined clays or lave, are used commercially and are less susceptible to contamination with pathogen (Whipps 2004). Various forms of AM fungi are commercially produced and available in various formulations for sale throughout the world.

8.4.3 On-Farm Production

As AM fungi are obligate symbionts, they require host plants to sporulate and colonise roots to complete their life cycles. Currently, AM fungi are multiplied in various ways like monoxenic/in vitro, pot culturing/greenhouse, aeroponic system and nutrient film technique (Fortin et al. 2002; Lee and George 2005). While inocula produced by these techniques are commercially available, the pot culture or conventional method is still widely used (Saito and Marumoto 2002). There are many steps including isolation of AM fungi, the use of substrate/potting mixture and subsequent maintenance and transportation which incur costs and limit commercialisation. On-farm multiplication of indigenous and resident AM fungi removes many steps, which reduce the cost and enhance the acceptability to the farmers (Douds et al. 2006). The on-farm technology is more appropriate since it uses the indigenous AM fungi already adapted to that site and environment. Apart from this, the technology can be used for producing introduced AM fungi (applied as starter culture in beds) using one or a succession of trap plants (Sieverding 1991). Under this method, the fungal inoculum is produced on raised/elevated beds in situ; in the farmer's own nursery or his kitchen garden, a space that he generally uses for growing seedlings for field transplantation (Sharma and Sharma 2008). The mycorrhizal roots can then be harvested and used in the field as inocula. The soil left in the nursery after removing the roots contains a many AM fungal propagules which will serve as the source of AM fungi for further multiplying the inocula in the subsequent cycles. This method can produce inoculum of the indigenous AM fungi already adapted to the site. This field-based method deals with preparing beds of sterilised (solarised by polythene) soils in which either the indigenous AM fungi community or introduced isolates are increased using one or a succession of trap plants (Sieverding 1991). An important consideration in producing AM fungi is the level of available phosphorus which is critical for inoculum production and needs to be analysed before multiplication. In general, under Indian conditions, the level of Olsen P (available P in tropical soils) is low (less than 10 ppm), but high available P level (beyond 20 ppm) could be detrimental to AM sporulation and hence should be determined prior to multiplication. A unique feature of such technique is that it will not only produce mycorrhizal spores, hyphae and highly colonised roots but at the same time beds can be used for preparing seedlings for field transplantation.

8.5 Need of Regulatory Mechanisms and Quality Assurance

Currently, large-scale production of AM fungi is not possible in the absence of a suitable host, and species cannot be identified in their active live stages (growing mycelium). As a consequence, quality control is often a problem, and tracing the organisms into the field to strictly relate positive effects to the inoculated AM fungus is nearly impossible (IJdo et al. 2011). Pringle et al. (2009) have also indicated the risks associated with the transport of AM fungi around the world and have detailed the problem that can arise with the introduction of exotic material. In India, registration of biofertiliser production units is compulsory and is being done by the Ministry of Agriculture and Cooperation through a nodal agency, National Centre of Organic Farming, Ghaziabad, India.

8.6 Conclusion

Oilseeds comprise both legumes and nonlegumes, and major oilseeds like groundnut, sesame and soybean are grown under rainfed conditions in the tropics and subtropics in the marginal lands with meagre amount of external application of fertilisers. Very often, the major oilseeds crop faces vagaries of weather conditions like erratic rainfall and mid- and end-of-season drought coupled with plethora of diseases and pests severely limiting the productivity. Thus, to enhance the productivity of the oilseed crops, management of nutrients is of utmost importance to enhance availability of nutrient in suboptimal conditions of cultivation. Therefore, there is great opportunity of application of microbes especially rhizobia, PGPMs and AM fungi alone or in combinations. Considering the plant genotype as a constant factor, microbial package should be developed based on climate, soil and microbe interactions. Furthermore, formulation of biofertiliser packages should be developed not only for enhancing nutrient availability and uptake but for managing soil-borne and foliar diseases, in addition to enhancing growth by production of plant growth regulators. Within the constraints of available resources, a large number of PGPMs and AM fungi have been identified with capability to enhance growth and yield of many oilseed crops, but effective strains tolerant to abiotic stresses are few. Therefore, ongoing effort is needed to identify efficient strains of PGPMs and AM fungi which can alleviate abiotic stresses and have potential biocontrol abilities, besides enhancing nutrient availability and uptake in suboptimal conditions of cultivation. Many studies have shown large amounts of hyphal biomass and higher indigenous AM fungi in crop rotations involving maize. The large-scale production of resident AM fungi is still in its infancy and the combined application of AM fungi and PGPMs are yet to be streamlined. Finally, potential commercial formulations need to be subjected to regulatory requirements and quality checks before they are eventually registered as a commercial formulation.

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Chapter 9
Arbuscular Mycorrhizal Diversity and Function in Grassland Ecosystems

Tomoko Kojima, Sasha Jenkins, Anjani Weerasekara, and Jing-Wei Fan

9.1 Introduction

Grasslands are widely distributed globally and occur on all continents except Antarctica. It is estimated that they make up one-fifth of the Earth's land surface (Parton et al. 1993). The majority of grasslands have resulted from anthropogenic activities where forests were cleared for domesticated animal grazing. Grasslands are important economically as they provide forage for livestock industry and a landscape for recreational and tourism activities. Grasslands are classified as natural, semi-natural or artificial. Natural and semi-natural grasslands are both grazed and unfertilised; natural systems do not receive any further agricultural improvements, whereas semi-natural grasslands are maintained by tillage, cutting, mowing or burning. Increased demand for grazing livestock has led to intensification and creation of artificial or improved grasslands that require regular reseeding and herbicide and fertiliser inputs. Some of the semi-natural grasslands are also used for hay and silage production. Artificial grasslands are generally more productive and profitable, whereas natural and semi-natural grasslands may have better soil quality and support a greater microbial biomass, species biodiversity and ecosystem function, including biogeochemical cycling, disease suppression and

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carbon sequestration (Grayston et al. 2001; Oehl et al. 2003). Grasslands are under threat from the impacts of climate and land-use change (O'Donnell et al. 2007) due to an expanding human population with increased demand upon land resources for agricultural, residential, waste treatment, recreational and industrial development (Firbank 2005; Kan 2009). Meeting these demands requires a better understanding of soil functioning in grasslands and, ultimately, the ability to manipulate the diversity-function relationships and microbial interactions such as mycorrhizas (O'Donnell et al. 2007, 2001).

9.2 AM Fungal Ecology in Grasslands

Arbuscular mycorrhizal (AM) fungi are ubiquitous in grasslands (van der Heijden et al. 1998) where they are thought to play a major role in ecosystem functioning and services (Gianinazzi et al. 2010; Johnson et al. 2004) To date, AM fungi have been characterised in a wide range of grasslands across the world with varying soil and vegetation types including tropical grasslands (Zangaro et al. 2008), arid grasslands (Pezzani et al. 2006), boreal grasslands (Eriksen et al. 2002) and temperate grasslands (Barni and Siniscalco 2000). Succession in plant communities has been related to the changes in mycorrhizal type and their diversity (Allen 1996; Johnson et al. 1992). In particular, AM fungi play a vital role in facilitating plant nutrient uptake in nutrient-deficient soils of arid and semi-arid grasslands. In the case of succession in the Mexican Chihuahuan Desert, pioneer grasses were mycorrhiza-independent species, and late-successional grasses were more responsive to AM fungi and supported a higher spore density (Pezzani et al. 2006).

As a key link between above- and below-ground plant biomass, AM fungi play an important ecological role in shaping plant communities by influencing plant growth, plant diversity and competitive ability (Johnson et al. 2004; Klabi et al. 2014; McCain et al. 2011; van der Heijden et al. 1998). AM fungi are generalists and are able to colonise the majority of vascular plant species, including many important crop species such as maize, wheat, rice and potato (Roy-Bolduc and Hijri 2011). Thus, they do not display host specificity that is characteristic of other plant-microbe symbioses (Smith and Read 2008). As absolute symbionts, AM fungi provide a range of benefits (Roy-Bolduc and Hijri 2011) in return for plant-assimilated carbon from their hosts. They promote plant growth by mining soil pore spaces that are inaccessible to plant roots and by significantly increasing the total volume of soil explored for both nutrients and water (Garg and Chandel 2010; Kaya et al. 2003; Rillig et al. 2003). AM fungi also improve the efficiency of plant P uptake and a range of other nutrients, including organic N via various mechanisms (Al-Karaki and Clark 1998; Harrison et al. 2002; Subramanian and Charest 1999). AM fungi can contribute to improvement of soil structure through the formation of soil aggregates which in turn increases water-holding capacity (Andrade et al. 1998; Augé 2001; Augé et al. 2001) and tolerance of host plants to fungal pathogens, nematodes and water stress (Borowicz 2001; Forge et al. 2001; Newsham

et al. 1995; Plenchette et al. 2005; Smith and Read 2008). They are also involved in alleviating effects of salinity on plant growth and ameliorating effects of heavy metal toxicity and pollution (Gianinazzi et al. 2010; Hildebrandt et al. 2001). These attributes enable AM fungi to have significant roles in maintaining sustainable levels of plant biomass in grasslands.

9.2.1 Host Plant Preferences

AM fungi can colonise a wide range of terrestrial plant species including grass species and do not display host specificity in the conventional sense of strict matching of host and symbiont (Smith and Read 2008). However, AM fungi can exhibit what has been termed 'ecological host specificity' (McGonigle and Fitter 1990) where roots of the same plant species may become preferentially colonised by some AM fungi in contrast to others when grown in the presence of the same community of AM fungi (Li et al. 2014; Scheublin et al. 2007; Vandenkoornhuyse et al. 2003). This may occur because of differences in root architecture or root proliferation. It may also arise due to variation in the infectivity of hyphae from spores of different AM fungi either arising from spores or from the common mycorrhizal network in soil (Fellbaum et al. 2014). Host preference for mycorrhiza formation can also be expressed in terms of the physiological effectiveness of the AM fungi (Klironomos 2003) that are present in the grassland community. Indeed, the indigenous assemblages of AM fungi associated with the native prairie grass *Andropogon gerardii* appeared to be functionally adapted to the local soil environment (Ji et al. 2013). However, species-specific interactions do not always occur (Santos et al. 2006), and this could be due to management and abiotic factors such as fertilisation and seasonality.

In experiments investigating interactions between AM fungi and plant species in grasslands compared with other agricultural systems, variation in the diversity and abundance of AM fungi associated with different plants has been demonstrated (Verbruggen et al. 2010). The same authors found that AM fungal species diversity was highest in grasslands compared to a cropping system. However, AM fungal communities of organically managed cropping systems were also more similar to those of grasslands compared to cropping systems where synthetic fertiliser had been applied (Verbruggen et al. 2010). For example, the spore number and diversity of AM fungi were both greater in soil collected from red clover or grassland than from under crops of barley or wheat (Menéndez et al. 2001). Hetrick and Bloom (1986) showed that there was higher spore production of AM fungi when sudangrass was grown as the host plant, compared with asparagus, tomato and red clover. Furthermore, in an experiment investigating the coexistence of two grass species, the distribution of phosphorus and nitrogen differed when plants were inoculated with different AM fungal species (van der Heijden 2003). Even when environmental conditions are similar, the AM fungal species composition or biomass can differ in rhizosphere soils of different plant species coexisting in the same

soil. This has been demonstrated in semi-natural grasslands, where the AM fungal spore density in rhizosphere soil associated with *Miscanthus sinensis* was higher than in soil associated with *Pleioblastus chino* (Murakoshi et al. 1998).

Different cultivars of the same grass species can differ in response to colonisation by AM fungi, as was shown for growth of orchard grass with different combinations of AM fungal species (Tsuchida and Nonaka 2002, 2003). For the legume alfalfa, the effects of AM fungal species also varied with cultivar (Douds et al. 1998). Furthermore, AM fungi might alter plant species diversity by increasing competitive intraspecific suppression and decreasing interspecific suppression of small plants surrounded by larger neighbour plants (Moora and Zobel 1996). In another study, AM fungi were found to maintain grassland community stability by regulating plant diversity through increased interspecific competition between the grazing grass *Elymus nutans* and the poisonous plant, *Ligularia virgaurea* (Jin et al. 2011). Where there are dominant and subordinate plant species in a grassland community, the dominant species could be negatively affected depending on the combination of AM fungi present, especially if the growth of subordinates is enhanced by mycorrhizas to a greater extent than that of the dominant species (Klabi et al. 2014). Klabi et al. (2014) found that the competitiveness of the dominant cool-season grass *Elymus trachycaulus* ssp. *subsecundus* was reduced in the presence of *Glomus cubense*. These effects have been demonstrated in pot experiments where it has been suggested that less favourable AM fungi could reduce the dominance of some plant species in grassland (Mariotte et al. 2013). In addition, seasonal changes in the composition of AM fungi in roots can occur (Santos et al. 2006), and AM fungal diversity can be affected by plant species composition (Johnson et al. 2004). Furthermore, mycorrhizal dependency is different among plant species, and there are differences in mycorrhizal response between C4 and C3 grasses (Hetrick and Wilson 1991; Hetrick et al. 1990). Indeed, C4 grasses had higher mycorrhizal dependency than did C3 grasses (Lugo et al. 2003). Therefore, in grasslands, the composition of a plant community could affect the AM fungal community and *vice versa*. Consequently, factors that contribute to these processes are complex in space and time, and site-specific studies are needed to identify the mechanisms involved before confident generalisations can be made.

9.2.2 AM Fungal Diversity and Analytical Methods

AM fungal diversity in grasslands was initially characterised using bioassays and traditional taxonomic approaches that identified and quantified AM fungal spores extracted directly from the field or trap cultures (Leal et al. 2009; Oehl et al. 2003, 2009). However, it is often difficult to characterise spores below the family level using traditional methodology due to the lack of discriminating morphological characters (Redecker and Raab 2006) and because sporulation is species and environment dependent (Young 2012). As AM fungi are obligate symbionts (Franz Lang and Hijri 2009), selective isolation in artificial media remains a

significant challenge. Consequently, the vast majority of the currently described taxa on the AM fungi database (www.amf-phylogeny.com) are uncultured morphospecies (Krüger et al. 2009, 2012). Since AM fungal communities influence plant competition, diversity and productivity, a greater taxonomic and phylogenetic resolution is needed for the understanding relationships between plant-fungi interaction and ecosystem functioning in grasslands (Klironomos 2003; McCain et al. 2011; Öpik et al. 2003; van der Heijden et al. 1998, 2003).

More recently, knowledge of AM fungi in grasslands has been based on the application of molecular methodologies with more rapid and precise identification of the fungi in both roots and soil (Montero Sommerfeld et al. 2013; Young 2012). PCR-based analysis of ribosomal RNA gene (rRNA) using primers that target the small subunit (SSU), large subunit (LSU) and the internal transcribed spacer (ITS) region has been extensively used as taxonomic biomarkers for characterising AM fungal communities in soil (Krüger et al. 2009, 2012; Lee et al. 2008). However, some primers (especially those targeting the SSU) have poor specificity and sensitivity for AM fungi making it difficult to distinguish between different species (Krüger et al. 2009; Redecker 2000; Schüßler et al. 2001). For better taxonomic resolution of AM fungi at the species level and a more unbiased determination community diversity, it is recommended that primers cover the ITS and LSU region (Stockinger et al. 2009). The ITS region is recognised as a general fungal bar-coding marker; however, it is difficult to differentiate between certain groups within *Glomeromycota* (Schocha et al. 2012). Recently, highly AM fungal-specific primers that amplify the SSU-ITS-LSU region (Krüger et al. 2009) have been described for better taxonomic resolution. Moreover, new phylogenetic reference data of AM fungi (Krüger et al. 2012) and databases (e.g. MaarjAM) (Öpik et al. 2010) containing representative sequences from published *Glomeromycota* sequence-based taxa and known morphospecies have been developed that improve DNA-based species characterisation of AM fungal communities in grasslands.

Initially, 'fingerprinting' technologies such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) were used to gain insights into how AM fungal community structure was shaped by environmental and management drivers (Lugo et al. 2003; Montero Sommerfeld et al. 2013; Yang et al. 2010). Microbial diversity was assessed by preparing clone libraries and analysing with Sanger sequencing methods (Chen et al. 2014; Liu et al. 2012; Santos-González et al. 2007; Yang et al. 2010). Data generated from these molecular techniques has revealed that the community structure and diversity of AM fungi in grassland are strongly influenced by seasonality, fertilisation and management practices (Chen et al. 2014; Montero Sommerfeld et al. 2013; Yang et al. 2010). With the advent of the next-generation sequencing (NGS), AM fungal diversity, function and distribution patterns are now being examined at levels that were unthinkable only a decade ago (Öpik et al. 2010; Schüßler and Walker 2010; Stürmer 2012) which has significantly improved our understanding of their role in grasslands and other environments (Dumbrell et al. 2011; Krüger et al. 2012; Redecker et al. 2013; Stockinger et al. 2010).

Glomus spp. are often dominant in grassland soil based on examination of spores. For example, sporocarpic *Glomus* spp. were dominant in a natural mountain grassland (Pampas) in Argentina (Lugo and Cabello 2002). *Glomus heterosporum* and *G. intraradices* were dominant in the tallgrass prairie of Kansas, USA (Eom et al. 2000), and unidentified *Glomus* spp. were dominant in grasslands of Central Europe (Oehl et al. 2003). *G. aggregatum* and *G. leptotichum* (*Ambicispora* sp.) were dominant in experimental plots in Minnesota, USA (Johnson et al. 1992), and *G. geosporum* was dominant in the Inner Mongolia steppe as determined using the method trap culture (Su and Guo 2007). In contrast, *Acaulospora colossica*, *Scutellospora calospora*, *Gigaspora gigantea*, *Archaeospora leptoticha* and several other species were common in grasslands of North Carolina, USA (Pringle and Bever 2002), and in several Japanese semi-natural grasslands, *Sclerocystis rubiformis* and an unidentified *Glomus* sp. (orange-dark red, about 100–150 μm) were the dominant species (Kojima et al. 2009; Fig. 9.1) along with Paris-type root colonisation (Fig. 9.2). Recently, DNA-based sequencing approaches have characterised AM fungal communities associated with the soil and roots of grassland plant species (Hiiesalu et al. 2014; Li et al. 2014). They revealed that grassland

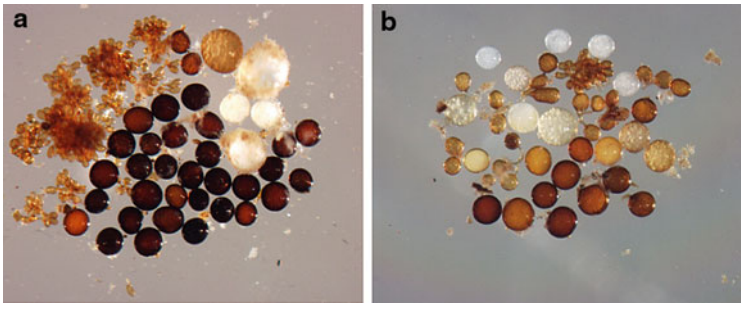

Fig. 9.1 AM fungal spores in the soil (10 g) collected from semi-natural: (**a**) *Zoysia japonica*-dominant or (**b**) *Miscanthus sinensis*-dominant grasslands

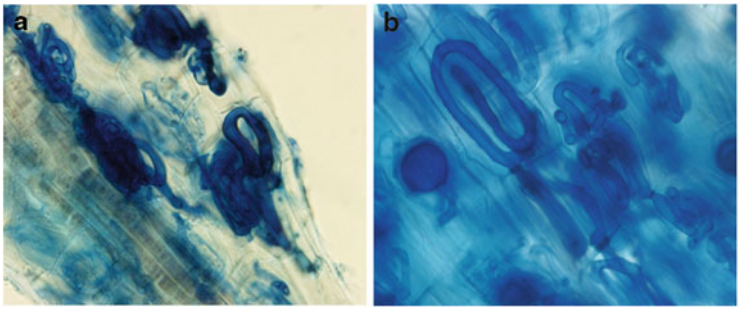

Fig. 9.2 AM fungal colonisation stained with Trypan blue in the roots of (**a**) *Zoysia japonica*, (**b**) *Pleioblastus chino* collected from semi-natural grasslands

communities are dominated by members of the families *Archaeosporaceae*, *Claroideoglomeraceae*, *Diversisporaceae*, *Glomeraceae*, *Gigasporaceae*, *Acaulosporaceae* and *Paraglomeraceae* (Hiiesalu et al. 2014; Li et al. 2014; Ohsowski et al. 2014). Members of the *Glomerales* (*Glomeraceae* and *Claroideoglomeraceae*) usually exhibit more r-selected traits, whereas members of the *Diversisporales* (*Gigasporaceae*, *Acaulosporaceae*, *Pacisporaceae* and *Diversisporaceae*) tend towards more K-selected traits such as large extraradical mycelium, spore size and density and increased hyphal diameter (Chagnon et al. 2013; Sýkorová et al. 2007). The most dominant AM fungal genus found in these grasslands was *Glomus*, followed by *Rhizophagus*, *Acaulospora*, *Scutellospora and Diversispora* which is in agreement with taxa recovered using traditional morphological methods (del Mar Alguacil et al. 2010; Hazard et al. 2014; Liu et al. 2012).

Conclusions about the composition of AM fungal species in grassland soils can differ depending on the methods used (Brundrett et al. 1999; Clapp et al. 2003). Sampling strategy, sampling intensity and the methodology used may further influence the detection and quantification of species diversity of AM fungi in roots or soils (Shi et al. 2012), including grasslands (Whitcomb and Stutz 2007). For example, when the AM fungal community colonising roots in a grassland was investigated using phylogenetic analysis, there was not a close correspondence with the morphotypes of AM fungi (Schnoor et al. 2011a). Furthermore, it has been shown that the treatment of root samples can influence conclusions related to AM fungal analysis (Renker et al. 2006). Molecular methods generally yield more information and have greater taxonomic resolution; however, assigning taxonomic affiliation is complicated by the fact that AM fungi reproduce asexually leading to higher diversity within species (Munkvold et al. 2004; Stockinger et al. 2009). This can be overcome by analysing molecular assays in combination with morphological-based methods that can identify different spore features (Wetzel et al. 2014). The integration of these methods will provide a greater insight into the AM fungal diversity and functional activities in grasslands.

9.3 The Dynamics of AM Fungi in Grasslands

In some grasslands, environmental factors such as altitude, nutrient availability, salinity, soil water content, temperature, pH as well as plant diversity were identified as key drivers of AM fungi diversity (An et al. 2008; Eason et al. 1999; Eom et al. 2001; Li et al. 2014). In other grasslands, light incidence, temperature and aluminium availability in the soil influenced AM fungal colonisation (Göransson et al. 2008; Zangaro et al. 2013) demonstrating the breadth of influence of local soil conditions. Intensification and changes in agricultural practice include fertilisation, manure application, pesticide usage, crop rotation, mowing and tillage (Barto et al. 2010; Binet et al. 2013; Birgander et al. 2014; Collins and Foster 2009; Kabir 2005; Mathimaran et al. 2007; Oehl et al. 2003), all of which could influence

colonisation of roots by AM fungi. For example, long-term pesticide use resulted in an 80 % reduction in mycorrhizal root colonisation (Smith et al. 2000). Livestock grazing is an example of a major land-use practice which influences plant production, plant species composition and nutrient cycling in soil, all of which could alter root colonisation by AM fungi and their relative abundance.

Temporal changes and seasonal fluctuations in AM fungal colonisation have been observed (Bentivenga and Hetrick 1992; Birgander et al. 2014; Escudero and Mendoza 2005; Lingfei et al. 2005; Lugo et al. 2003). These changes are most likely to be a consequence of seasonal differences in rainfall and temperature that alter soil water content and metabolic activity, respectively (Lingfei et al. 2005; Lugo et al. 2003). In a number of studies, a distinct warm to cold seasonal shift in AM fungi community composition was observed, and root colonisation rates were greater during the growing season (Dumbrell et al. 2011; Hazard et al. 2014; Lugo et al. 2003; Montero Sommerfeld et al. 2013). In a seasonal investigation of the interaction between AM fungi and dark septate endophytic (DSE) fungi, AM fungi were most abundant during the peak growing season with dominant C4 plants, whilst DSE fungi were most abundant during the early part of the growing season (Mandyam and Jumpponen 2008). In contrast, higher AM fungi colonisation rates occurred during the growing season, but DSE colonisation was positively correlated with AM fungi colonisation suggesting they share an ecological niche in the grasslands of southwest China which were studied (Lingfei et al. 2005).

Interestingly, temporal variation in AM fungi colonisation or diversity is not always observed in grasslands. Saito et al. (2004) showed that the AM fungi in roots of *Zoysia japonica* remained relatively unchanged throughout a year. Other studies have shown that the composition and diversity of AM fungal communities do not change seasonally (Santos-González et al. 2007). Also, in grassland dominated by *Pleioblastus chino* and *Miscanthus sinensis*, the vegetation changed over a 4-year period, but the AM fungal community were relatively stable (Kojima et al. 2009). Sanders and Fitter (1992) showed colonisation in some plant species in a grassland in England changed both within and between years, but colonisation in other species was more constant. Thus, whilst the community structure of AM fungi in roots of some perennial plant species is relatively constant (McGonigle and Fitter 1990; Read et al. 1976), variability observed elsewhere may depend on location, soil characteristics or grassland composition.

9.3.1 Soil and Management Practices

In grasslands, colonisation of roots by AM fungi can occur through interception of hyphae from nearby roots, as well as from hyphae from germinated spores (Allen and Allen 1980). Thus, the network of AM fungi in grasslands is important not only for transfer of nutrients but also for colonisation of new roots (Wilson and Trinick 1983). Tilling or ploughing has been shown to reduce overall AM fungal abundance, spore density and species richness (Allison et al. 2005; Helgason et al. 2010;

Kabir 2005). Disking or tillage alters AM fungal dynamics in grasslands by disturbing the hyphal network and interacting with other soil organisms (Kabir et al. 1997; Murugan et al. 2013). In order to fully investigate these processes, many facets of AM fungi need to be considered. For example, AM colonisation was lower in a 3-year-old disked site compared to the natural prairie, but spore density was similar (Allen and Allen 1980). In another study, tillage increased the relative abundance of saprotrophic fungi at the expense of AMF and bacteria (Murugan et al. 2013). AM fungal biomass in roots and soil were both decreased by ploughing and rotavation in a sandy grassland (Schnoor et al. 2011a), and it was found that carbon allocation to the AM fungi decreased with soil disturbance. Furthermore, for a semi-natural grassland, soil disturbance with replicated ploughing reduced phylotype richness and changed the AM fungal community composition (Schnoor et al. 2011b). Disturbance of the soil structure is thought to make soil organic nitrogen accessible for mineralization which was otherwise protected from degradation (Kristensen et al. 2000). In another example, the reduction of woody species in a simulated grassland by burning decreased mycorrhizal colonisation (O'Dea 2007). Overall, management practices that disturb plant vegetation or AM fungal communities are likely to decrease AM fungal diversity in grasslands. Mechanical disturbances can also affect soil aggregation. Helgason et al. (2010) found that AM fungi were particularly affected by tillage disturbance with increases of 40–60 % among aggregate-size classes in non-tilled system compared to the conventional tilled system.

9.3.2 Soil Nutrients and Fertiliser Regime

Intensive management of grasslands is negatively correlated with AM fungal diversity, and this is thought to be due to management-induced changes in nutrient availability following the application of lime, synthetic fertiliser or manure (Jenkins et al. 2009). Moreover, since AM fungi play a role in mitigating nitrous oxide in soils, disruption of the mycorrhizal symbiosis through agricultural intensification may further contribute to increased N_2O emissions (Bender et al. 2014).

In general, increasing levels of fertilisation, whether they are synthetic or manures, is usually associated with reduced diversity of AM fungi compared to unfertilised grassland soils (Chen et al. 2014; Christie and Kilpatric 1992; Murugan et al. 2013; Wuen et al. 2002). However, the AM fungal community response to fertilisation is largely dependent upon the loading amount, fertiliser type and combination and the method of application (Chen et al. 2014; Lin et al. 2012). For example, AM fungal colonisation in two wild plant species was higher in the unfertilised compared to the grassland receiving phosphate fertilisation (Wuen et al. 2002). In another study, the genus *Acaulospora* dominated in the unfertilised treatment, whereas the genus *Glomus* prevailed in the fertilised soils, but this was largely dependent on the amount of P fertiliser applied (del Mar Alguacil et al. 2010). Overall, spore densities of AM fungi and mycorrhizal colonisation

rates are higher in the unfertilised soil compared to fertilised soils. Nevertheless, fungal biomass and AM colonisation rates were higher in organically managed soils (receiving manure applications) compared to soils receiving synthetic fertiliser (Bittman et al. 2005; Eason et al. 1999; Kabir et al. 1997). Also, the age of the plant can influence the impact of fertilisation on AM fungal communities. For example, the formation of mycorrhizas in seedlings of *Plantago lanceolata* in managed grasslands were affected by fertilisation, but the adult plants were unaffected (Šmilauerová et al. 2012). In this case, fertilisation decreased total colonisation and relative arbuscular frequency in seedling roots. These differences might be dependent mainly on the phosphate and nitrate availability in the grassland.

The addition of nitrogen fertiliser has been reported to cause a reduction in species diversity of AM fungi (Chen et al. 2014; Egerton-Warburton and Allen 2000; Jumpponen et al. 2005), but AM fungi abundance is often unaffected (Chen et al. 2014). For example, Jumpponen et al. (2005) found that clades within *Glomus* spp. were associated with either the fertilised and unfertilised treatments in tallgrass prairie. The decreased species richness could reflect a reduction in translocation of C from the plant to the AM fungi under N-rich conditions and the roots becoming colonised by a few nitrophilic AM fungi taxa. The addition of phosphate fertiliser has been shown to reduce AM fungal abundance, in particular mycorrhizal colonisation rate, arbuscule formation and hyphal length density (Chen et al. 2014; Khaliq and Sanders 2000; Liu et al. 2012). Indeed, grasslands receiving high P fertiliser inputs can be less productive because under these non-limiting conditions, mycorrhizas can reduce plant growth when not contributing to the symbiosis (Kaeppler et al. 2000). Other examples of site-specific effects include studies where P fertilisation altered AM fungi community structure in some grasslands (del Mar Alguacil et al. 2010; Liu et al. 2012), but other grasslands were unaffected (Chen et al. 2014).

9.3.3 Grazing Pressure

Grazing pressure by domesticated animals in grasslands can alter the extent to which roots are colonised by AM fungi. In most studies, grazing or defoliation has been shown to decrease AM fungal colonisation in grasslands or in grassland species grown under controlled conditions in a glasshouse (Bethlenfalvay and Dakessian 1984; Eom et al. 2001; Grime et al. 1987; Saravesi et al. 2014). However, grazing has also been shown to increase AM fungal colonisation (Frank et al. 2003) or have no influence on colonisation of roots by AM fungi (Yang et al. 2013). A negative effect of defoliation on mycorrhizal colonisation has usually been ascribed to reduced photosynthetic capacity of plants which in turn, limits the carbon supply to roots and mycorrhizal fungi, particularly for heavy grazing conditions (Barto et al. 2010). However, different responses between AM fungi and their hosts during defoliation could also be associated with variation in plant genotype, soil and climatic factors, fertilisation and/or differences in relative

growth rates of roots and hyphae of AM fungi inside roots and in the surrounding soil (Barto and Rillig 2010).

One possible reason for the inconsistent findings between studies investigating the effects of grazing on AM fungal community is due to methodological constraint. In a study of mountain grasslands in Argentina, grazing did not affect AM fungi when they were assessed as spores alone (Lugo et al. 2003). However, when the interaction between grazing management and AM fungi was investigated in a desert steppe in Inner Mongolia, China, using sporulation, colonisation and diversity measures, differences were found between control and grazed treatments (Bai et al. 2013). In another system, differences in plant species had more of an impact. Yamane et al. (1999) found that grazing pressure and the defoliation of shoots decreased AM fungi colonisation in roots of *Miscanthus sinensis* but not in roots of *Zoysia japonica*. Further, molecular analysis of AM fungi colonising roots of the same plant species showed that defoliation had a greater impact on the community composition of AM fungi in roots of *M. sinensis* than in roots of *Z. japonica* (Saito et al. 2004). Also, when the effect of overgrazing on AM fungal diversity was investigated in a grassland in Inner Mongolia by trap culture (Su and Guo 2007), both spore diversity and species richness of AM fungi were significantly decreased by long-term overgrazing. Grazing can alter root morphology, plant community composition and soil properties (Eom et al. 2001; Hiiesalu et al. 2014; Su and Guo 2007) which could all alter mycorrhiza formation to varying degrees. Hiiesalu et al. (2014) found that AM fungi species richness was associated with both above- and below ground plant species richness.

Increased grazing pressure can induce a plant succession; for example, in a Japanese semi-natural grassland, tallgrass species such as *Pleioblastus chino* and *Miscanthus sinensis* were replaced by turf grass species, *Zoysia japonica,* following long-term grazing (Numata 1961). In another study it was found the number of plant species and plant community cover in a grassland decreased with long-term overgrazing and affected AM mycorrhizal colonisation (Su and Guo 2007). Similarly, the changes in both AM fungal and plant community composition were more pronounced in a tallgrass prairie after agricultural disturbance than in a comparable natural system (Stover et al. 2012). Furthermore, AM fungal diversity was higher under light to moderate grazing pressure compared to heavy grazing, and these differences were attributed to changes in plant diversity, in particular the density of tillers of the dominant grass *Leymus chinensis* (Ba et al. 2012). Such a reduction in above-ground plant biomass would reduce the capacity of plants to supply carbon to roots. Reduced carbon supply to roots could fail to meet the demands of AM fungi, leading to reductions in mycorrhizal colonisation (Barto et al. 2010). Simultaneously, a change in root morphology induced by grazing (especially, hyphal density and area) could also influence root colonisation by AM fungi (Allsopp 1998; Lugo et al. 2003). Another factor that requires consideration is that species of AM fungi can differ in their tolerance of grazing pressure and in their competitive ability which is evident by the observation that non-*Glomus* species were particularly affected by overgrazing in a study in an Inner Mongolia steppe grassland (Su and Guo 2007).

Grazing has been shown to increase carbon storage in C4-dominated grasslands (Sanjari et al. 2008), whereas grazing decreased soil organic carbon in C3-dominated grasslands (Li et al. 2008). It has been suggested that changes in plant species composition as a result of grazing may be more important than direct or indirect effects of grazing on soil carbon (Yates et al. 2000). Indeed, sheep overgrazing for 17 years did not significantly alter soil organic carbon level and total soil N content (Raiesi and Asadi 2006) indicating that variation in decomposition rates of different plant species may be greater than direct impacts of grazing on carbon dynamics in the studied ecosystem. As AM fungi can make important contributions to soil aggregation, nutrient cycling and carbon sequestration (Rillig and Mummey 2006), grazing could influence a range of soil processes that vary in the extent to which they are mediated by mycorrhizas. Although the dynamics of soil carbon in grasslands is expected to influence AM fungi, this could be site specific and depend on differences in hyphal growth associated with soil organic matter, as well as on relationships between the various AM fungi present and the extent to which each one colonises roots of the plant species that are present (Allsopp 1998). In other words, the effects of grazing on AM fungi need to be interpreted within a very complex and dynamic soil-root environment.

9.3.4 Interactions with Other Soil Organisms

There are considerable interactions between AM fungi and other soil organisms in grasslands. For example, earthworms can be important agents in the distribution of mycorrhizal fungi and influence plant establishment in early succession (Gange 1993). Earthworm-AM fungal interactions affected plant diversity and productivity in a model grassland ecosystem, and different earthworm species were shown to have different influences (Zaller et al. 2011). Both earthworms and AM fungi play roles in the formation of soil aggregates (Rillig and Mummey 2006; Rillig et al. 2002; Spurgeon et al. 2013; Wright and Upadhyaya 1998) and that the amount of glomalin formed by AM fungi that is likely to contribute to soil aggregation changes with land use (Rillig et al. 2003). Although tillage can decrease the amount of glomalin in soil (Wright et al. 1999), it is an uncommon practice in most grasslands, so glomalin may have greater persistence in stable grassland ecosystems. Furthermore, Lutgen et al. (2003) showed that glomalin present in grassland soil changed seasonally with arbuscular colonisation in plant roots. There are multiple interactions between diverse communities of soil organisms and mycorrhizal fungi in grasslands, including those related to root-feeding nematodes. For example, it has been demonstrated that AM fungi can control nematodes feeding in the dune grass *Ammophila arenaria* (De La Peña et al. 2006) and protect plants from pathogens or parasites (Gworgwor and Weber 2003; Newsham et al. 1995).

9.4 Conclusions

Many studies on AM fungi in grassland ecosystems have been reported where species diversity, colonisation dynamics and biomass were investigated. Most studies have focused on the ecological aspects of AM fungi in grasslands, with less emphasis on their function. In addition to field observations, pot experiments have been used to simulate grassland environments to determine underlying processes affecting interactions between AM fungi and grassland plant species. Although it is difficult to investigate the function and effectiveness of unidentified AM fungal species, future studies need to establish new approaches for evaluating the function of unidentified and unculturable AM fungi which dominate grasslands.

AM fungal diversity in grasslands generally does not appear to change greatly within a few years after management practices are implemented. However, the full extent of how communities of AM fungi function in grasslands is not understood. In some situations, AM fungi in grasslands have been compared with those in crop fields, and usually AM fungal diversity was higher in the grassland than in the crop. Clarification of interactions between AM fungi and other soil organisms would improve understanding of how AM fungi contribute to grassland ecosystems. This information is important from both agricultural production and ecological perspectives. It is also important for protection of threatened plant species that occur in natural and semi-natural grasslands.

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Chapter 10
Application of AM Fungi to Improve the Value of Medicinal Plants

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10.1 Introduction

Medicinal herbs are known to be a source of phytochemical constituents or bioactive compounds (Toussaint et al. 2007). Unlike synthetic medicines, natural medicinal products are claimed to be safe to humans and the environment, and some can play a significant role in the treatment of cancer (Nema et al. 2013). The use of plants medicinally has a tradition in many cultures. In Europe, apothecaries stocked herbal ingredients for their medicines. Traditional Chinese medicine, Ayurvedic (Indian) medicine, and herbal medicine are examples of medical practices that incorporate the medical uses of plants. The well-known Chinese *Materia Medica* documented over 600 medicinal plants and recorded the first use of medicinal herbs in China as early as 1,100 BC (Cragg et al. 1997). It has been estimated that more than 30 % of known plant species have been or are being used medicinally in at least one medicinal tradition (Joy et al. 1998).

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Like many other terrestrial plants, most medicinal plants are capable of forming mycorrhizal associations. Mycorrhizal symbioses play crucial roles in contributing to soil structure formation, plant nutrient acquisition, growth, productivity, and biodiversity in both agricultural and natural ecosystems (Smith and Read 2008). Among all kinds of mycorrhizas, arbuscular mycorrhizas are the most widely distributed association (Liu and Chen 2007; Smith and Read 2008). However, research on mycorrhizal relationships with medicinal plants began much later and varies among regions and countries. Colonization by AM fungi has been confirmed in many medicinal plants, and two major advances relate to (1) AM fungal community and diversity in the rhizosphere of medicinal plants (e.g., Kumar et al. 2010; Wubet et al. 2003; Zeng et al. 2013) and (2) improved medicinal values by AM fungal colonization (e.g., Copetta et al. 2006; Yuan et al. 2007; Morone-Fortunato and Avato 2008; Toussaint et al. 2008; Sasanelli et al. 2009).

Taber and Trappe (1982) pioneered and observed the presence of AM fungi in medicinal plants in Figi Islands and Hawaii, America. Waheed (1982) conducted field surveys on medicinal plants and their mycorrhizal status in Murree Hills and Kaghan Valley, Pakistan. Observations confirmed mycorrhizal associations were commonly present in medicinal plants in Pakistan (Gorsi 2002; Haq and Hussain 1995; Iqbal and Nasim 1986). The occurrence of AM associations in medicinal plants has also been reported from China (Wei and Wang 1989) and Japan (Udea et al. 1992). Many studies concerning AM fungi in medicinal plants emerged from the late 1980s.

With the development of Chinese, Indian, Arabian, and other traditional medicines, production systems have made extensive use of wild medicinal plants. At an earlier time, the traditional healthcare practice mainly depended on harvesting of wild medicinal plants. Increases in population, inadequate supply of drugs, side effects of several allopathic drugs, and development of resistance to the currently used drugs for infectious diseases have focused attention on the use of plant materials as a source of medicines for a wide variety of human ailments. Therefore, attempts to explore wild resources and to develop cultivation technologies for large-scale plantations have been made across Asian countries. In the traditional cultivation process, pests and diseases have direct impacts on the yield and quality of medicinal plants. Simultaneously, pesticide abuse involving harmful heavy metals affects the quality of plant-origin medicinal products and has led to environmental pollution. Development of innovative methods and technologies for cultivation and plantation of medicinal plants are needed.

Inoculation with AM fungi during an early stage of plant growth has become an alternative strategy for improved plant survival and growth (Kothamasi et al. 2001). During the establishment of AM symbioses, a range of chemical and biological reactions could occur in the rhizosphere and plant tissues, including producing plant secondary metabolites. AM associations have been reported to have functions in improving the growth of medicinal plants and the productivity of medicinal compounds (Chandra et al. 2010; Karthikeyan et al. 2009; Gupta and Janardhanan 1991).

10.2 Resource and Diversity of AM Fungi in Rhizosphere of Medicinal Plants

10.2.1 AM Fungal Species Diversity

Hundreds of plant species are reported for use as complementary medicines. Among these, a large proportion of medicinal plants have mycorrhizal associations. However, the diversity of AM fungal communities in rhizospheres of medicinal plants and the extent of colonization may vary depending on host plant species, sampling season, soil properties, and locality.

Gorsi (2002) reported that 76 medicinal plants were associated with AM fungi in the Azad Jammu and Kashmir areas in Pakistan. In Bangladesh, the AM fungal status of 40 medicinal plants was investigated on the Rajshahi University Campus (Zaman et al. 2008). Gautam and Roy (2009) investigated the seasonal variation of AM fungi associated with some medicinal plants in India, and Radhika and Rodrigues (2010) found that 30 out of 36 medicinal plant species were mycorrhizal in Goa region, India. Modern molecular approaches are now used to identify AM fungal diversity in rhizosphere soil and roots of medicinal plants. By analyzing phylogenetic data of 5.8S ribosomal DNA, Ethiopian researchers investigated the molecular diversity of AM fungi associated with *Prunus africana* (Wubet et al. 2003). They revealed 109 sequences belonging to members of the Glomeromycota. Subsequent 5.8S/ITS2 rDNA sequence analysis indicated high AM fungal diversity and dominance of *Glomus* type. Similarly, Appoloni et al. (2008) analyzed the community of AM fungi in roots of *Dichanthelium lanuginosum* and claimed that 16 rDNA phylotypes belonged to the genera *Archaeospora*, *Glomus*, *Paraglomus*, *Scutellospora*, and *Acaulospora*. The most diverse and abundant lineage was *Glomus* group A, with the most frequent phylotype corresponding to *Glomus intraradices*. Using nested PCR techniques, Cai et al. (2009) reported that the molecular diversity of the AM fungal community in the rhizosphere of *Phellodendron amurense* in Northeast China had three general groups in related to *Glomus*, *Scutellospora*, and *Hyponectria*. We list some most commonly used medicinal plant species capable of forming AM associations in Table 10.1.

10.2.2 AM Morphology

AM fungi in the rhizosphere of medicinal plants are abundant, but their colonization status is greatly influenced by plant species and environmental factors. Both biotic and abiotic factors affect the population of fungal species and distribution although abiotic/edaphic factors may be relatively more important than biotic factors for establishing and maintaining population pattern (Panwar and Tarafdar 2006). The vegetative stage of plants exhibits higher colonization rates compared to

Table 10.1 List of some commonly used medicinal plants and associated AM fungal species reported in the literature

Plant family	Plant species	AM fungal species	Source
Acanthaceae	*Andrographis paniculata*	*Acaulospora scrobiculata*, *Glomus aggregatum*	Radhika and Rodrigues (2010)
Amaranthaceae	*Amaranthus spinosus*	*A. denticulata*, *A. scrobiculata*, *A. tuberculata*, *G. claroideum*, *G. fecundisporum*, *G. monosporum*	Yang et al. (2002)
		Scutellospora pellucida, *Sclerocystis clavispora*	
Apocynaceae	*Hemidesmus indicus*	*Ambispora leptoticha*, *G. maculosum*, *G. geosporum*, *G. multicaule*, *G. fasciculatum*	Radhika and Rodrigues (2010)
Araliaceae	*Panax ginseng*	*A. cavernata*, *A. spinosa*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum*, *G. microaggregatum*, *G. mosseae*	Xing et al. (2000)
			Cho et al. (2009)
Araliaceae	*Panax notoginseng*	*G. versiforme*, *G. monosporum*, *G. mosseae*, *G. constrictum*, *G. claroideum*	Ren et al. (2007)
			Zhang et al. (2011)
Asteraceae	*Arnica montana*	*G. geosporum*, *G. constrictum*, *G. intraradices*, *G. mosseae*, *G. macrocarpum*, *G. fasciculatum*, *G. versiforme*	Jurkiewicz et al. (2010)
			Chaudhary et al. (2008)
Asteraceae	*Echinacea purpurea*	*G. intraradices*	Araim et al. (2009)
Caprifoliaceae	*Lonicera japonica*	*G. constrictum*, *G. geosporum*, *G. mosseae*, *G. versiforme*	Gai et al. (2000)
Cercidiphyllaceae	*Cercidiphyllum japonicum*	*G. aggregatum*, *G. constrictum*, *G. dimorphicum*, *G. fasciculatum*, *G. flavisporum*, *G. intraradices*, *G. mosseae*, *S. aurigloba*, *Archaeospora leptoticha*	Wang et al. (2008)
Compositae	*Carthamus tinctorius*	*A. rehmii*, *G. claroideum*	Zhao (2006)
Compositae	*Xanthium sibirlcum*	*G. claroideum*, *G. mosseae*	Zhao (2006)
			Zhang and Tang (2006)
Elaeagnaceae	*Elaeagnus sarmentosa*	*G. etunicatum*, *G. mosseae*, *G. caledonium*, *G. constrictum*	Lin et al. (2003)
Elaeagnaceae	*Hippophae rhamnoides*	*G. albidum*, *G. claroideum*, *G. constrictum*, *G. coronatum*, *G. intraradices*	Tang et al. (2004)
Gentianaceae	*Gentiana scabra*	*G. Mosseae*, *G. geosporum*	Wang et al. (1998)

(continued)

Table 10.1 (continued)

Plant family	Plant species	AM fungal species	Source
Ginkgoaceae	*Ginkgo biloba*	*G. mosseae*, *G. aggregatum*, *G. geosporum*, *G. versiforme*, *G. caledonium*, *S. heterogama*, *Gigaspora gigantea*, *Gi. margarita*	Chen and Han (1999)
Labiatae	*Salvia miltiorrhiza*	*A. bireticulata*, *G. aggregatum*, *G. mosseae*, *G. clarum*, *G. reticulatum*	He et al. (2010)
Labiatae	*Scutellaria baicalensis*	*G. geosporum*, *G. versiforme*	Zhang and Tang (2006)
Leguminosae	*Robinia pseudoacacia*	*G. aggregatum*, *G. albidum*, *G. claroideum*, *G. constrictum*, *G. fasciculatum*, *G. mosseae*, *G. reticulatum*	Hu (2006)
Leguminosae	*Prosopis cineraria*	*G. fasciculatum*, *G. aggregatum*, *G. mosseae*	Verma et al. (2008)
Leguminosae	*Pueraria lobata*	*G. constrictum*, *G. geosporum*, *G. mosseae*, *G. reticulatum*	Gai et al. (2000)
Leguminosae	*Albizia julibrissin*	*G. mosseae*, *G. etunicatum*	Lin et al. (2003)
Liliaceae	*Allium macrostemon*	*G. caledonium*	Gai et al. (2000)
Liliaceae	*Aloe vera*	*G. maculosum*, *G. multicaule*, *G. geosporum*	Radhika and Rodrigues (2010)
Liliaceae	*Paris polyphylla*	*A. appendicula*, *A. brieticulata*, *A. excavate*, *G. albidum*, *G. ambisporum*, *G. luteum*, *Gi. albida*, *S. calospora*, *S. gilmorei*	Zhou et al. (2009)
Meliaceae	*Azadirachta indica*	*A. scrobiculata*, *G. fasciculatum*, *Gi. albida*, *S. calospora*	Radhika and Rodrigues (2010)
Meliaceae	*Naregamia alata*	*A. scrobiculata*, *Am. leptoticha*, *A. nicolsonii*, *G. rubiforme*, *G. maculosum*, *G. fasciculatum*, *S. verrucosa*	Radhika and Rodrigues (2010)
Plantaginaceae	*Plantago asiatica*	*G. intraradices*	Zhang and Tang (2006)
Rhamnaceae	*Ziziphus jujuba* Mill. var. *inermis*	*G. coronatum*, *G. intraradices*, *G. monosporum*, *G. reticulatum*	Tang et al. (2004)
Rosaceae	*Crataegus cuneata*	*G. constrictum*, *G. caledonium*	Di et al. (2006)
Rosaceae	*Prunus armeniaca*	*G. constrictum*, *G. geosporum*	Di et al. (2006)
Solanaceae	*Physalis minima*	*A. rehmi*, *G. fasciculatum*, *G. multicaule*, *G. maculosum*, *G. geosporum*, *G. rubiforme*	Radhika and Rodrigues (2010)

(continued)

Table 10.1 (continued)

Plant family	Plant species	AM fungal species	Source
Solanaceae	*Solanum nigrum*	*Gigaspora margarita*, *G. caledonium*	Gai et al. (2000)
			Zhang and Tang (2006)
Solanaceae	*Lycium barbarum*	*Gi. margarita*, *G. albidum*	Tang et al. (2004)
Taxaceae	*Taxus chinensis*	*G. aggregatum*, *G. ambisporum*, *G. clarum*, *G. constrictum*, *G. fasciculatum*, *G. geosporum*, *G. magnicaule*, *G. reticulatum*, *G. verruculosum*, *G. viscosum*, *A. denticulata*	Wang et al. (2008)
Trochodendraceae	*Euptelea pleiosperma*	*G. ambisporum*, *G. constrictum*, *G. fasciculatum*, *G. geosporum*, *G. hyderabadensis*, *G. intraradices*, *S. verrucosa*	Wang et al. (2008)
Umbelliferae	*Centella asiatica*	*G. multicaule*, *G. clarum*, *G. fasciculatum*, *A. delicate*, *S. scutata*	Radhika and Rodrigues (2010)
Umbelliferae	*Angelica dahurica*	*G. Mosseae*, *G. caledonium*, *G. constrictum*, *A. spinosa*, *S. calospara*	Cao et al. (2007)
Zingiberaceae	*Alpinia galanga*	*G. caledonium*, *G. mosseae*, *G. fasciculatum*, *G. geosporum*, *Am. leptoticha*	Radhika and Rodrigues (2010)

flowering and fruiting stages. In addition, herbaceous plants showed more entry points of hyphae into roots than did the shrubby and woody plants (Gorsi 2002). AM fungal spore density in soil around medicinal halophytes (*Suaeda fruticosa*, *Salsola baryosma*, *Haloxylon recurvum*) had a strong positive correlation with soil pH and organic carbon content but a negative correlation with soil phosphorus (Mathur et al. 2007). AM fungal spore density is often higher in association with wild medicinal plants compared with cultivated species, which may be due to the undisturbed nature of the ecosystem in wild habitats (Radhika and Rodrigues 2010). Experimental evidence of physical and functional selectivity in AM symbiosis has been demonstrated in field soils where diverse communities of AM fungi form associations with individual hosts (Helgason et al. 2002; Smith et al. 2000).

AM fungal morphology has been classified as either Arum type or the Paris type (Smith and Smith 1997). The physiological and functional disparity between Arum type and Paris type remains unclear. The development of Arum type is faster than that of Paris type (Cavagnaro et al. 2001). Zubeck and Blaszkowski (2009) reported that AM colonization rates in roots of *Mentha citrata*, *Origanum majorana*, *Salvia officinalis*, and *Thymus vulgaris* ranged from 67 to 100 % and were often Arum type. Both Arum and Paris types have been found in medicinal plants in Lamiaceae (Burni and Hussain 2011). Three wolfberry (*Lycium barbarum* L.) cultivars in arid

Table 10.2 AM morphologies and mycorrhizal status of medicinal plants

Plant family	Plant species	Morphology types	References
Acanthaceae	*Andrographis paniculata*	Arum	Muthukumar et al. (2006)
Amaranthaceae	*Amaranthus spinosus*	Arum	Muthukumar et al. (2006)
Araceae	*Pinellia ternata*	Intermediate	Cheng et al. (2009a)
Asteraceae	*Echinacea purpurea*	Arum	Zubek and Blaszkowski (2009)
Asteraceae	*Rhizoma Atractylodis Macrocephalae*	Paris	Cheng et al. (2009b)
Caesalpiniaceae	*Cassia siamea*	Arum	Muthukumar et al. (2006)
Campanulaceae	*Platycodon grandiflorus*	Arum	Cheng et al. (2009b)
Caprifoliaceae	*Lonicera Japonica*	Paris	Cheng et al. (2009b)
Compositae	*Vernonia cinerea*	Paris	Muthukumar et al. (2006)
Crassulaceae	*Sedum aizoon*	Arum	Cheng et al. (2009b)
Hypericaceae	*Hypericum perforatum*	Arum	Zubek and Blaszkowski (2009)
Labiatae	*Leucas aspera*	Arum	Muthukumar et al. (2006)
Lamiaceae	*Ocimum americanum*	Arum	Burni and Hussain (2011)
Lamiaceae	*Ocimum basilicum*	Paris	Burni and Hussain (2011)
Lamiaceae	*Rosmarinus officinalis*	Paris	Burni and Hussain (2011)
Lamiaceae	*Salvia lanata* Roxb	Intermediate	Burni and Hussain (2011)
Magnoliaceae	*Michelia champaca*	Paris	Panna and Highland (2010)
Solanaceae	*Lycium barbarum*	Paris	Zhang et al. (2010)
Umbelliferae	*Centella asiatica*	Intermediate	Muthukumar et al. (2006)
Umbelliferae	*Radix bupleuri*	Arum	Cheng et al. (2009b)

Northwestern China had the same Paris-type AM fungal associations, but colonization rate within a cultivar varied (Zhang et al. 2010). The Arum-type association was observed more often in medicinal plants than the Paris type (Table 10.2; Muthukumar et al. 2006).

10.3 Effect of AM Fungal Inoculation on Plant Growth

Several studies confirm that mycorrhizal medicinal plants generally have greater nutrient contents and grow better than non-mycorrhizal plants (e.g., Karagiannidisa et al. 2011; Nisha and Rajeshkumar 2010). For example, mycorrhizal inoculation increased dry matter accumulation in five medicinal plants (*Abelmoschus moschatus*, *Clitoria tematea*, *Plumbago zeylanica*, *Psoralea corylifolia*, and *Withania somnifera*) grown in five different soil types (Chandra et al. 2010). AM fungal associations improved the shoot height growth and root biomass of *Poncirus trifoliata*, *Piper longum*, *Salvia officinalis*, and *Plectranthus amboinicus* (Geneva et al. 2010; Gogoi and Singh 2011; Rajeshkumar et al. 2008; Wang et al. 2006).

10.3.1 Nutrient Uptake and Plant Biomass

The mycorrhizal hyphal network provides a larger absorptive surface than root hairs alone. The positive effects of AM fungal inoculation on plant growth are generally attributed to improved acquisition of nutrients of low mobility, especially phosphorus. It has been demonstrated that external hyphae of AM fungi are able to increase NH_4^+ and NO_3^- uptake and to assimilate these molecules into free amino acids (Johansen et al. 1996). Mycorrhizal symbioses stimulate plant uptake of nutrients such as P, Zn, Cu, Mn, and Fe in deficient soils (Chen and Zhao 2009), and mycorrhizal hyphae play an important role in nutrient uptake, convinced by labeling nutrient elements in controlled experiments (e.g., Hosamani et al. 2011). However, Zhao and Yan (2006) reported that leaf nitrogen contents were lower in mycorrhizal *Camptotheca acuminata* than in its non-mycorrhizal counterpart. Shoot dry weight was four times greater in *Withania somnifera* colonized by *Glomus fasciculatum* than in uninoculated plants (Hosamani et al. 2011). Glasshouse experiments showed that inoculation of palmarosa (*Cymbopogon martinii*) with *G. aggregatum* enhanced biomass production threefold compared to non-mycorrhizal plants (Gupta and Janardhanan 1991). Many studies of AM associations have shown that the effectiveness of AM fungi differs with plant species, soil nutrient level, and plant growth environments (e.g., Smith and Smith 2011).

10.3.2 Stress Tolerance

Compared to non-mycorrhizal plants, AM plants often show greater tolerance to several biotic and abiotic stresses, such as toxic metals, root pathogens, drought, high soil temperature, saline soils, adverse soil pH, and transplanting shock (Evelin et al. 2009; Lu et al. 2003; Tang et al. 1999; Turkmen et al. 2008). Where salt stress

is a major threat to plant growth and productivity, AM fungi have been shown to promote plant growth and salinity tolerance. For example, AM fungi were able to colonize *Bacopa monnieri* roots effectively under high salinity levels (Khaliel et al. 2011). Inoculated plants significantly enhanced dry mass production, and this occurred to a greater extent when plants were grown at high salinity levels. AM fungi increased Na^+ and Cl^- uptake and reduced rhizosphere NaCl level. AM fungi can induce a buffering effect on the uptake of Na^+ when the content of Na^+ is within the permissible limit (e.g., Allen and Cunningham 1983), and mycorrhizal plants may employ mechanisms to promote plant tolerance to salinity. These may include enhanced nutrient acquisition, maintenance of the K^+/Na^+ ratio, biochemical changes (accumulation of proline, betaines, polyamines, carbohydrates, and antioxidants), physiological changes (photosynthetic efficiency, relative permeability, water status, abscisic acid accumulation, nodulation, and nitrogen fixation), molecular changes (the expression of genes: PIP, Na^+/H^+ antiporters, Lsnced, Lslea, and LsP5CS), and ultrastructural changes (Evelin et al. 2009).

High bicarbonate (HCO_3^-) content and associated high pH of irrigation water are detrimental to plant growth. Inoculation with AM fungi enhanced the tolerance of *Rosa multiflora* to HCO_3^- as indicated by greater nutrient uptake and leaf chlorophyll and lower root iron reductase activity and alkaline phosphatase activity (Cartmill 2004). When exposed to drought, AM plants exhibited a higher level of proline and activity of two antioxidant enzymes, superoxide dismutase and peroxidase. In addition, mRNA abundance of four genes involved in reactive oxygen species homeostasis and oxidative stress battling was higher in the mycorrhizal compared with non-mycorrhizal plants (Fan and Liu 2011). These findings illustrate the possible participation of drought-induced genes in the enhanced tolerance of AM plants to water deficit. It has been claimed that activation of physiological, biochemical, and molecular alterations may be involved in the improvement of the growth and drought tolerance of mycorrhizal *Poncirus trifoliata* seedlings (Fan and Liu 2011; Ruiz-Lozano et al. 2008).

Potential roles of AM associations in alleviating metal stress of plants have been demonstrated, but the mechanisms involved in the metal tolerance of AM fungi are still poorly understood (Hildebrandt et al. 2007). Heavy-metal-tolerant AM fungi isolated from polluted soils showed capability in binding heavy metals (Joner et al. 2000). *Datura metal* plants inoculated with AM fungi showed increased tolerance to heavy metals (Salvaraj and Kim 2004). A pot experiment with sweet basil (*Ocimum basilicum*) under heavy metal (Cr, Cd, Pb, and Ni) stress showed that the AM symbiosis could be used as a novel approach to enhance yield and maintain the quality of volatile oil under metal-contaminated soils (Prasad et al. 2011). Sweet basil has been traditionally used for the treatment of headaches, coughs, and diarrhea (Jayasinghe et al. 2003).

10.4 Effect of AM Fungi on Medicinal Composition

Secondary metabolites of medicinal plants are the critical resources in natural medicinal products used for pharmacological and therapeutical purposes. Terpenoids, phenolics, and alkaloids are the three major groups of secondary plant metabolites. There are a few attempts being made to study the relationship between the occurrence of AM fungi and improved secondary metabolite contents in medicinal plants. The improved phosphorus status or an altered hormonal balance of the plants may contribute to the AM effects on the secondary metabolites, but the reasons for such effects remain largely unknown (Toussaint 2007).

10.4.1 Terpenoids

The effects of AM fungi on terpenoid concentration in medicinal plants have received more attention in recent years. Essential oils are volatile, lipophilic mixtures of secondary plant compounds, mostly consisting of monoterpenes, sesquiterpenes, and phenylpropanoids, which are often used as flavors and fragrances, as antimicrobials and antioxidants, and as medicines (Deans and Waterman 1993). AM fungi increased the content of essential oil and alterations of its composition, such as in medicinal basil (*O. basilicum*) (Copetta et al. 2006).

Andrographis paniculata, commonly known as "king of bitters," has been used for centuries in Asia to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat, and other chronic and infectious diseases. The primary medicinal component of *A. paniculata* is andrographolide, a colorless diterpene lactone with bitter taste. The high concentration of andrographolide in the leaf extracts of *A. paniculata* inoculated with *Gi. albida* showed that the AM symbiosis can enhance the production of this secondary metabolite (Radhika and Rodrigues 2011). The concentration of andrographolide in mycorrhizal *A. paniculata* plants reached the highest level at the flowering growth stage.

10.4.2 Phenolic Compounds

Apart from terpenes and essential oil constituents, the relationship between mycorrhizal associations and other secondary plant metabolites such as phenolic compounds have been investigated. Phenolic compounds include phytoalexin, wall-bound phenol, flavonoids, and isoflavonoids and their derivatives. A significant increase of total phenolic content was detected in leaves and flower heads of *Cynara cardunculus* inoculated with *Glomus intraradices*, either alone or in a mixture with *G. mosseae* under both greenhouse and field conditions (Ceccarelli et al. 2010). Isoflavone levels were altered in roots of legume plants locally and

systemically when colonized by AM fungi (Catford et al. 2006). The shoot flavonoid levels in white clover (*Trifolium repens*) increased when roots were colonized by AM fungi (Ponce et al. 2004). An increased content of rosmarinic acid, a highly antioxidant phenolic compound, was detected in AM-colonized basil (Toussaint et al. 2008). The content of flavonoids in *Bupleurum chinense*, *Ginkgo biloba*, and *Astragalus membranaceus* (Meng and He 2011) and that of total coumarin and imperatorin in *Angelica dahurica* (Zhao and He 2011) were significantly higher in mycorrhizal compared with non-mycorrhizal plants of the same species. AM fungal colonization induced two different signaling pathways in the accumulation of phenylpropanoid metabolism: one through induction of phenylalanine ammonia-lyase and chalcone synthase and the other through suppression of isoflavone reductase (Zhao and Yan 2006).

There are controversial conclusions concerning mycorrhizal effects on phenolic contents in medicinal plants. Zeng et al. (2013) showed neutral effects of AM colonization on the composition of phenolic ingredients. The AM symbiosis did not alter the total concentrations of phenolic and rosmarinic acid in roots of *Salvia officinalis* (Nell et al. 2009) or the polyphenolic profile in leaves and stems of basil (Lee and Scagel 2009) after AM fungal inoculation.

10.4.3 Alkaloids

Vinca (*Catharanthus roseus*) is an important medicinal plant from which antineoplastic alkaloids (e.g., vinblastine) are extracted. AM fungal inoculation significantly enhanced plant growth and the total content of vinblastine in Vinca leaves (Rosa-Mera et al. 2011). A positive correlation was found between mycorrhizal colonization and castanospermine content in field-grown *Castanospermum australe* seeds and in greenhouse-grown leaves in inoculated plants (Abu-Zeyad et al. 1999). This finding is interesting because castanospermine is effectively used in the treatments against AIDS and cancers (Spearman et al. 1991). Sweet basil has been traditionally used for the treatment of headaches, coughs, and diarrhea (Jayasinghe et al. 2003). AM fungal colonization increased the production of rosmarinic acid (antioxidant activity) in sweet basil shoots (Toussaint et al. 2007). Mycorrhizal *Ocimum basilicum* and *Coleus amboinicus* possessed higher amounts of the total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids, and tannins in the root and leaf than non-mycorrhizal plants (Hemalatha 2002). The AM symbiosis also played a positive role in the accumulation of camptothecin in *Camptotheca acuminata* and vinca alkaloids in Vinca; both are important anticancer compounds (Rosa-Mera et al. 2011).

10.5 Conclusion

The quality of herbal materials in terms of active ingredients is largely influenced by abiotic and biotic factors, and AM fungal colonization can play an important role in improving the medicinal values of medicinal plants (Szakiel et al. 2011a, b). Their positive role in plant growth, disease resistance, and both yield and quality of medicinal materials make AM fungi potential alternatives to existing methods for promoting the growth of some important medicinal plants. With the increased demands in plant-oriented medicines, there is increasing scientific interest in the study of the interaction between medicinal plant production and their associated mycorrhizal symbioses. The advantages, prospects, and feasibility of introducing AM fungi into the process of cultivation of medicinal plants have been recognized (Xiao et al. 2011; Yang et al. 2008). AM fungal diversity and its significance in medicinal plant nutrient acquisition and secondary metabolite alteration have been investigated, and its application provides a sustainable method to enhance the agricultural and pharmaceutical outcomes of medicinal plants. AM fungi are not host specific, but their affinity to a particular host can be preferential (Rogers et al. 1994). Thus, selecting efficient AM fungi for a particular plant is essential for the cultivation of medicinal plants. Advanced mycorrhizal technologies in agriculture can be adopted to study medicinal plants and their production. To ensure a better understanding of the diversity and function of AM fungi and a wider application of AM fungi in the plantation of medicinal plants, the following research areas are recommended: (1) exploiting the diversity and distribution of AM fungi in important medicinal plants, (2) identifying the relationship between genetic structure and functional diversity of AM fungal species and mechanisms of signal perception and plant growth regulators in mycorrhizal establishment under diverse ecosystems, and (3) selecting and using efficient AM fungi for improved active secondary plant metabolites.

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Chapter 11
Arbuscular Mycorrhizas and Their Role in Plant Zinc Nutrition

Timothy R. Cavagnaro

11.1 Introduction

The majority of higher land plants form arbuscular mycorrhizas (AM). The formation of AM can result in improved plant nutrition, growth, disease resistance, and drought tolerance (Smith and Read 2008). It is for these reasons that AM are increasingly recognized as having an important role in many ecosystem processes and as an integral part of sustainable agroecosystems (Jackson et al. 2008). While it has been found that a large proportion of the P in plants can be delivered via the mycorrhizal pathway (Smith et al. 2004), AM also contribute significantly to plant acquisition of other nutrients including Zn, NH_4^+, NO_3^-, Cu, K, and others (Cavagnaro et al. 2006; Frey and Schuepp 1993; Johansen et al. 1993; Marschner and Dell 1994; Tanaka and Yano 2005). Despite the significance of AM in the uptake of nutrients other than P, our understanding of the underlying processes lags considerably behind that of P. Although significant advances have been made, this still remains a significant knowledge gap.

The importance of AM in plant nutrient acquisition, especially in low-fertility soils (Hetrick 1991; Menge 1983), is well recognized (Cardoso and Kuyper 2006; Jackson et al. 2008; Ryan and Angus 2003). This is especially true for nutrients with a low mobility in the soil (e.g., P, Zn, NH_4^+) (Tinker and Nye 2000). It has been estimated that up to 10 % of plant Zn is delivered by the extra-radical hyphae AM fungi (AMF) (Marschner and Dell 1994), although values vary considerably between studies (see Cavagnaro 2008 for review). Furthermore, the formation of AM by plants is particularly important where nutrients are distributed heterogeneously in the soil, due to the ability of arbuscular mycorrhizal (AM) fungi to forage for nutrients effectively (see Tibbett 2000 for review).

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AM have an important (and fundamentally interesting) dual role in plant Zn physiology. While the formation of AM can increase the capacity of plants to acquire Zn from soils with low Zn concentrations, they can also have a protective role against Zn accumulation to toxic levels in plant grown under high soil Zn conditions. Indeed it appears that there may be a critical soil Zn concentration below which Zn uptake is enhanced and above which it is reduced (Cavagnaro et al. 2010; Christie et al. 2004; Hildebrandt et al. 2007; Watts-Williams and Cavagnaro 2012). While of considerable interest, this latter protective role is not reviewed here (see Christie et al. 2004 for review).

Zinc is an essential element for plant growth. It is an important component of over 300 enzymes and plays important role in the catalytic activity and/or structure of many enzymes (Christie et al. 2004; Hacisalihoglu and Kochian 2003; Marschner 1995). While Zn is abundant in the Earth's crust, much of it occurs in forms unavailable to plants. Consequently, approximately 30 % of the world's soils are considered Zn deficient (Kochian 2000). Zinc deficiency in many important crops is, therefore, a common problem. This has flow on effects for consumers of those crops, with much of the world's human population not meeting its daily Zn requirements. This can have serious consequences for human health which can result in diminished human potential, felicity, and worker productivity (Brown and Wuehler 2000). Thus, understanding how plants acquire and utilize Zn is very important. While the importance of increasing the density of Zn in staple crops is widely recognized (Brown and Wuehler 2000; Burns et al. 2010), the role of AM in this has received little attention. If this is to change, we must develop a sound understanding how plants (and AM) acquire Zn. In addition to an understanding of the physiological and molecular aspects of plant uptake of Zn via the mycorrhizal pathway, an understanding of the effects of different land management practices on the formation and functioning of AM is also required.

This chapter will discuss the role of AM in plant Zn acquisition, with a strong emphasis on agroecosystems. To this end, the impacts of agricultural management on AM and their consequences to plant uptake of Zn via the mycorrhizal pathway will be explored. In order to do this, it is first necessary to revisit the physiological basis of Zn uptake, translocation and transfer to plants, by AMF; this is a subject that has been dealt with more depth in my earlier review (Cavagnaro 2008), and so only key details are represented here. Finally, emerging trends and future directions will be identified and discussed with a view to stimulating research on Zn-AM dynamics, especially where the aim is to capitalize on the benefits that AMF may afford crop plants, and those who depend upon them.

11.2 Zn Uptake by AM: Evidence from Isotope Studies

The importance of AMF in plant Zn nutrition has long been recognized (Cavagnaro 2008; Cavagnaro and Jackson 2007; Cavagnaro et al. 2006; Gao et al. 2007; Kothari et al. 1991; Marschner and Dell 1994; Ortas et al. 2002; Smith and Read 2008;

Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2013). Understanding how, and to what extent, plants acquire Zn via the mycorrhizal pathway will be important in studying AM/Zn interactions in agroecosystems. A wide range of experimental approaches has been used to demonstrate how AMF help plants acquire Zn, and indeed other nutrients. Studies that have used ^{65}Zn as a tracer have been particularly important. Unequivocal evidence for Zn uptake by AMF and its subsequent translocation and transfer to plants have come from studies where a ^{65}Zn tracer is supplied to the plant in compartments accessible to the AMF alone (Bürkert and Robson 1994; Cooper and Tinker 1978; Jansa et al. 2003). This approach makes it possible to partition root and AMF contributions to plant Zn acquisition. Using such an approach, it has been found that Zn supply to plants by AMF (via direct hyphal uptake, not effects of AMF on plant uptake) ranges widely among studies (two orders of magnitude). For example, Jansa et al. (2003) reported that almost 9 % of the Zn supplied to plants in a compartment accessible to hyphae only was transported to the plant over 25 days after Zn supply to the plant. While other studies have found smaller amounts of Zn supplied to the plant using a range of experimental systems (e.g., split-plate culture-based system Cooper and Tinker 1978), these studies demonstrate unequivocally the uptake, translocation, and transfer of Zn to plants by AMF.

11.3 Zn Uptake by AM: Field Studies

While the use of ^{65}Zn as a tracer has been highly valuable in demonstrating Zn uptake by AMF and transfer to plants, this approach has not, to my knowledge, been applied in a field setting, as with radioisotopes of P (e.g., Schweiger and Jakobsen 1999) and stable isotopes of N (Cavagnaro et al. 2012). Be that as it may, various other experimental methods have been used to demonstrate the role of AM in plant Zn nutrition, both in the laboratory and the field. These approaches include:

1. Crop rotations that reduce AMF inoculum potential (systems-based approach) and, hence, AMF colonization of target crops (Ryan and Angus 2003; Ryan and Ash 1999; Sorensen et al. 2005; Thompson 1987, 1996)
2. Mycorrhiza-defective mutants (genotypic approach) to establish AM controls (Cavagnaro et al. 2006; Ruzicka et al. 2010)
3. Fumigation of soils (fumigation-based approach) to eliminate AMF in situ

Emphasis here is placed on the first two of these approaches.

The inclusion of plants in crop rotations that do not form AM has been shown to be linked to a decrease in the inoculum potential of soils and, thence, levels of colonization in subsequent crops. This systems (or rotation)-based approach to reducing the inoculum potential of soils allows for identification of the role of AM in plant Zn nutrition under conditions that are commonly encountered in agricultural ecosystems. The growth and Zn and P acquisition of crops have been reported to be lower in crop rotations that include the non-mycorrhizal plant canola

(*Brassica napus* (L.)) or include long periods without plant cover (12–18 months fallow) (in southern Queensland, Australia) (Thompson 1987, 1996). In the case of the mycorrhizal crop linseed (*Linum usitatissimum* L.), inoculation of seedlings (in the glasshouse) with AMF ameliorated deficiencies in Zn and P when grown in soils that have been subjected to a long fallow period (and hence reduce AMF inoculum potential) (Thompson 1996). Also using a systems-based approach, increases in colonization of roots by AMF and Zn uptake in wheat (*Triticum aestivum* L.) (Ryan et al. 2002) and leek (*Allium porrum* L.) (Sorensen et al. 2005), following mycorrhizal cover crops, have been reported (see Cavagnaro 2008 for more detailed review). Together, these studies illustrate the importance of AMF in plant Zn acquisition in "real world" agricultural settings. Such an on-farm approach to research also permits study of the impacts of various agronomic practices (see below) on AM functioning and inoculum potential.

Irrespective of the ecosystem system, establishing non-mycorrhizal controls is one of the biggest challenges that we face in the study of AM. While a fumigation-based approach to establishing non-mycorrhizal controls in a field setting is effective at suppressing colonization of roots by AMF, and often the only practical approach, nontarget effects on other soil biota cannot be entirely discounted. In an attempt to overcome this issue, a genotypic approach to controlling for the formation of AM can be used. For example, we have compared the growth, nutrition, and soil ecology of a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonization (Barker et al. 1998) (named *rmc*) with that of its mycorrhizal wild-type progenitor (cv. Peto 76R). This genotypic approach for controlling colonization of roots by AMF makes it possible to study the contribution of AM to plant Zn nutrition, with the wider soil biota "intact." For example, in tomato plants grown on an organically managed farm, we found that Zn contents in both the vegetative and edible aboveground biomass was up to 50 % higher in the mycorrhizal genotype (Cavagnaro et al. 2006). This highlights the importance of AM in plant Zn nutrition in an on-farm setting.

The establishment of non-mycorrhizal controls "lies at the heart of difficulties of experimenting with AMF at the ecosystem scale" (Rillig 2004). Thus, in this chapter I have provided selected examples that have tried to overcome some of these challenges. In particular, emphasis has been placed on approaches that allow studies to be undertaken with minimal deviations from normal farming practices or indirect effects of establishing non-mycorrhizal controls on other members of the soil biota. While further research is required, it is clear that AM have an important role to play in plant Zn nutrition in a range of agroecosystems.

11.4 Zn Uptake by AM: The Physiological Basis

Uptake of nutrients, including Zn, by AM involves three core processes:

1. Nutrient acquisition by the AMF

2. Nutrient translocation within the AMF to the intra-radical plant-fungal symbiotic interface
3. Nutrient transfer from the AMF into the interfacial apoplastic space from which it is taken up by the plant

Each of these processes, in the context of Zn acquisition, is now considered.

As is the case with other nutrients, AMF access nutrients not necessarily otherwise accessible to the roots by growing beyond the rhizosphere depletion zones that commonly from around roots (see Tinker and Nye 2000). Zinc uptake by AMF at distances of 40–50 mm from the root surface has been reported (Bürkert and Robson 1994; Jansa et al. 2003). While only a handful of studies have focused on the impacts of Zn addition on intra- and extra-radical growth of AMF, positive (Seres et al. 2006), negative (Liu et al. 2000), and neutral (Toler et al. 2005) responses have been reported (see Cavagnaro 2008 for detailed review). Each of these will likely impact the uptake of Zn by AMF in different ways. Be that as it may, as noted above, an increase in plant ^{65}Zn uptake has been related to the hyphal length density in the compartment containing the labeled Zn (Jansa et al. 2003). This suggests that AMF are able to "forage" for Zn, as is the case for other nutrients (Cavagnaro et al. 2005; Tibbett 2000). In addition to increasing hyphal length density in Zn "patches" in the soil, AMF may also increase Zn uptake via other mechanisms, such as enhanced levels of expression of genes implicated in Zn uptake (as with P Maldonado-Mendoza et al. 2001). This, however, is speculative and requires further consideration (see below).

The mechanisms underlying the long-distance translocation of Zn in the extra-radical hyphae of AMF in the soil, to the intracellular symbiotic interface within the root cortex, remain elusive (see below). Irrespective of how Zn is translocated within the extra-radical hyphae of AMF, any Zn delivered to the plant-fungal symbiotic interface needs to be unloaded into interfacial apoplastic space and thence taken up by the plant. To this end, some important insights have been gained. A cation diffusion facilitator (named *GintZnT1*) has been identified in the AMF *G. intraradices* (Gonzalez-Guerrero et al. 2005). *GintZnT1* has been suggested as having a role in Zn storage or efflux within hyphae (e.g., from an internal storage compartment involved in long-distance Zn translocation), or the efflux of Zn into to the plant/fungal interfacial apoplast (Gonzalez-Guerrero et al. 2005). Once inside the apoplast, the Zn then needs to be taken up across the plant plasma membrane. MtZIP2 is a plasma membrane-localized Zn transporter in *Medicago truncatula* (Gaertn); its expression is influenced by the effects of AM on the Zn status of the plant (Burleigh et al. 2003). Further studies of the molecular basis of Zn uptake via the mycorrhizal pathway will provide important insights into the functioning of AM; these factors are considered in more detail in an earlier review of this topic (Cavagnaro 2008). While such studies will be important, those that begin to integrate Zn uptake via the mycorrhizal pathway, with other aspects of plant and AM biology, are likely to be especially important. To this end, interactions between plant P and Zn nutrition and acquisition via the AM pathway seem to be an obvious, but little considered, starting point (Jansa et al. 2003; Marschner

1995; Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2014; Zhu et al. 2001a, b).

11.5 Zn Uptake by AM: Agroecosystems

It is clear that AM have a role to play in the Zn nutrition of plants. But do they have a place in the modern agricultural paradigm? There is no doubt that both the global population and pressures upon ecosystems are increasing. Indeed achieving global food security in a sustainable manner is one of the largest challenges society currently faces. To this end, we need to carefully consider the options available to us to increase both the yield and nutritive value of crops (Burns et al. 2010). AMF occur in the soils of most arable regions (see Read 1991; Treseder and Cross 2006) and, therefore, are essentially "freely available" to (most) agricultural producers. This, coupled with their role in improving plant growth, nutrition (including, but not limited to, Zn), plant disease resistance, and drought tolerance, suggests that AMF should be an important element of any such debate.

Agricultural yields have increased rapidly in recent decades due to a number of reasons, including the advent and widespread use of pesticides and fertilizers, the mechanization of agriculture, and the breeding of improved plant varieties. However, many of these factors can reduce the AMF inoculum potential of soils and/or the responsiveness of crops to AMF. For example, the application of fungicides can result in a decrease in AM colonization (see Cavagnaro and Martin 2011; Miller and Jackson 1998; Smith et al. 2000). Colonization of roots by AMF is often (but not always) reduced with high levels of fertilizer application. For example, Bolan et al. (1984) reported that at low soil P concentrations, colonization of roots by AMF was inhibited and that small additions of P to the soil increased colonization slightly. However, larger additions of P to the soil can result in a reduction in colonization. Importantly, the magnitude of the effect of soil P on colonization differs between plant and fungal combinations studied (Baon et al. 1992; Oliver et al. 1983). Similar reductions in colonization have been reported in response to N and Zn addition also (see Cavagnaro 2008). In addition to effects of agricultural practices on the formation of AM, plant breeding programs may have also inadvertently selected varieties with low mycorrhizal dependency and/or responsiveness by screening varieties in sterile growth media with high rates of nutrient addition. Be that as it may, these factors, alone and together, may have resulted in decreased mycorrhizal responsiveness and/or dependency upon AMF in many agricultural ecosystems.

Organically managed farms generally have higher AMF inoculum potential than conventionally managed farms (see Cavagnaro et al. 2006 and references therein). Furthermore, organic farming systems typically have higher soil organic matter, microbial biomass, and enhanced rates of N cycling and tend to support more diverse microbial communities (see Jackson et al. 2008 for recent review). These factors suggest that AMF may be more abundant and functionally important in

these farming systems compared to conventionally managed farming systems. Many of the advances in agriculture mentioned above (pesticides and synthetic fertilizers) have not been readily available to farmers in the developing world (due largely to cost and access to distribution networks), where much of the future global increase in population is projected to occur. In other words, most farmers in the developing world can be considered to be using "organic" management practices (Cardoso and Kuyper 2006). Thus, AM are likely to be especially important in these agroecosystems (see Burns et al. 2012; Cardoso and Kuyper 2006). Disruption of hyphal networks due to soil cultivation, which is common place in most agroecosystems, may reduce AMF inoculum potential and, therefore, needs to be considered. These factors aside, there is a paucity of studies that have focused on Zn uptake by plants via the AM pathway in a field context. Given the benefits that plants can accrue due to forming AM, such studies should be of high priority, especially in the context of subsistence farming systems.

In response to widespread global inadequacies in dietary Zn intake (Brown and Wuehler 2000), much effort has focused on the development of Zn-efficient genotypes, that is, genotypes that can grow and yield well Zn-deficient soils. Although some studies have shown the Zn efficiency of some genotypes is the same irrespective of the presence of AMF, such responses are inconsistent across genotypes (see Hacisalihoglu and Kochian 2003 for review). Nevertheless, most crop species form AM, and AMF are found in the soils of most arable regions of the world (see work by Read 1991; Treseder and Cross 2006). This, coupled with the significant increases in plant Zn concentrations reported in many of the studies highlighted here, suggests that studies of Zn efficiency should consider (as has been the case in many, but not all, examples) the role of AM in enhancing the Zn efficiency of crops.

Much of our knowledge (with many important exceptions) on the physiology (including Zn nutrition) of AMF has come from studies using a reductionist approach (Johnson et al. 1997). For example, plants are often grown with single or limited numbers of isolates of AMF and (to a lesser extent) plant species. While important model systems, this must be balanced against the functional diversity that exists in AM (Cavagnaro et al. 2005; Smith et al. 2004), and the need to challenge plants with the AMF with which they naturally occur (Johnson et al. 2005). This point is especially relevant, but challenging, where new crop varieties are being screened on a large scale.

11.6 Zn Uptake by AM: Future Research

Our understanding of the uptake of Zn via the mycorrhizal pathway lags behind that of P. Given the widespread global deficiency of Zn in human diets, I argue here that there is an urgent need to better understand the role of AM in improving plant Zn nutrition. To this end, a number of future research opportunities are identified. These broadly fall under the themes of mechanisms, methods, and management.

This section is neither exhaustive nor complete, but it does, nevertheless, aim to stimulate further research in this area.

1. Molecular mechanisms. Identification of the molecular mechanisms by which plants take up Zn via the mycorrhizal pathway will be an important advance. Little is known about long-distance translocation of Zn in AM. For example, a motile vacuolar system as described for P (Uetake et al. 2002) may be important, especially if Zn can act as a counterion to polyP (Christie et al. 2004), but this is speculative (see Cavagnaro 2008 for more detailed discussion). Identification of additional, and further studies of already identified, genes involved in the Zn physiology of AM is also needed.
2. Functional diversity. Estimates of Zn uptake by AM (using ^{65}Zn) vary by approximately two orders of magnitude (see Jansa et al. 2003 and references therein). The underlying reasons for this variation remain unknown. Furthermore, studies that identify the proportion of plant Zn taken up via the mycorrhizal pathway, which in the case of P can be as high as 100 % with certain plant/fungal combinations (Smith et al. 2004), will be of particular value.
3. Isotopic studies. Studies that employ isotopic techniques in the field are likely to be important. Similar such studies have been important in helping to understand the role of AM in plant P nutrition in the field (e.g., Schweiger and Jakobsen 1999).
4. Field relevant studies. Since most people who experience Zn deficiency are in the developing world, their farming is essentially "organic," and the formation of AM can increase plant Zn nutrition, research on this topic should be of high priority. To this end, studies of agricultural management impacts on AM functioning (and other aspects of the biology/ecology of AM) are needed. The need for this is exemplified by the work on long fallow disorder in northern Australia (Thompson 1987, 1996). Genotypic and systems-based methods for the study of AM in the field may be of particular benefit. In studies using a genotypic-based approach, careful selection of mutant/wild-type genotypes should be undertaken so as to ensure that the pairs are as closely "matched," as is practicable. Where a systems-based approach is employed, appropriate crop rotations should be selected.
5. Pre-inoculation. The potential for pre-inoculation of crops with AMF inoculants also deserves further attention; high-value horticultural crops may benefit in particular. Although the importance of "matching" plants and AMF (Johnson et al. 2005), and edaphic and environmental conditions, need to be taken into account. Equally, the identification of land management practices that limit the formation and functioning of AM also need to be undertaken in parallel.

11.7 Conclusions

AM have an important role to play in plant Zn acquisition. Here emphasis has been placed on the role of AM under low soil Zn conditions. In this chapter I have sought to highlight some important advances that have been made and insights gained. If

we are to capitalize on the benefits of AM in agroecosystems, we must have a strong knowledge base. Further, while there is a need for highly focused and detailed studies, there is also a need to integrate these findings in a wider context. This will necessarily involve studies undertaken under both laboratory and field conditions. While it is a significant challenge, it is sure to be rewarding.

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Chapter 12
Function of Mycorrhizae in Extreme Environments

Catherine A. Zabinski and Rebecca A. Bunn

12.1 Extreme Environments: What and Why

The most compelling story of mycorrhizae in extreme environments is that the symbiosis extends the ecological niche for host plants when environments are at their most limiting. And although research on symbioses in extreme environments is sparse, studies in marginal environments show that mycorrhizae can ameliorate harsh conditions for host plants (Bothe et al. 2010). However, mycorrhizal function is dependent on the identity of both symbionts, as well as environmental characteristics, and does not uniformly benefit host plants (Johnson et al. 1997). Further, when we consider the potential for a "file drawer" bias (Casada et al. 1996), that is, the publication of positive results (mycorrhizae benefiting host plants) while studies with negative results (mycorrhizae neutral or detrimental to host plants) get relegated to the file drawer, it makes the compelling story of mycorrhizal amelioration of extreme stresses tenuous or at least too simplistic. Here we clarify the current understanding of mycorrhizal effects on host plants in extreme environments and discuss why stress tolerance by mycorrhizal fungi is more likely due to acclimation rather than adaptation, but more research is required for conclusive proof. Finally, we suggest avenues for future research that could increase our understanding of the biology and ecology of this symbiosis.

Extreme environments are characterized by conditions that make survival difficult for most organisms (Rothschild and Mancinelli 2001). Research in extreme environments has increased dramatically as scientists study adaptations present in these environments (Gostinčar et al. 2010; Tiquia and Mormile 2010), search for

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organisms and molecules with potential biotechnology applications (Morozkina et al. 2010), and address the potential for life in other planetary environments (Canganella and Wiegel 2011; Harrison et al. 2013). While the harshest environments are primarily inhabited by prokaryotes, eukaryotes also colonize sites that are generally limiting for higher forms of life. Understanding mechanisms by which eukaryotes persist in extreme environments can inform fundamental science of stress physiology and evolutionary ecology, along with increasing the toolbox for managers restoring heavily disturbed sites (Smith et al. 2010). Furthermore, insight into plants' tolerance of heat, drought, and salinity is of critical import as agricultural food production strains to meet increasing demand under changing climate conditions.

Early terrestrial environments were extreme for the first land plants, which required a consistent water supply, a relatively narrow temperature range, and readily available nutrients. As plants transitioned into land-based habitats, they were exposed to damaging UV radiation, desiccating air, and high temperature fluctuations (Waters 2003). The mycorrhizal symbiosis evolved multiple times over the history of plant evolution, and evidence suggests that the ancestral form of this symbiosis, arbuscular mycorrhizae (AM), was present in the roots of the first plants colonizing terrestrial habitats (Wang and Qiu 2006). Given the increasing evidence that symbioses between plants and microorganisms, including mycorrhizae and fungal endophytes, can enhance survival and fitness in extreme environments (Rodriguez and Redman 2008; Chalk et al. 2010), it is likely that mycorrhizae have played an important role for plants' adaptations to extreme environments since the evolution of the symbiosis.

12.2 Symbiosis Function

Mycorrhizal function in non-extreme environments varies, but the most consistently observed phenomenon is enhanced nutrient uptake by the host plant in exchange for host carbon provided to the fungus. The mycorrhizal symbiosis has broad taxonomic and structural diversity, but in this chapter, we will focus on the two most commonly studied forms: ectomycorrhizae (EM), which form between fungi of the *Basidiomycota* and *Ascomycota* and woody plants that may be either angiosperms or gymnosperms, and arbuscular mycorrhizae (AM), between fungi from the *Glomeromycota* and primarily herbaceous plants (but also some woody gymnosperms and angiosperms). In addition to the taxonomic diversity, the structural differences between AM and EM mycorrhizae are most pronounced on the fungal fruiting bodies and at the fungal-root interface. EM fungi produce relatively large fruiting bodies, and EM hyphae form a fungal sheath surrounding the host plant roots, both of which require a large carbon investment from the host plant. The sheath provides a physical barrier between the root and the soil, thereby conferring some level of pathogen protection for the root. In contrast, AM fungi do not construct an external sheath, and the fruiting bodies are generally a small spore

formed at the end of soil hyphae. AM fungal carbon requirements are more modest, and protection mechanisms occur biochemically, rather than physically.

The relationship between a host plant and its mycorrhizal fungi has been measured via a cost/benefit framework (Koide and Elliott 1989; Schwartz and Hoeksema 1998). Typically, the net benefit to the host plants is equated to biomass, which is used along with flowering, nutrient status, and seed production to estimate fitness of the host plants. With these measurements, the symbiosis varies from mutualistic to parasitic depending on the species and life stage of host plant, the fungal species, and the soil conditions (Johnson et al. 1997; Hoeksema et al. 2010). However, even without biomass differences, mycorrhizal fungal nutrient uptake pathways are utilized over those of the host plant (Smith et al. 2009), and there may be an advantage for the host plant not always evidenced by increased biomass (Smith et al. 2010) but with long-term implications for host plant fitness. From the perspective of the fungal partner, the symbiosis is clearly beneficial for AM fungi, as a result of their dependence on host plant carbon, and probably comparably so for EM fungi, although some species of EM fungi are able to obtain carbon from decomposing organic matter. An analysis of the costs and benefits of the symbiosis in extreme environments may be in simple terms of survival and the persistence of symbiont populations.

The relative costs of the mycorrhizal symbiosis can increase for the host plant in marginal or extreme environments if the plant has stress-induced constraints on photosynthesis. Alternatively, if a mycorrhizal symbiont supplies nutrients that are limiting in the extreme environment or reduces the host plant's contact with toxic compounds, the benefit of the symbiosis increases relative to benefits in non-extreme environments. Mechanisms of stress tolerance or avoidance conferred by the fungus include: (1) enhancing host plant access to resources, which indirectly affects the host plant's ability to tolerate a suboptimal environment, (2) protecting the host plant from the stress, and (3) altering the plant biochemistry apart from enhanced nutrient concentrations. Because enhancement of nutrient status has cascading effects for the host plant, the three mechanisms are not exclusive of one another.

12.2.1 Mycorrhizal Effects on Host Plant Access to Resources

Mycorrhizal fungal hyphae occur both inside roots and in the soil surrounding the roots. Fungal hyphae are more than two orders of magnitude smaller in diameter than are fine roots and thus able to penetrate soil pores that are inaccessible to roots (hyphal diameter is ~2–20 μm, while fine roots are ~2 mm diameter; Friese and Allen 1991). In this manner, mycorrhizal hyphae greatly increase the volume of soil explored and thereby increase the uptake of immobile nutrients, such as phosphorus. Uptake of other immobile soil ions, including zinc and copper, is also well

documented in AM fungi (Smith and Read 2008). Some EM fungi are also capable of accessing nutrients in decomposing organic matter (Plassard and Dell 2010).

Besides enhancing nutrient uptake, mycorrhizal plants may increase host plant water uptake by maintaining a plant/soil continuum or accessing water otherwise unavailable to roots. The small diameter of fungal hyphae suggests that it is unlikely that mycorrhizae have a significant effect on large amounts of water movement, but it is possible that even a small enhancement of water uptake could be crucial to plants under drought conditions (Boyd et al. 1986; Stahl et al. 1998). Mycorrhizae can also improve water status directly via the increased surface area afforded by extraradical hyphae or through the regulation of aquaporins, the membrane proteins that control water intake (Porcel et al. 2006; Lehto and Zwiazek 2011). Indirectly, mycorrhizae can improve water access through their effects on soil structure and aggregate stability (Rillig and Mummey 2006).

In marginal and extreme environments, if mycorrhizal fungi can tolerate abiotic conditions that host plants cannot, the symbiosis can augment root function in those portions of the soil profile. For example, fungal hyphae appear to have a higher tolerance for high soil temperatures and are present in thermal soils where there are no roots, thus expanding the host plant's access to resources (Bunn et al. 2009). However, fungal tolerance for environmental extremes is not always higher than the host plant. For instance, AM hyphal growth is inhibited in saline soils with roots present, suggesting that the AM hyphae have lower tolerance to the saline solution than roots (McMillen et al. 1998).

12.2.2 Mycorrhizal Effects in Soils with Toxic Compounds

The mycorrhizal interface represents increased linkage between the root and the soil matrix. This increase allows for additional uptake of nutrients as discussed above but also could result in an increase in the uptake of toxic compounds. The role of mycorrhizae in toxic, and particularly metal-contaminated, soils has received intense interest with its immediate applicability to remediation and restoration projects. A significant body of literature reports mycorrhizal plants have greater metal tolerance than non-mycorrhizal plants, but these results are by no means universal (Smith and Read 2008), and rarely have studies separated the benefits of enhanced nutrient uptake from enhanced metal tolerance (Meharg and Cairney 1999; Meharg 2003). Additionally, the published literature includes reports of mycorrhizae both increasing and decreasing toxic element concentration in the host plant. From a remediation perspective, either result can be positive, depending on whether the management objective is to move the element of consideration out of the soil and into the biota or whether the objective is to stabilize sediments without introducing contaminants into the biota via trophic transfer.

EM plants (mainly conifers) are typically some of the first plant species to colonize mine spoils (Meharg and Cairney 1999). Physically, the fungal sheath encasing EM roots acts as a barrier between the roots and soil contaminants.

Because high metal concentration in the soil can damage root apical zones, this physical protection contributes to enhanced growth of mycorrhizal plants (Marschner 1995). Biochemically, EM fungi may protect the host plant from high concentrations of metals extracellularly, when metals are immobilized in exuded ligands or on fungal cell surfaces; intracellularly, when metals are immobilized in the cytosol also via ligands; or transcellularly, via increased efflux of metals from fungal cells or storage in vacuoles (Bellion et al. 2006).

AM hyphae can either increase or decrease the uptake of metals from the soil (Weissenhorn et al. 1995; Leyval et al. 1997; Neagoe et al. 2013) but generally reduce the transfer of metals to the shoots (Joner and Leyval 1997; Chen et al. 2005). Reduced element transfer to the plant in phytotoxic soils can be the result of enhanced binding, adsorption, or chelation of metals by mycorrhizal fungal tissues or fungal exudates (Evelin et al. 2009) and sequestration of metals into structures to minimize cellular damage (Ferrol et al. 2009) as well as increased efflux of metals from the cytoplasm (Colpaert et al. 2011). AM fungi can actually avoid metals by changing the direction of hyphal growth or growing through patches of higher metal concentration with reduced branching (Ferrol et al. 2009). The variable effects of the symbiosis on the host plant result from the net effect of enhanced element uptake in conjunction with binding, adsorption, chelation, sequestration, and efflux of excess elements (Audet and Charest 2009). A better understanding of the shifts in mycorrhizal function with changing element concentration would improve our ability to utilize these symbionts in remediation efforts (Hildebrandt et al. 2007).

Similarly, for organic contaminants, mycorrhizae can either increase or decrease host plant uptake (Gunderson et al. 2007). AM hyphae can take up polycyclic aromatic hydrocarbons (PAHs) in soils and transport them to the host plant (Gao et al. 2010), a positive result for those interested in phytoextraction, and can also increase the degradation of PAHs in the soil as a result of positive interactions with soil biota that occur in the rhizosphere and mycorrhizosphere (Yu et al. 2011).

12.2.3 Mycorrhizal Effects on Host Plant Biochemistry

The formation of the mycorrhizal symbiosis involves a complex array of communication between the host plant and the fungus (Maillet et al. 2011). Some of the biochemical responses between the plant and fungus may allow the host plant to better respond to stress. For example, plants produce reactive oxygen species as a signaling mechanism to regulate a number of cellular processes in response to stress (Nanda et al. 2010). These chemicals react with cell constituents in an irreversible way, especially when produced in high concentrations, and lead to aging and death of the cell. Reactive oxygen species are also produced in normal physiological processes, such as oxidative phosphorylation or photosynthesis. Cells regulate both low-level steady-state concentrations and high-level stress-response concentrations

of reactive chemicals with antioxidants, a broad class of compounds that can neutralize chemically reactive molecules (Gill and Tuteja 2010).

Mycorrhizal enhancement of growth in environments with limiting conditions is correlated with higher levels of antioxidant enzymes in host plant tissues, suggesting improved oxidative stress regulation for plants in stressful conditions (Bressano et al. 2010). This phenomenon of elevated levels of antioxidants in mycorrhizal plants occurs following exposure to elevated metals (Schützendübel and Polle 2002; Azcón et al. 2010), temperature stress (Zhu et al. 2010), salinity (Garg and Manchanda 2008; Estrada et al. 2013), and drought (Alvarez et al. 2009). The benefit conferred by the mycorrhizal symbiosis may be the augmentation of the biochemical pathways to produce compounds that the plant uses in response to a variety of abiotic stress conditions.

12.3 Adaptation or Acclimation?

Discovering mycorrhizal fungi adapted to extreme environments could have wide ranging applications in agriculture and restoration. And, at first glance, extreme environments present seemingly ideal conditions for adaptation. Limiting environmental parameters promote strong evolutionary and ecological responses, which should enable organisms to adapt to marginal environments, expanding their ecological niche and geographic distribution (Kawecki 2008). But, while selection coefficients for adaptations that enhance survival and reproduction are high, small population size and low genetic variation limit evolutionary potential (Parsons 1991). Whether adaptation occurs depends on the balance between selection pressure and availability of genetic variation. In contrast, acclimation is the adjustment to a new environment that occurs without a change in the genetic profile of a population. It can be difficult to distinguish instances of acclimation from those of true adaptation. Adaptation requires that populations exhibit genetic variation that results in differential ability to occur in conditions particular to that site.

Species occurring in adverse environments may be found only in sites with those conditions, with a consequently narrow ecological distribution, or may occur across a wide range of site conditions with a resulting wider distribution. Mycorrhizal fungi appear to favor the second strategy, exhibiting a high degree of functional plasticity (Lekberg and Koide 2008) along with a high level of genetic variation (Ehinger et al. 2012). For AM fungi this variation is distributed within populations, as opposed to between populations (Koch et al. 2004; Rosendahl 2008). And, most of the variation within a population is represented within a single multinucleate spore (Pawlowska and Taylor 2004), which may give rise to genetically different variants (Ehinger et al. 2012). Similarly, EM fungi exhibit broad genetic variation within species nearly equal to that found between species (Smith and Read 2008).

Evidence for acclimation in AM fungi exists from studies of cold temperature tolerance (Addy et al. 1998) and Ni tolerance (Amir et al. 2008). Tolerance can be induced in a period of months (Amir et al. 2008) and can be lost in isolates that are

not maintained under the same stress conditions (Sudova et al. 2007). For AM fungi, the combined evidence of the fungi's functional plasticity, genetic variation, and impermanent tolerance to stresses supports the hypothesis that mycorrhizae acclimate rather than adapt to extreme environments. There are several reports of ecotypic variation in EM fungi, relative to metal tolerance (Colpaert et al. 2011) and serpentine soils (Jourand et al. 2010). Further studies may reveal evidence that populations of EM fungi are adapted to site conditions and particularly to extreme sites.

Adaptation within a symbiosis is especially problematic to discern as the response of each symbiont is mediated by the presence of the other. To circumvent this issue, assessment of both host and fungal traits with combinations of potentially adapted and non-adapted symbionts is needed (Johnson et al. 2010). Patterns of response would serve as the basis for research on mechanisms resulting in the enhanced performance of either symbiont in harsh environments. Comparison across host plants is critical, since host plants adapted to marginal or extreme environments may not perceive that environment as extreme. A comparable study across a climatic gradient shows that host plants differentiated between AM fungi isolated from different climatic regimes only when growing under temperature regimes that they were not accustomed to (Antunes et al. 2011). While comparison across fungal isolates is also important, our understanding of fungal adaptations will be limited until we distinguish between induced responses versus evolutionary differentiation of races or ecotypes.

12.4 A Case Study in an Extreme Environment: Thermal Soils of Yellowstone National Park

Yellowstone National Park (YNP) is underlain by an area of high volcanic activity, where the heat from the subterranean magma chambers is transferred via the groundwater to the surface in the form of geysers, fumaroles, mudpots, hot springs, and thermal vents. The soils in the thermal areas are generally poorly developed, low in nutrients, and with temperatures that increase with depth. Plants growing in thermal areas are generally small in stature and shallow rooted to avoid high soil temperatures (Fig. 12.1), and AM are present in soils with temperatures in the rooting zone as high as 56 °C (Bunn and Zabinski 2003).

The thermotolerant grass, *Dichanthelium lanuginosum* (Elliott) Gould (hot springs panic grass), occurs in YNP thermal areas (Stout et al. 1997; Bunn and Zabinski 2003). We tested the effects of AM for this and two other host plants, *Agrostis scabra* Willd. (rough bent grass) and *Mimulus guttatus* DC. (yellow monkeyflower), both of which are widely distributed in the area, either on or off thermal soils. Plants were grown in the greenhouse with one of two sources of whole-soil AM inoculum, one from YNP thermal soils and the second from a nonthermal soil outside of YNP but in a similar climate regime. All combinations

Fig. 12.1 Thermal site in Yellowstone National Park, in the Midway Geyser Basin

of treatments were subjected to a soil temperature treatment, either ambient greenhouse temperature or with pots growing on a heat blanket, resulting in soil temperatures near 50 °C at the base of the pot and 30 °C at the soil surface (Bunn et al. 2009).

Our first question was whether mycorrhizae affect host plant growth in high-temperature soils. The three host plants responded to the soil temperature treatment differently. *Dichanthelium lanuginosum*, which is only present on thermal sites, barely grew at ambient soil temperature and was far more robust at high soil temperature, whereas the biomass of the two species that occur on and off thermal sites was twice as high when growing on soils at ambient temperature as compared to high temperature (Fig. 12.2). Additionally, mycorrhizal effects for facultatively thermal plants were neutral in regard to biomass, at either ambient or high soil temperatures, while *D. lanuginosum* grew much better in the presence of mycorrhizae. Therefore, mycorrhizal effects on host plant growth in high-temperature soils were dependent on the host species, and for *D. lanuginosum*, its growth on high-temperature soils was dependent on the symbiosis.

Our second question was whether symbiosis function in ambient versus high-temperature soils varies depending on the source of AM inoculum. We found no evidence for differences in mycorrhizal function with whole-soil inoculum from thermal versus conventional soils, when measuring both host plant response traits (biomass, flowering, and root characteristics) and fungal traits (internal colonization rates and extraradical hyphae). Those results are consistent with the general finding that mycorrhizal diversity is high and distributed within populations rather than between populations.

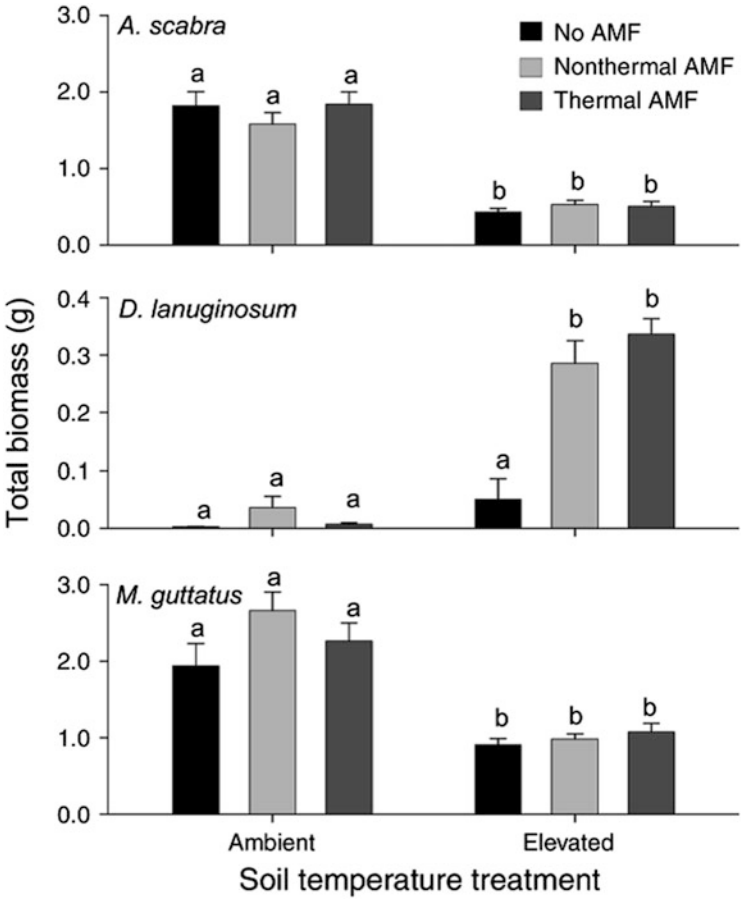

Fig. 12.2 Biomass of three host plants grown with three mycorrhizal treatments either at ambient soil temperature or elevated soil temperatures. *Lowercase letters* represent significant differences ($P < 0.05$) between least squares means of temperature treatments within each species. $n = 18$ for all factor combinations, except for *D. lanuginosum* in ambient temperatures, where $n = 10$, 10, and 8 for no, nonthermal, and thermal AMF, respectively

While plant species seem to be specifically adapted to high-temperature soils, AM fungi do not, which raises interesting questions in regard to the potential for adaptation of a symbiosis to extreme environmental gradients. The AM fungi present in thermal soils include both species that are widely distributed and species that are unique to thermal areas (Appoloni et al. 2008; Meadow and Zabinski 2012). While comparison of whole-soil inoculum from contrasting sites can tell us broadly about function of AM fungal communities, a comparison of isolates of the same species from thermal versus nonthermal sites would allow us to determine whether genetic differences exist between isolates. An additional limitation of this research is the difficulty of extrapolating greenhouse results to the field, due to the

complexity of the natural environment relative to experimental conditions. We measured adaptation to a single environmental trait, soil temperature, when in fact organisms in the field are responding to a complex environment. In the greenhouse we used the same field soil and generated a temperature treatment with the use of heat blankets at the base of the pots. In the field, however, soil temperature differences are confounded with soil pH and accompanying chemical differences (Lekberg et al. 2011). Multifactor environmental gradients comparing isolate and host plant combinations become logistically difficult but may be necessary to measure symbiosis function and the potential for adaptation and acclimation of the symbionts to extreme environments.

12.5 Conclusions and Application to Management

The long history of the mycorrhizal symbiosis since plant colonization of terrestrial environments suggests that this symbiosis has functioned to reduce stress for the host plant, and as environmental conditions ameliorate, to increase host plant fitness via enhanced nutrient uptake. If mycorrhizae contribute to plant species' ability to tolerate fluctuating and extreme environmental conditions, as evidenced by enhanced antioxidant levels, contaminant sequestration, and enhanced nutrient status, then managing for changing environments should include recognition of the importance of soil biota, including mycorrhizal fungi, on plant growth.

From an application perspective, land managers would like to know how best to use mycorrhizae for revegetation of disturbed environments that have characteristics in common with extreme environments. Addition of inoculum to restoration sites or managed lands could potentially benefit both aboveground and belowground community developments if the site had depleted mycorrhizal fungal communities or if the inoculum was specifically adapted to site conditions. While there is some evidence for EM fungal ecotypes, there is a need for more studies across a wider taxonomic range of EM fungi to be able to estimate under what conditions site-adapted fungi should be used. For AM fungi, future research should address environment effects on gene expression and the potential for AM fungal species to acclimate to novel environmental conditions. Research that measures symbiont function across time and contrasting gradients, along with gene regulation relative to environmental conditions, will help to elucidate the potential for adaptation and acclimation of fungal symbionts to extreme environments.

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Chapter 13
Alleviation of Soil Stresses by Arbuscular Mycorrhizal Fungi

Obed F. Madiba

13.1 Introduction

It is well known that arbuscular mycorrhizal (AM) fungi form symbiotic associations with a range of plants (Killham 1994; Marschner 1995; Smith and Read 2008). The extent of colonisation is not only controlled by P concentration in the soil solution but also by P content in the plant (Marschner 1995). A large amount of carbon from the host plant is needed by the fungi (Ryan et al. 2002). Mycorrhizal roots experience high respiration and thus have high carbon loss (Killham 1994; Marschner 1995; Calderon et al. 2012). Arbuscules (primarily for transfer of nutrients) and vesicles (primarily as storage organs) are crucial structures in the transfer of P and other nutrients, e.g. immobile nutrients (Zn, Cu), to the plant (Smith and Read 2008). The likely mechanism involves P (in the form of phosphate) uptake from the soil solution directly through the root epidermis and root hairs or via the AM fungi pathway (Marschner 1995; Solaiman and Saito 2001). Nutrients are then delivered to shoots and leaves by the transpiration stream.

Phosphorus (P) is generally a limiting nutrient in many agricultural systems especially in sandy soils (Evans et al. 2006). As a result, large application of P fertilisers has been administered to increase crop production, some of which results in P leaching (Fertiliser Working Group 2007) or fixation in soil in acid soils (Lujerdean et al. 2004) and alkaline soils (Aliasgharzad et al. 2010; Cardarelli et al. 2010). The consequences of fixation of P in soil result in the reduction of P availability to crops and consequently crop production will decrease (Lujerdean et al. 2004). Leaching of P from fertiliser does not only decrease nutrient availability, but can also cause algal blooms, thus polluting waterbodies (Lehmann et al. 2003). In addition, the raw material used to make P fertilisers (rock phosphate)

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Table 13.1 Examples of soil stresses effect on arbuscular mycorrhizal fungi

Soil stress	Mycorrhizal response	References
Soil disturbance	Disturbance affects AM fungal colonisation	Evans and Miller (1988), Miller (2000)
	Disturbance does not affect AM fungal colonisation	Duan et al. (2011)
Soil compaction	Increase in soil aggregation	Rillig et al. (2001), Schimel et al. (2007), Miransari et al. (2008)
Soil pH (alkalinity)	Reduce alkalinity effect	Smith and Read (2008), Cardarelli et al. (2010)
Soil pH (acidity)	Reduce acidity effect	Marschner (1995), Aliasgharzad et al. (2010)
Drought	Increase P uptake	Quilambo et al. (2005)
Salinity	Increase P uptake	Miransari and Smith (2007), Schimel et al. (2007), Daei et al. (2009)
Flooding	Low AM fungi colonisation	Solaiman and Hirata (1995), Sah et al. (2006)
	High AM fungi colonisation	Sivaprasad et al. (1990), Secilia and Bagyaraj (1992), Mendoza and Garcia (2005), Matsumara et al. (2008)

is a non-renewable resource and needs to be conserved. It has been estimated that mining of rock phosphate will reach 'peak P' around 2030 as rock phosphate reserves are predicted to be exhausted by 2060 (Cordell et al. 2009). Strategies are therefore required to increase crop production while at the same time protecting the environment.

Soil stresses, such as acidity and alkalinity, compaction, moisture, salinity, extreme soil temperatures and flooded soils, reduce plant growth and yield and hence reduce production (Miransari 2010). However, the production of rice increases with flooding (Sivaprasad et al. 1990; Secilia and Bagyaraj 1992) probably when colonisation by AM fungi increases. The presence of both commercial inocula of AM fungi and AM fungi indigenous to the agricultural system had potential to alleviate these stresses (Quilambo et al. 2005; Miransari 2010). However, examples of the impact of soil stresses on AM fungi show inconsistencies among studies (Table 13.1).

13.2 Soil Physical Constraints

No-tillage is defined as a cropping system in which there is minimal soil disturbance and can be practiced with diverse crop rotation to increase carbon sequestration for potential mitigation of climate change (Macvay et al. 2006). Additionally, no-tillage practices require that the soil must have a permanent plant cover for organic matter build-up (Diacona and Montemurro 2010). However, these requirements are not possible for many countries largely due to costs

associated with the equipment needed, climate and other factors including government policies. No-tillage is an agricultural system which has been adopted in many countries, including Australia (D'Emden and Llewellyn 2006; Triplett and Dick 2008). For example, D'Emden and Llewellyn (2006) indicated that 86 % of farmers in Western Australia and 42 % in Southern Australia responded that they are using no-till in their cropping system. The main reason for this adoption is probably because no-tillage provides more benefits than negatives. The main benefit of no-tillage is that it reduces soil erosion either by water or wind erosion, thus increasing nutrients for crop growth (Diacona and Montemurro 2010). Furthermore, soil compaction, which prevents seedlings and water infiltration into the soil, is reduced by controlling traffic in the paddock. The retention of organic matter associated with no-tillage will enhance the ease of water movement and availability and the enhancement of soil biological activities (Diacona and Montemurro 2010). Where possible, weeds, pests and diseases can be controlled biologically (by using cover crops) and chemically using herbicides and by crop rotation (e.g. legumes), thus avoiding escalating costs of pesticides and nitrogen fertilisers. Again, by promoting diverse biological activities including those related to AM fungi, nutrient uptake, especially P, will be more efficient (Killham 1994; Marschner 1995; Smith and Read 2008; Verbruggen and Kiers 2010).

The role of AM fungi in nutrient uptake is influenced by crop rotation and agricultural practices, including tillage (Jasper et al. 1989; McGonigle and Miller 2000; Jansa et al. 2006). Evans and Miller (1988) reported that soil disturbance can cause destruction of the AM fungal hyphae network thus disrupting the flow of nutrients to roots (Miller 2000). However, in other circumstances, Duan et al. (2011) demonstrated that soil disturbance did not affect AM fungal colonisation as evidenced by high P uptake and increased growth but Jansa et al. (2003) reported that soil tillage affected the community structure of AM fungi in maize roots.

The timing of tillage can maintain the activity of AM fungi. For example, Maiti et al (2011) reported that although less soil disturbance results in high AM fungal colonisation of roots, the timing of tillage may play a role as well. Their findings indicate that no soil disturbance (no-tillage) in the off-season (fallow period) in rain-fed systems (in this case about 8 months after harvest) can have a positive effect on the indigenous AM fungal population. This suggests that the fallow period without tillage after harvest (in summer) and sowing another crop (in winter) is effective for AM fungal proliferation. Thus, although soil disturbance may disrupt AM fungal hyphae, the extent of destruction may depend on the soil type, the AM fungal species and the schedule of tillage. Agricultural practices such as cultivation exposes organic matter and aerates the soil which leads to oxidation of organic matter (Killham 1994). Subsequently, aggregate stability is reduced and soil bulk density increases, and this can lead to soil compaction (Miransari et al. 2008). Furthermore, the increased abundance of micro-aggregates can also contribute to soil compaction. If AM fungi are present in the soil in sufficient quantities, the hyphal network will contribute to binding micro-aggregates into macroaggregates (Miransari et al. 2008). Glomalin, a glycoprotein, as defined by Rillig et al. (2001)

is a 'glue-like' structure released from hyphae which protects hyphae of AM fungi from losing water due to their hydrophobicity. Due to the coating of AM fungal hyphae with glomalin, aggregates can be stabilised during wetting and drying cycles can occur whereas these cycles usually destroy aggregation (Schimel et al. 2007). Rillig et al. (2001) indicated that glomalin can exist in the soil from 6 months to 40 years, thus making it a crucial component in soil aggregation. Furthermore, AM fungi can reduce compaction impacts through their interactions with roots, providing strength in penetrating soil for exploration of nutrients and water (Miransari et al. 2008). AM fungal hyphae and glomalin contribute to soil organic carbon in grasslands, and due to their decomposition, more cementing agents, such as polysaccharides, are produced (Rillig et al. 2001). It is doubtful whether glomalin contributes significantly to aggregation in semiarid climates due to low production of biomass.

13.3 Soil Water Constraints

Stress caused by shortages of water can be challenging for microbial functioning and survival. In order to minimise water stress, microorganisms, including AM fungi, alter their physiology and cellular mechanisms (Schimel et al. 2007). Microorganisms shift their resources from growth to survival mode as a strategy in water-limiting environments (Schimel et al. 2007). In addition, under dry conditions, water potential of AM fungi decreases and therefore accumulation of osmolytes is needed to prevent dehydration which usually comes at a cost to the environment (Auge 2001). However, upon re-wetting they have to dispose of the accumulated osmolytes and that usually occurs with respiration, releasing CO_2 from the ecosystem and thus contributing to large carbon losses. Furthermore, when the soil dries, substrate diffusion decreases due to discontinuation of pores, and this make substrates less available due to slow diffusion (Schimel et al. 2007).

Both indigenous and commercial AM fungi differ in their ability to increase crop growth under water stress conditions. Quilambo et al. (2005), working on low-P coastal soils of Mozambique, showed that indigenous AM fungi provided higher root colonisation compared to commercial AM fungi. Although AM fungi act as a pathway for increased uptake of nutrients and water to the plant, these are not the only requirements for the plant to grow.

By definition, waterlogged soils are those that most of the year or several months of the year are ponded with water (Marschner 1995). These soils can be submerged during and after heavy rain or excessive and frequent irrigation in poorly structured soils; paddy soils are good examples of such soils (Marschner 1995). In some cases, clearing forests for agriculture results in waterlogging (Cramer et al. 2004). The replacement of trees with shallow-rooted crops results in less interception of water by roots, and this increases ground water recharge, resulting in shallow water tables associated with waterlogging (Cramer et al. 2004).

AM fungi are thought to be aerobic, and any condition which results in O_2 deficiency (such as flooding) is detrimental to their development and survival (Atwell and Steer 1990). Less mycorrhizal colonisation occurs in reducing conditions where redox potential are lower compared to oxidising environments with high redox (Khan 1993). Lack of aeration in roots results in lower concentrations of N, K and P in shoots (Atwell and Steer 1990). Under waterlogged conditions, the reduction of iron Fe^{3+} to Fe^{2+} increases (Kirk et al. 1990), and in plant species such as rice, this reduction of Fe encourages the solubility and availability of phosphates to the rice plant (Kirk et al. 1990). Solaiman and Hirata (1996, 1997a) showed that rice grown in paddy soil had a higher plant dry matter compared to non-flooded soil. However, AM fungal colonisation in flooded soil was lower compared to non-flooded soil. Thus, under anaerobic conditions, AM fungal development is restricted due to O_2 unavailability (Atwell and Steer 1990).

Earlier research showed that AM fungi may be totally absent or temporarily absent in waterlogged conditions and become available in dry conditions (Solaiman and Hirata 1995, 1997b; Sah et al. 2006). In contrast, studies by Sivaprasad et al. (1990), Secilia and Bagyaraj (1992) and Matsumara et al. (2008) revealed that AM fungal colonisation and development was increased under flooded conditions. The findings of Matsumara et al. (2008) indicated that hyphal density and AM fungal colonisation of orange roots increased in a waterlogged soil where oranges were grown intercropped with bahia grass. This was attributed to the intercropping of oranges with bahia grass and inoculation with *G. margarita*. It was thought that the oranges were exposed to O_2 through the well-developed aerenchyma of bahia grass. Furthermore, Mendoza and Garcia (2005) indicated that the mycorrhizal fungal network increased in seedlings of a legume in a flooded soil.

13.4 Soil Chemical Constraints

Strongly saline soils (e.g. electrical conductivity (EC) >1.87 dS/m; McKenzie et al. 2004) can reduce crop production due to loss of water through osmosis (Daei et al. 2009). Under salty environments, water moves from plant cells to the soil solution and as a result, cells plasmolyse (shrink) and ultimately collapse and die (Brady and Weil 2008). Under saline conditions, P uptake is reduced and high ion toxicity from Na and Cl is experienced (Miransari and Smith 2007). Due to low uptake of these ions by AM fungi in salty conditions, their transportation to the plant will be restricted, thus alleviating salinity stress (Daei et al. 2009). Although AM fungi are capable of existing in saline soils, their development is inhibited in extreme saline conditions (Al-Karaki 2000). In addition, extreme salinity (EC of >1.87 dS/m) reduces crop growth by inhibiting spore germination and hyphae development for AM fungi (Juniper and Abbott 2006), thus reducing arbuscular development (Miransari 2010). These limitations will reduce the effectiveness of AM fungi in facilitating nutrient and water uptake by crops in saline conditions. However, under moderate salinity conditions (EC of 0.15–0.7 dS/m, McKenzie

et al. 2004), AM fungal inoculation will alleviate saline stress (Miransari and Smith 2007). The mechanisms underlying salt-sensitive and salt-tolerant AM fungi are different depending on the species (Daei et al. 2009). Those species that are resistant enhance leaf respiration and transpiration, and as a result CO_2 and water exchange increases which will affect the water use efficiency (WUE) of the crop and consequently crop yield will increase (Daei et al. 2009; Miransari and Smith 2007). Unlike the case for water stress, AM fungi alleviate salinity stress by increasing osmolytes (carbohydrates) in host plants, and due to this mechanism, root and shoot growth can be reduced (Daei et al. 2009). Most soils in Mediterranean environments experience high evaporation during summer resulting in high salt accumulation on the surface of soils and reduced crop production. AM fungal inoculation could be beneficial in those situations, but not without other remediation practices.

Soil pH is an important soil characteristic, and AM fungi can differ substantially in their pH tolerance range. For example, Hayman and Mosse (1971) demonstrated enhanced colonisation and plant growth stimulation by AM fungi in soil of pH 5.6 and 7.0, but not in more acid soils of pH 3.3–4.4. Supporting this, soil pH was regarded as a constraint to the distribution of *A. laevis* and *Glomus* sp. (WUM 3) in southwestern Australia (Porter et al. 1987). In this environment, *Glomus* sp. (WUM 3) colonised roots well in the pH range of 5.3–7.5; *A. laevis* was tolerant of acidic soil up to pH 6.2, while *S. calospora* formed mycorrhizae at pH 5.3. Soil pH is one of the soil characteristics that is likely to determine the distribution and relative abundance of AM fungal species in soil (Robson and Abbott 1989).

Cardarelli et al. (2010) observed that inoculation of AM fungi in alkaline soil conditions increased P, K, Fe and Zn content in zucchini plants and decreased the toxic levels of Na with associated increased fruit yield and quality. Bacteria generally thrive well in soils with a high pH (Rousk et al. 2009) although Aliasgharzad et al. (2010) showed that AM fungi were more abundant in deeper soil layers (20–30 cm) with increasing pH.

Phosphorus is likely to be the soil nutrient that has the greatest influence on the extent of root colonisation by AM fungal species. Formation of hyphal entry points into the root, hyphal growth within the root, hyphal growth in soil and sporulation are highest at phosphorus levels suitable for optimal growth of particular hosts but are reduced at very low or very high phosphorus host levels (Thingstrup et al. 1998; Thomson et al. 1986). In high-P soil, the extent of colonisation varied substantially among plant families, genera and among closely related genotypes of the same species (Graham et al. 1991; Krishna et al. 1985; Manske 1989; Toth et al. 1990). Increasing nitrogen fertilisers of both ammonium and nitrate have also been reported to reduce root penetration and colonisation of subterranean clover by AM fungi (Chambers et al. 1980). The negative effect was more marked in the case of nitrogen fertilisers than that of P fertilisers (Jensen and Jakobsen 1980). However, these effects depend on the rate added and the available nutrient in the soil (Sharma and Adholeya 2000).

13.5 Conclusions

Phosphorus is a limiting nutrient in most agricultural systems especially in sandy soils. The practice of applying large amounts of P fertilisers to increase production has negative environmental consequences. Soil stresses, such as alkalinity and acidity, compaction, drought, salinity and extreme temperature and waterlogging, can reduce crop production. AM fungi are to some extent resistant to these stresses, but there may be negative influences on AM fungal development and survival.

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Chapter 14
Mechanisms for Alleviation of Plant Water Stress Involving Arbuscular Mycorrhizas

Bede Mickan

14.1 Introduction

Water is often a limiting factor in many dry land agricultural cropping systems, with many global climate change projections predicting an increase in drought frequency and duration. Furthermore, water deficits are now spreading to many agricultural regions where drought was uncommon in the past (Anwar et al. 2013). This poses a significant global challenge to maintain or increase food production from semiarid agricultural zones (Tscharntke et al. 2012). However, this reduction in rainfall has led to many innovative farm management practices such as no-tillage (Jemai et al. 2013), precision seeding, and reduced traffic (Boizard et al. 2012), which can increase soil water retention and water infiltration. In parallel with these developments, activities of arbuscular mycorrhizal (AM) fungi have been claimed to include mechanisms that increase both plant water relations directly (Manoharan et al. 2010) and indirectly increase the soil water retention and infiltration by the enhancement of soil aggregation (Rillig and Mummey 2006).

AM fungi are obligate symbionts. The fungi have an internal phase inhabiting roots and an external phase comprising the extra-radical hyphal network that forms a branching mass of hyphae with potential to acquire nutrients and water beyond the root depletion zone (Allen et al. 2003; Khalvati et al. 2010). The potential of AM fungi to enhance water relations of plants and soil is claimed to be related to a degree of alleviation of water stress, and the mechanisms involved have been widely investigated by a multidisciplinary group of specialists including ecologists, botanists, agricultural scientists, and more recently plant geneticists. Key reviews discussing the role of AM fungi in plant water relations include those of Augé

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(2001) and Ruiz-Lozano et al. (2012) and for AM fungal influences on soil structure a review by Rillig and Mummey (2006). Here I investigate how AM fungi can (1) directly alleviate water stress directly through nutrient and water acquisition and enhanced plant water physiology and (2) indirectly through enhancement of the structure of the soil/water interface.

14.2 Direct AM Fungi Benefits to Host Plants Under Water Stress

The ability of AM fungi to alleviate water stress directly in its most obvious mechanism is through an increase in the amount of nutrients and water acquired through the AM fungi hyphal network. However, there is also evidence to state that AM fungi are able to alter plant biochemical composition to increase antioxidant production and beneficial osmoregulator (proline) that directly alleviate a degree of water stress through drought-avoidance mechanisms (for key papers, see Table 14.1). Partitioning which mechanism is the largest contributor in alleviating water stress has not been determined, though it is most likely the combination of direct mechanisms in unity that are responsible for alleviating a degree of plant water stress.

14.2.1 Plant Nutritional Benefits from Mycorrhizal Symbiosis

Enhanced plant growth associated with the AM fungal symbiosis was first claimed to be through a direct increase in nutrient uptake, giving the host plant a greater tolerance to water stress in the early 1970s (Safir et al. 1971, 1972). Initially, these early authors claimed AM fungi were able to decrease water resistance from the root to leaves, and this was correlated with an increase in the growth of shoot mass (Safir et al. 1971). Later, they reported water resistance between AM-fungi- and non-AM-fungi-colonised plants was negligible once the addition of nutrients (Hoagland solution) was applied. Thus, non-AM fungi host plants are able to match water transpiration rate and shoot growth when there are sufficient nutrients for plant growth (Safir et al. 1972). Subsequently, it has been widely reported that colonisation of roots by AM fungi can increase the growth of the host plant through an enhanced acquisition of P and, more recently claimed, through the acquisition of N (Veresoglou et al. 2012). This increase can at least partly alleviate water stress of AM-fungi-colonised plants (Smith and Read 2008). Plant roots are able to acquire nutrients through a direct pathway, through the soil root interface, or through the extra-radical phase of AM fungi from the hyphal network path (Smith and Smith 2011). Thus, once the N and P concentrations in the soil have been exploited by a

Table 14.1 Key experimental papers on direct effects of AM fungi in plant water relations

Focal point	Experimental procedure	Experimental outcome	Reference
AM fungi effect on water resistance through soybean roots, stem, and leaves	Comparison of plant water resistance between non-AM-fungi- and AM-fungi-colonised soybeans	AM-fungi-colonised soybean reduced water resistance in roots compared to non-AM fungi soybean; this was related to an enhanced nutrient uptake proposed through the hyphal network	Safir et al. (1972)
AM fungi effect on leaf water potential, transpiration rates, and correlation with hyphal entry points into soybean	Allen MF calculated the plant water transpiration between AM-fungi- and non-AM-fungi-colonised soybeans	AM fungi soybean had 50 % lower leaf resistance with no change in leaf or root water potentials, thus AM fungi increased transpiration by 100 %. Allen correlated this increase in plant water to hyphal entry points to calculate direct water access through the hyphal network	Allen (1982)
Evaluated six AM fungi isolates' ability to alter rates of root water uptake under soil water-deficit conditions	Monitored soil-drying rates of non-AM fungi control plants of comparable size with nutritional status of AM-fungi-colonised lettuce	AM-fungi-colonised lettuce was able to deplete soil moisture significantly more than control non-AM-fungi-colonised lettuce. Furthermore some AM fungi species were able to deplete soil moisture than others, and this was directly correlated with hyphal mass production	Marulanda et al. (2003)
Quantification of plant water uptake through the hyphal network	Split chamber microcosm—using high-resolution on-line water content sensors	Plant water uptake through the hyphal network was estimated to be potentially up to 20 %. This value contradicts studies showing the contribution to be negligible	Ruth et al. (2011)
Antioxidant production and transpiration rates during drought stress on AM-fungi-/non-AM-fungi-colonised rice	Investigate drought tolerance mechanisms in rice induced through AM fungi colonisation	AM-fungi-colonised rice induced the accumulation of the antioxidant molecule glutathione whilst reducing oxidative damage to lipids. Combined with an AM fungi increase in leaf gas exchange aided to alleviate water stress above non-AM-fungi-colonised rice controls	Ruiz-Sánchez et al. (2010)

plant within the root depletion zone through the direct pathway, plants that have AM fungi associations are able to exploit nutrients through the hyphal network path because this pathway extends beyond the root depletion zone. Under these circumstances, when the exploitable limiting nutrient resource shifts from a direct pathway to a hyphal pathway, it is likely the AM fungi growth response will be positive (Allen et al. 2003). Under low soil-nutrient conditions, it is likely that the growth response associated with the AM fungi will be larger than that of a non-AM fungi counterpart, giving the AM-fungi-colonised plant the 'big plant, little plant advantage' arising from an increase in nutrient acquisition (Augé 2001).

AM fungal-associated increases in plant growth and phenology allow roots to develop deeper and more extensively, enabling greater access to exploitable water in soil. Furthermore, larger plants have an added advantage of a greater reserve of sugars to draw on under water-stressed conditions (Porcel and Ruiz-Lozano 2004). However, whilst there may be an increase in drought avoidance due to an increase in plant size attributed to AM fungi when the AM fungi growth response is positive, there is evidence that AM-fungi-colonised plants of similar size and nutrition status still show signs of enhanced plant tolerance to water stress (Augé 2001; Porcel and Ruiz-Lozano 2004).

14.2.2 Water Access Through the AM Fungi Hyphal Network

Pioneer studies by Allen (1982) claimed AM fungi hyphae were able to exploit water resources and transport water to the host plant. Allen reported mycorrhizal plants had 50 % lower leaf resistance with no change in leaf or root water potentials. More recently, the most widely claimed benefit of AM-fungi-colonised plants under water stress is through a superior water allocation mediated by the hyphal network of AM fungi, giving the plant access to water in a lower soil water potential as compared to comparative non-AM fungi plants (Marulanda et al. 2003; Ruiz-Lozano et al. 2012). It is currently widely accepted that AM fungi hyphae are able to exploit water and nutrients beyond the root depletion zone in both distance and space (Ruth et al. 2011; Ruiz-Lozano et al. 2012).

Investigations into the function and physiology of AM fungi hyphal network have incorporated split chamber microcosms, separated by fine nylon mesh (usually around 38 μm) allowing hyphae to pass whilst blocking roots (Al-Karaki et al. 2004). This allows empirical quantification of hyphal network services provided to the host plant, which is important, as AM fungi cannot survive without plant roots and they occupy the same volume of soil (Khalvati et al. 2005). Furthermore, natural abundance stable isotopes C^{13} and N^{15} along with deuterium have also been used to trace the movements of these nutrients from the soil to plant leaves, allowing definitive contributions of water, N, and P provided through the hyphal network (Egerton-Warburton et al. 2007).

The amount of AM fungi hyphae within pot experiments is variable with ranges from 1 to 40 m/g of soil, but this can be highly dependent on species identity (Smith and Smith 2011). In natural settings, the hyphal length can explore a much greater soil volume (e.g. 111 m/cm^3 of soil for one particular prairie community (Rillig et al. 2001)). The rate of hyphal spread through soil is also considerable. Jakobsen et al. (1992) in a glasshouse experiment showed *Acaulospora laevis* had extended through the soil about 80 mm by 28 days at a rate 3.0 mm/day from the host plant root. The quantity of hyphal network-derived water to the host plant values ranges from 0.1 μL/h (Allen 1982) for each hyphal penetration point to 0.37 μL/h (Faber et al. 1991); this represents in some experiments between 4 % (Khalvati et al. 2005) to 20 % of total water uptake through the hyphal network (Ruth et al. 2011). Marulanda et al. (2003) demonstrated that AM fungi *G. mosseae* when in association with *Lactuca sativa* under water-stressed conditions were able to deplete volumetric soil moisture by 0.95 %, equating to an additional 4.75 mL/plant/day compared to a comparative non-AM fungi control. Moreover, there was a direct correlation between the species (*G. intraradices*) of AM fungi that depleted the most volumetric soil moisture also produced the largest amount of hyphal network and frequency of root colonisation (Marulanda et al. 2003).

Alleviation of water stress has also been reported through mycorrhizal networks able to connect two or more plants through the hyphal network; this allows resource allocation by a source–sink relationship (Eason et al. 1991). Briefly, if plant A is deficient in water and plant B has sufficient water, then water is able to be transported from plant B via the hyphal network to plant A along the source–sink gradient (Simard et al. 2012). This has been shown to be especially pronounced in hydraulically lifted water from plants with deeper roots to plants with shallower roots. However, when Querejeta et al. (2012) quantified how much water can be transported from donor oak trees to receiver seedlings in shallower soil, they reported that a greater soil water potential gradient was more of a driving force in the vertical redistribution of water from deeper soil profiles to shallower soil profiles than the mycorrhizal network. The significance of mycorrhizal networks alleviating water stress from a host plant to another is still not fully ascertained and represents an exciting area of future research (Prieto et al. 2012).

Plant physiological evidence supporting AM fungi-associated alleviation of some degree of water stress in colonised plants has been shown by increases in (1) water uptake rate, (2) transpiration, and (3) stomatal conductance when compared to non-AM-fungi-colonised host plant controls (Marulanda et al. 2003; Khalvati et al. 2005). The widely claimed direct benefit to the host plant arises from AM fungi external hyphal network able to acquire both nutrients and water past the root depletion zone, and accessing microsites of water locked within the soil pores is probably the most substantive water-alleviating mechanism of AM fungi (Subramanian and Charest 1999; Khalvati et al. 2005; Ruth et al. 2011; Ruiz-Lozano et al. 2012).

14.2.3 AM Fungi Influence on Plant Biochemical Properties

There is evidence that AM fungi are able to alter biochemical properties of roots and shoots inside the host plant. AM fungi colonisation can increase in antioxidant and proline production, and this is claimed to be most beneficial to the host plant through a better osmotic adjustment potential and reduction in oxidative stress (Ruiz-Sánchez et al. 2010). Whether the claimed increase in beneficial biochemical compounds is directly related to the increased nutrient and water acquisition or if AM fungi are altering plant compounds independently of resource acquirement has not been determined.

14.2.4 AM Fungi Production of Antioxidant Compounds

Water-stressed plants display signs of oxidative damage as a result of increased production of degenerative free radical reactive oxygen species (ROS) (Ruiz-Lozano et al. 2012). These free radical ROS include superoxide (O_2^-), hydroxyl radicals, and others such as hydrogen peroxide (H_2O_2). The detrimental effects of O_2^- and H_2O_2 are in their ability to initiate reactions that cause the production of hydroxyl free radicals under water-stressed conditions (Porcel et al. 2003). Hydroxyl free radicals are among the most reactive species known to chemistry, indiscriminately causing oxidative damage to plant biomolecules and lipid membranes, denaturation of proteins, and mutation of DNA (Ruiz-Sánchez et al. 2010). It is claimed AM fungi are able to suppress oxidative damage by mediating antioxidant ROS scavenging enzymes into the host plant under water-stressed conditions (Porcel and Ruiz-Lozano 2004; Ruiz-Sánchez et al. 2010).

Experimental evidence in the antioxidant production in AM fungi plants has shown increases, decreases, and stabile concentrations in water-stressed plants. These variable results are highly dependent on plant, AM fungi species, and also the type of antioxidant (Kohler et al. 2008). Investigations by Porcel et al. (2003) showed that three out of four antioxidants within AM-fungi-colonised plant roots remained stable or slightly lower under water-stressed conditions between AM fungi and comparative non-AM fungi treatments. However, these authors reported antioxidant glutathione concentration increased 534 % in AM fungi plant roots under water stress compared to corresponding non-AM fungi treatment. Ruiz-Sánchez et al. (2010) reported similar results with the antioxidant glutathione reductase (reduced form). For water-stressed conditions, shoot concentration of antioxidant glutathione reductase in AM fungi plants increased by 436 % as compared to corresponding non-AM fungi plants (Ruiz-Sánchez et al. 2010). AM fungal stimulation of antioxidants in commercially grown lettuce (*Lactuca sativa* L.) is widely claimed to be beneficial for reduction of water stress to a certain degree via alleviation of oxidative stress (Porcel et al. 2003). Additionally, the

increased nutritional status of the leaf has potential implications for increasing human health nutrient uptake (Baslam and Goicoechea 2012).

14.2.5 AM Fungi-Induced Accumulation of Proline

Water-stressed plants are also known to accumulate organic osmolytes such as proline (amino acid) and sugars that contribute to the host plant tolerance under water-stressed conditions through enhanced osmoregulation (Trotel-Aziz et al. 2000). Proline is a nonprotein amino acid that accumulates in plant tissues under water stress together with sugars, and after water stress recovery, it is readily metabolised (Singh et al. 2011). During water-deficit or saline conditions, plants accumulate proline to maintain osmotic balance under low water potentials (Ruiz-Lozano et al. 2012). During water stress, sugar and proline content in roots colonised by AM fungi has been shown to increase as compared to non-AM fungi roots, giving evidence that osmotic adjustment is occurring, enhancing the ability of the host plant to cope with water stress (Porcel et al. 2003; Kohler et al. 2008). Proline also acts as a reservoir of energy and N during water stress and has been found to increase when the plant is colonised by AM fungi. Whilst it is widely claimed proline accumulation in plant roots enhance plant tolerance to water stress through better osmoregulation at the root soil interface, there are also complex responses that show proline levels can be AM fungal species dependent (Ruiz-Lozano et al. 2012).

14.2.6 AM Fungi Influence on Plant Gas Exchange

There is growing evidence that shows AM-fungi-colonised plants maintain higher gas exchange rates to comparative non-AM fungi plants of similar size and plant nutrition status (see reviews by Augé 2001; Ruiz-Lozano et al. 2012). Colonisation of roots by AM fungi in response to water stress has been claimed through an observed increase in stomatal conductance (g_s), g_s maintenance (drought tolerance), and early g_s closure (drought avoidance) in soils with lower water potential (Khalvati et al. 2005). High plant g_s translates into higher rates of transpiration/gas exchange which have often increased in AM fungi plants in well-watered, water-stressed, and also after exposure to salinity-affected water (Wu and Xia 2006; Sheng et al. 2008). Saline and water-deficit conditions have a common osmotic component as they reduce water uptake by roots causing dehydration of plant tissues, referred to as an osmotic stress. The observed higher g_s rates in AM-fungi-colonised plants have been in strong correlation with lower xylem-sap abscisic acid and lower abscisic acid fluxes to leaves in AM fungi host plants (Ruiz-Lozano and Aroca 2010). However, g_s in AM-fungi-colonised plant could be argued to be through an enhanced direct ability to deplete soil moisture through AM

fungi hyphal network (Augé et al. 2008) and/or increased root branching caused by AM fungi, giving the plant greater access to water (Kothari et al. 1990). Interestingly, AM-fungi-colonised soils have the ability to increase the g_s of non-AM fungi plants under water-stressed conditions through the ability of the hyphal network to enhance soil moisture potential through increasing soil structure (Augé et al. 2007).

14.3 Indirect AM Fungi Benefits to Host Plants Through Enhanced Rhizosphere Processes

Whilst there is a direct role of AM fungi in alleviating water stress to the host plant, there is also an indirect benefit of AM fungi through buffering water stress within the rhizosphere (Audet 2012). There has been considerable interest in reports that AM fungi hyphal networks can enhance soil aggregation through direct physical effects or through AM fungi production of biochemicals (Martin et al. 2012). This indirect soil structure effect has potential to improve the water holding capacity of soil (Augé 2004). Soil aggregation is the arrangement or structure of soil particles held in a single mass or cluster, commonly defined using a hierarchal model. Soil organisms influence soil structure by physically binding soil particles together increasing the quantity and size of the aggregates improving the habitat for microfauna (Tisdall and Oades 1982). Whilst recognising the greatest influence on soil aggregation is probably through plant roots, AM fungi influence on soil aggregation can be seen at the plant community and plant hyphal levels and is influenced by AM fungi either directly through the hyphal network or indirectly through altering plant root physiology (Rillig and Mummey 2006). Moreover, non-AM fungi symbiont-forming plants have shown increased resistance to water stress in AM fungi-infected soil. This has been directly correlated with AM-fungi-colonised soil having a greater water retention capacity through enhanced soil aggregation as compared to non-AM-fungi-colonised soil (Augé et al. 2007).

14.3.1 AM Fungi Biochemical Influence on Soil Aggregation

Biochemical compounds including glomalin, mucilages, polysaccharides, and hydrophobins are exudated from hyphal tips and are also secreted on hyphal walls in the mycorrhizal rhizosphere (Singh et al. 2012). Glomalin is a stable glycoprotein that can be measured directly from the soil as a glomalin-related soil protein, which is also deposited on the hyphal walls of the hyphal network and on adjacent soil particles (Wilson et al. 2009). Glomalin-related soil protein acts to bind soil particles together, forming a stable soil aggregation that can enhance the C storage in soil. The benefit of high amounts of glomalin-related soil protein in soil is in the enhanced water retention capacity, as soil aggregation protects C-rich detritus

from microbial decomposition (Rillig and Mummey 2006; Wilson et al. 2009; Verbruggen et al. 2012). The difficulty in quantifying AM fungi-produced glomalin-related soil protein is in the soil extraction process, as it is not clear whether glomalin-related soil protein is of AM fungi origin. In fact, there is evidence suggesting that glomalin-related soil protein also originates from other fungal species (Wilson et al. 2009). However, it is widely claimed AM fungi-produced glomalin, polysaccharides, and other related proteins exuded from the hyphal network act to bind soil particles of various sizes enhancing soil aggregation which correlates with increased water holding capacity of soil (Rillig and Mummey 2006; Wu et al. 2008; Hallett et al. 2009; Audet 2012).

14.3.2 AM Fungi Physical Influence on Soil Aggregation

Physical enmeshment and entanglement of AM fungi hyphae with soil particles to organic matter increase aggregate stability of soil (Daynes et al. 2013). Similar to plant roots, AM fungi hyphae form branching structures with glomalin acting to physically bind microaggregates with macroaggregates (Singh et al. 2012). Hyphal branching morphology is highly variable within and between AM fungi species, with dynamic hyphae lengths, chemistry, and thicknesses, which affects the enmeshment capability of hyphal network. Additionally, although the tensile strength of hyphae is unknown (owing to the narrowness of hypha), it may play an important role in stabilising soil aggregates under disturbance (Rillig and Mummey 2006). The hyphal network persistence in soil is also variable with turnover rates ranging from 5 to 6 days; hyphae runner have been recorded to last 32 days and also stabilise soil several months after plant death (Hallett et al. 2009). As for growing plant roots, AM fungi hyphae are also capable of aligning primary particles such as clay and organic matter together, exerting physical pressure on soil particles leading to a macroaggregate formation which has potential to increase the water holding capacity of soil (Singh et al. 2012). However, Daynes et al. 2013 reported the most pronounced influence on soil aggregation was the presence of plant roots, with AM fungi further stabilising soil structure. These authors reported the self-organising structure of soils to form aggregates in the absence of plant roots, AM fungi hyphae, and/or organic matter (Daynes et al. 2013). Thus, physical soil aggregate stability is dependent on soil characteristics and can also be influenced by soil fauna.

14.3.3 Biological Influence on Soil Aggregation

Biological alterations in soil by the AM fungi hyphal network have also been reported to alter soil aggregation through changes in the soil microbial food web. Changes to the prokaryotic communities induced by AM fungi alteration in soil

Table 14.2 Key papers on AM fungi-induced benefits to soil water through enhancing rhizosphere processes

Focal point	Procedure	Outcome	Reference
Developing a hierarchal model of soil structure, and its relationship with land management practices	Comprehensive review on soil management practices can alter soil aggregation	Soil management effect on soil structure through plant roots and AM fungi hyphae stabilise macroaggregates, thus land management influences the growth of plant roots and the oxidation of organic carbon	Tisdall and Oades (1982)
		The water stability of microaggregates depends on the persistent organic binding agents and appears to be a characteristic of the soil, independent of management	
The role of AM fungi hyphae in soil's effect on non-AM fungi symbiosis plants	Experiments using non-AM forming plants in AM-fungi-colonised soil with a drought stress treatment	AM fungal colonisation of soil may play as important a role as colonisation of plant roots. AM fungi hyphae affect the water relations of host plant through enhancing soil water status or potential by increasing soil aggregation	Augé (2004)
AM fungi influence soil structure at the plant community, individual root, and the soil hyphal network	Comprehensive review on mechanisms of AM fungi influence on soil structure at various scales	AM fungi can influence soil aggregation at each of the plant community, individual root, and soil hyphal network levels. Through physical, chemical, and biological mechanisms to different degrees. Understanding these relationships will require analyses emphasising feedbacks between soil structure and AM fungi	Rillig and Mummey (2006)
The role of AM fungi in ecosystems using soil aggregate stability	Large-scale field manipulations, using fungicide application as non-AM fungi control	Field manipulations that increased AM fungi hyphae increased water stable aggregates and glomalin-related soil proteins. Fungicide	Wilson et al. (2009)

(continued)

Table 14.2 (continued)

Focal point	Procedure	Outcome	Reference
		application that decreased AM fungi, this correlated significantly with decreasing soil aggregate stability	
The role f AM fungi hyphae and Collembola in soil aggregation independent of plant roots	Split chamber microcosms, partitioning plant roots with hyphae and Collembola present	Collembola can enhance soil aggregation, which complement effects of AM fungi hyphae, and that these effects are independent of plant roots. Even though AM fungi hyphae food quality is regarded as lesser than saprobic fungi hyphae	Siddiky et al. (2012a)
Mechanisms that underpin the development and stabilisation of soil structure	Manipulative pot experiments of severely disturbed mine spoil soil, with additions of AM fungal isolates, organic matter, and plants	Organic matter, living plant roots, and AM fungi are required for stable soil structure in complex ways. The presence of adequate organic matter and plant roots as key contributors to the development of soil structure are further enhanced by AM fungi hyphae	Daynes et al. (2013)

may influence changes to soil aggregation at the microaggregate level. Exudates from the AM fungal hyphal network act as a substrate for bacterial growth, where bacteria such as *Paenibacillus* spp. have been reported to enhance microaggregate soil structure (Hildebrandt et al. 2002). Changes in rhizodeposits through AM fungi have also been shown to alter community composition of bacterial populations which have variable functional attributes (Toljander et al. 2007). AM fungi-induced alteration of soil structure leads to changes in available pore space in soil, which logically leads to changes in environments made habitable to soil organisms (Rillig 2004).

The AM fungi hyphal network also forms the basis of the soil food web by being a valuable food source for micro-arthropods even though this resource is a lesser quality than saprophytic fungi. Siddiky et al. (2012a) conducted an experiment investigating how collembola and AM fungi hyphae together increase water stable aggregation independent of plant roots. Interestingly AM fungi hyphae decreased 6 % in length from collembolan grazing, but the collembolan population increased by 20 %. It is claimed that collembola are able to increase soil aggregation through their faecal pellets. The understanding of mechanisms of how AM fungi hyphal

network influences the soil food web and soil biological diversity and abundance is still in its infancy, but it presents an exciting area of future research (Siddiky et al. 2012b) (Table 14.2).

14.4 Conclusion

There is evidence that AM fungi colonisation in plants has the ability to directly alleviate water stress through physical, chemical, and biological drought-avoidance and tolerance mechanisms. Directly, AM fungi are able to exploit a greater amount of soil in both space due to the narrowness of hypha and distance through the hypha's ability to extend beyond the root depletion zone. Thus, separating traits of AM fungi that enable alleviation of water stress is difficult because variables of AM fungi are closely linked to one another. The conclusion most supported by evidence is that AM fungi are able to alleviate water stress to the host plant through multiple processes by enhancing (1) nutrient/water acquisition and antioxidant production, (2) proline accumulation, and (3) soil structure, thereby increasing soil water retention. The successful management of rainfed dry land agricultural production, whether it be cropping or grazing systems, will benefit from management practices that increase the diversity and abundance of AM fungi populations in water-limiting environments.

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Chapter 15
Role of Mycorrhizal Fungi in the Alleviation of Heavy Metal Toxicity in Plants

Hamid Amir, Philippe Jourand, Yvon Cavaloc, and Marc Ducousso

15.1 Introduction

Studies on the interactions between mycorrhiza and heavy metals are relatively recent. One of the first reports, by Gildon and Tinker (1981), highlighted a heavy metal tolerance of arbuscular mycorrhizal (AM) fungi in metal-polluted soil. These authors also suggested a possible role of AM fungi in increasing heavy metal uptake by plants. Since this report, about 500 articles have been published on this subject. Of these, about 150 articles deal with the influence of mycorrhizas on heavy metal absorption by plants and the alleviation of heavy metal toxicity; 78 % of these studies have been published during the last decade. Indeed, it is becoming more and more clear that mycorrhizal fungi can be used for the bioremediation of metal-polluted sites caused by industrial activities (Hildebrandt et al. 2007; Khade and Adholeya 2007; Orlowska et al. 2011a; Rajkumar et al. 2012). Several studies have suggested a role of these symbionts in the adaptation of plants to naturally metal-rich environments, i.e. mining areas and ultramafic (serpentine) soils (Ma et al. 2006; Leung et al. 2007; Jourand et al. 2010a, b; Lagrange et al. 2011; Amir et al. 2013).

Mycorrhizal symbioses occur in more than 80 % of the vascular plants (Brundrett 2009). Fungal symbionts constitute an important interface between the soil and the plant and induce physicochemical and biological changes in the rhizosphere (Hinsinger et al. 2009; Lambers et al. 2009; Smith and Smith 2010).

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It is well known that they have a considerable influence on the mineral nutrition of plants. Generally, they improve the absorption of mineral elements in relation to the increase of the soil-plant interface which is due to the abundant mycelium colonizing a large volume of soil and to their weathering effects on minerals (Marschner and Dell 1994; Smith and Read 2008). However, the effects of mycorrhizal fungi on the absorption of mineral elements vary according to the type and concentration of the element, indicating that the mycorrhizal root is highly selective; this is especially the case for heavy metals which can be necessary at very low concentrations and toxic at higher levels (Leyval and Joner 2001).

Heavy metals such as Cd, Pb, Zn, Cu and Ni are naturally present in the soil solution as trace elements, but their concentrations can be considerably enhanced by industrial activities such as mining, automobiles, industrial wastes and pesticides (Joshi and Luthra 2000). There are also naturally metal-rich soils, such as ultramafic soils characterized by high contents of heavy metals, especially Ni, Co, Cr and Mn. The toxicity of these metals depends on their bioavailability which is influenced by physicochemical soil characteristics such as pH, clay and organic matter content and microbial activities, including that of mycorrhizal fungi (Berthelin et al. 1995; Leyval et al. 1995; Leyval and Joner 2001; Amir and Pineau 2003).

This review aims to synthesize research on the influence of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi on plant absorption of heavy metals and on the alleviation of their toxicity. Prior to discussing this central topic, a few points should be noted about heavy metal tolerance and adaptation of these symbionts to metal-rich soils.

15.2 Presence of Mycorrhizal Fungi in Metal-Rich Soils

Mycorrhizal fungi have been found in all heavy metal-polluted soils, even when these soils are highly contaminated (Vallino et al. 2006; Gamalero et al. 2009). They are also relatively abundant in naturally metalliferous soils, such as ultramafic soils, with high contents of Fe, Mn, Ni, Co and Cr (Amir et al. 1997; Turnau and Mesjasz-Przybylowicz 2003; Perrier et al. 2006; Gonçalves et al. 2007; Jourand et al. 2010b). In these environments, soils under nickel hyperaccumulating plants, with up to 1,500 μg g^{-1} of DTPA-extractable Ni, contained viable AM fungal spores; however, root colonization by AM fungi was partly, or (in rare cases) totally, inhibited (Amir et al. 2007).

The diversity of AM fungi in heavy metal-polluted soils is generally lower than in other soils (Pawlowska et al. 1996; Regvar et al. 2003; Hassan Sel et al. 2011). *Glomus* species are clearly the most abundant (Griffioen 1994; Khade and Adholeya 2007; Ortega-Larrocea et al. 2010). The most reported species of this genus in these soils are *G. fasciculatum*, *G. intraradices*, *G. etunicatum* and *G. mosseae*; some *Acaulospora* and *Gigaspora* species are also present at low frequencies (Khade and Adholeya 2007; Ortega-Larrocea et al. 2007, 2010; Wu

et al. 2010). *Scutellospora* and *Sclerocystis* have been rarely reported (Khade and Adholeya 2007).

In ultramafic soils, *Glomus* species also seem to be highly dominant (Perrier et al. 2006; Gustafson and Casper 2006; Schechter and Bruns 2012), with *G. etunicatum* and *G. fasciculatum* being the most commonly observed (Gustafson and Casper 2006; Lagrange et al. 2011). Ji et al. (2012), using spore morphology, compared the AM fungal communities of two ultramafic and two non-ultramafic soils and reported differences in soil chemical characteristics, but without differences in AM fungal diversity. Perrier (2005) and Branco and Ree (2010) found diversity of ECM fungi was not limited in ultramafic soils.

Hrynkieiuicz et al. (2008) studied the structure of the ECM fungal community associated with *Salix caprea* in former silver-mining sites in Germany after 33 years of revegetation. Fungal diversity was represented by four families: Thelephoraceae, Cortinariaceae, Tricholomataceae and Tuberaceae, with Thelephoraceae the most frequent.

15.3 Heavy Metal Tolerance of Mycorrhizal Fungi

Several studies have indicated that isolates of AM fungi from heavy metal-polluted soils are more tolerant to metals than are those isolated from other soils (Leyval et al. 1995; Diaz et al. 1996; Gonzalez-Chavez et al. 2002; Tullio et al. 2003). The same conclusion has been reported for AM fungal isolates from ultramafic soils (Amir et al. 2008). This latter study showed that AM fungi isolated from roots of Ni-hyperaccumulating plants were more tolerant to Ni than were those isolated from ultramafic soils under non-hyperaccumulating plants, with these latter isolates being more tolerant than those from non-ultramafic soils. The same authors also found that AM fungal tolerance to Ni can be induced by the presence of high concentrations of this metal in the substrate where the symbiont has been grown.

The maximum metal concentrations tolerated by AM fungi vary greatly according to the type of metal, the type of substrate used and the type of propagule tested (Tullio et al. 2003; Khade and Adholeya 2007; Amir et al. 2008; Wu et al. 2009, 2010). For example, the spores of two *Glomus* spp. isolates were able to germinate in sand with up to 50 μg g^{-1} of Ni, whereas the less tolerant isolate did not germinate at 15 μg g^{-1} Ni (Amir et al. 2008). Wu et al. (2009, 2010) reported that spore germination of *G. mosseae* isolate from heavy metal-contaminated soils tolerated up to 5 μg g^{-1} Zn and 15.5 μg g^{-1} Pb. Considering the heterogeneity of heavy metal concentrations at the scale of soil aggregates and microsites in metal-rich soils, the tolerance to heavy metals by AM fungi is generally sufficient to colonize plant roots. However, colonization can be reduced by high concentrations of heavy metals in soil (Lingua et al. 2008; Amir et al. 2007; Gamalero et al. 2009).

The effects of heavy metals on ECM fungi have been widely reviewed (Leyval et al. 1997; Hartley et al. 1997; Jentschke and Goldbold 2000; Meharg 2003). Investigations at ECM fungal species and community levels have revealed wide

inter- and intraspecific variation in sensitivity to metals (Hartley et al. 1997). Fungi belonging to genera *Amanita*, *Cenococum*, *Laccaria*, *Lactarius*, *Paxillus*, *Pisolithus*, *Scleroderma*, *Suillus* and *Thelephora* have been shown to tolerate metals such as Al, Cd, Cu, Ni, Pb and Zn. Ray et al. (2005) showed that fungi belonging to genus *Hysterangium* were able to tolerate Al, Cd, Cr and Ni. In vitro solid agar or liquid medium metal tolerance tests allowed determination of specific EC_{50} (effective concentration of metal which reduces growth by 50 %) or IC_{50} (concentration that inhibits rates of growth by 50 %) varying in a range from μM up to mM (Hartley et al. 1997; Blaudez et al. 2000; Ray et al. 2005).

Several studies on ultramafic soils have highlighted ECM fungal community tolerance to heavy metals. In such soils, ECM fungal communities presented a high biodiversity and an adaptive tolerance to heavy metals, especially Ni (Perrier et al. 2006; Gonçalves et al. 2007, 2009; Urban et al. 2008; Jourand et al. 2010a, b). Majorel et al. (2012) on *Pisolithus albus* found five genes acting as markers of Ni-tolerance.

15.4 Role of Mycorrhizal Fungi on the Alleviation of Heavy Metal Toxicity of Plants

The role of mycorrhizal fungi in heavy metal-rich soils was first suggested by Gildon and Tinker (1981) and has been investigated in the two last decades with two objectives and approaches. One group of studies focused on the phytoextraction of heavy metals and the phytoremediation of polluted soils. These studies included extraction of metals from soil by heavy metal-accumulating plants (Khade and Adholeya 2007; Hildebrandt et al. 2007; Gamalero et al. 2009). Other studies investigated the ecological restoration of degraded areas (Leung et al. 2006; Ma et al. 2006; Chen et al. 2005; Amir et al. 2013). The conclusions of these studies are complex and vary in relation to the type of approach, the experimental conditions, the group of the plants studied, the characteristics of the soils used and the type of metal concerned and its concentration (Weissenhorn et al. 1995; Hildebrandt et al. 2007; Gamalero et al. 2009).

It is now clear that AM and ECM generally induce adaptation of plants to high metal concentrations in soil, and this occurs despite the complexity of the results obtained and difficulties in comparing different studies. Indeed, to demonstrate that mycorrhizas alleviate metal toxicity of plants or improve plant tolerance, it must be shown that, in comparison with a control without heavy metals, the negative effects of heavy metals on plant growth and plant health are less important in the presence of mycorrhizal fungi than in the non-mycorrhizal treatment. However, some experiments did not use a control without heavy metals and only evaluate the effects of the symbionts on plant growth and nutrition in metal-rich soils.

Experiments cover a large number of plant species and families, different metals (Cd, Cu, Zn, Pb, Cr, Mn, Ni, Al and As) and different experimental conditions. As

there were no clear conclusions specific to plant taxa, type of metal or particular conditions and considering the large number of publications, conclusions are synthesized here without detailed reference to these variables. Out of 44 publications, 43 % reported a better tolerance of the plant to heavy metals in the presence of AM fungi (Hildebrandt et al. 1999; Zhang et al. 2005; Lin et al. 2007; Lingua et al. 2008, Andrade et al. 2009; Cavagnaro et al. 2010; Orlowska et al. 2011b; Aloui et al. 2012; Amir et al. 2013, etc.), and 52 % showed an increase in growth and/or an improvement in mineral nutrition under the same conditions (Sadeque et al. 2006; Janouvska et al. 2007; Redon et al. 2008; Cabala et al. 2009; Dubkova et al. 2012; Neagoe et al. 2013, etc.). Overall, 86 % of studies showed a better adaptation of mycorrhizal plants to heavy metal-rich soils, and only 12 % did not report any positive effects of AM fungi on plant growth in the presence of heavy metals (Carvalho et al. 2006; Marques et al. 2006; Sudova et al. 2008, etc.). Boulet and Lambers (2005) showed that AM fungal inoculum from ultramafic soil did not improve the growth of *Hakea verrucosa*, but enhanced its mineral nutrition in an ultramafic soil.

The effects of ECM on heavy metal toxicity of plants are generally clear, although there are fewer publications than those concerning AM fungi. ECM protection against Cu, Cd, Zn and Ni toxicity of *Pinus sylvestris* has been demonstrated (Ahonen-Jonnarth and Finlay 2001; Adriaensen et al. 2006; Colpaert et al. 2011). *Eucalyptus globulus* plants inoculated with *P. albus* from ultramafic soils were clearly tolerant to Ni (Jourand et al. 2010a). Improvement of plant biomass and mineral nutrition by ECM in the presence of toxic heavy metal concentrations has been reported for Ni and Cr (Aggangan et al. 1998), Mn (Walker et al. 2004) and Zn (Adriaensen et al. 2006). Only one study (Dučić et al. 2008) did not show positive effects of ECM on plant growth when exposed to heavy metal stress (Mn), but the tolerance of the ECM fungi isolate to Mn was not tested.

To estimate the effects of ECM fungi on plant tolerance, Jentschke and Goldbold (2000) suggested considering the sensitivity of seedling growth and plant mineral nutrition, especially N and Ca/Mg uptake, which could be influenced by heavy metal toxicity. These effects depend on fungal species and, for the same species, on fungal isolates that can show different levels of tolerance to the metal. There is evidence that there is a relationship between fungal ecotype and amelioration of plant host heavy metal tolerance (Adriaensen et al. 2003, 2006; Jourand et al. 2010a).

15.4.1 Influence of Mycorrhizas on Heavy Metal Absorption by Plants

It is important to stress that the alleviation of plant heavy metal toxicity by AM fungi, or the improvement of plant growth in the presence of heavy metals by these symbionts, is not necessarily induced by a reduction of heavy metal absorption by

the plant. About half the studies on this topic showed an increase in heavy metal absorption by mycorrhizal plants in comparison to non-mycorrhizal controls. Some studies reported an increase in heavy metal concentrations or heavy metal accumulation in roots and shoots (Marques et al. 2006; Deram et al. 2008; Tseng et al. 2009; Redon et al. 2009; Andrade et al. 2009). Other studies showed an increase in heavy metal concentrations or heavy metal accumulation only in the roots (Joner and Leyval 2001; Rufyikiri et al. 2004; Carvalho et al. 2006; Honglin et al. 2006; Redon et al. 2008; Li et al. 2009; Bissonnette et al. 2010, Orlowska et al. 2012). About a third of the studies reported a clear reduction of heavy metal concentrations in the whole plant (Vivas et al. 2005; Sadeque et al. 2006; Andrade et al. 2010; Amir et al. 2013), and about 20 % showed a variation (increase or reduction) in heavy metal concentrations in the plant, depending on heavy metal concentrations in soil (Diaz et al. 1996; Audet and Charest 2007; Janouskova et al. 2007; Wu et al. 2009, 2010), AM fungal species or isolates (Zhang et al. 2005; Janouskova et al. 2007; Redon et al. 2009) and the metal considered (Guo et al. 1996; Zhang et al. 2005; Dubkova et al. 2012). These complex relationships between the heavy metal accumulation in plant organs and the positive effects of mycorrhizas on plant tolerance to these metals can be easily understood when considering the diversity of physiological adaptations of plants to high heavy metal concentrations in soils. These include metal excluders, metal indicators and different types of metal accumulators (Whiting et al. 2004; Kazakou et al. 2008; Fernando et al. 2008), the diversity of heavy metal neutralization mechanisms (Khan et al. 2000; Meharg 2003; Hildebrandt et al. 2007;) and, more generally, the diversity of factors affecting these processes in the rhizosphere (Hinsinger et al. 2009; Lambers et al. 2009). Thus, high concentrations of heavy metals in roots and shoots do not indicate a high level of toxicity, as these metals are generally stored in inactive forms.

A few studies have dealt with the influence of ECM on heavy metal absorption by plants. Needles of *Picea abies* associated with *Laccaria laccata* showed a Cd content 2.5 times lower than for non-mycorrhizal plants (Galli et al. 1993). *Suillus bovinus* reduced Zn content in plant tissues of *P. sylvestris* (Adriaensen et al. 2006). Bojarczuk and Kieliszewska-Rokicka (2010) studied the effects of various ECM on Cu and Pb accumulation in leaves of *Betula pendula* grown in heavy metal-contaminated soil. Heavy metal concentrations in leaves varied inversely with the abundance of ECM fungi. Walker et al. (2004) reported lower concentrations of Mn in *Betula lenta* seedlings when inoculated with *Pisolithus tinctorius* on coal mine spoil and a Ni-tolerant isolate of *P. albus* from New Caledonian ultramafic soil significantly reduced Ni transfer into plant tissues of *E. globulus* (Jourand et al. 2010a). Baum et al. (2006) reported both a decrease and increase in different heavy metal contents in stems and roots of *Salix* plants, depending on heavy metal concentrations, type of heavy metal and fungal isolate.

15.4.2 Combined Effects of Mycorrhizal Fungi and Other Factors on the Alleviation of Heavy Metal Toxicity and Metal Accumulation in Plants

Most studies aimed at improving phytoextraction/phytoremediation have shown combined effects of AM fungi and other treatments. The combination of *G. mosseae* and a Plant Growth Promoting (PGP) bacterium (*Brevibacillus brevis*), both isolated from Cd-contaminated soil, increased AM colonization, plant growth and plant Cd tolerance (Vivas et al. 2005). These effects were related to an increase in P and K and a decrease in Cd, Cr, Mn, Cu, Mo, Fe and Ni in plant tissues. The co-inoculation of Eucalyptus plants with *Glomus deserticola* and *Trichoderma koningii* was more effective for Cd uptake and plant growth than was each treatment considered separately (Arriagada et al. 2007). Ma et al. (2006) tested the combined effects of AM fungi and earthworms on *Leucaena leucocephala* in topsoil amended mine tailings and showed additional positive effects on plant growth, plant nutrition and a reduction in Pb and Zn mobility. The combined effects of AM fungi and organic amendments were also tested. Inoculation of *Trifolium repens* with AM fungi in heavy metal-contaminated soil amended with *Aspergillus niger*-treated sugar beet stimulated bacterial diversity, plant growth and the phytoextraction process (Azcon et al. 2009). Medina et al. (2010) showed that the combination of AM fungi and *A. niger*-treated dry olive cake increased *T. repens* growth and its tolerance to Cd. The association of ECM fungi and bacteria can also improve the adaptation of pine to metal-polluted soils (Krupa and Kozdroj 2007), resulting in a higher accumulation of the metals, especially Zn, in the roots and a reduction of metal translocation to the shoots.

15.5 Mechanisms Involved in the Role of Mycorrhizas in Alleviation of Heavy Metal Toxicity of Plants

During the two last decades, a relatively large number of studies have focused on the mechanisms which can explain the influence of mycorrhizas on the alleviation of heavy metal toxicity of plants. Several processes have been highlighted. Direct mechanisms concern extracellular heavy metal inactivation, heavy metal binding in fungal wall, enhanced efflux of heavy metals through cellular membranes, intracellular inactivation and adaptive response to oxidative stress. Indirect mechanisms act through the improvement of mineral nutrition, which enhances the growth and influences plant tolerance to environmental stress. The plant and associated mycorrhizal fungi have different strategies to cope with heavy metal toxicity; some are common and act in together; others are different and operate independently (Meharg 2003). This text focuses mainly on fungal strategies.

15.5.1 Extracellular Heavy Metal Inactivation Mechanisms

Different mechanisms of heavy metal exclusion by mycorrhizal fungi have been suggested, among them extracellular chelation, cell wall binding and heavy metal accumulation in extraradical mycelium (Colpaert et al. 2011). Mycorrhizas can inactivate heavy metals through the exudation of complexing agents into the soil solution. According to Meharg (2003), organic acid exudation has a clear role in mycorrhizal adaptation to metal-contaminated sites. Citric, malic and oxalic acids are known to be produced by mycorrhizal fungi (Ahonen-Jonnarth et al. 2000; Meharg 2003); they can mobilize or immobilize metals by complexation, depending on various factors, especially rhizosphere pH (Gimmler et al. 2001; Hinsinger et al. 2009). Phenolic compounds produced by ECM are also involved in metal immobilization in soil (Schützendübel and Polle 2002). Machuka et al. (2007) highlighted different metal-chelating compounds in in vitro culture of ECM fungi collected from pine plantations (species of *Scleroderma*, *Suillus* and *Rhizopogon*). Oxalic, citric and succinic acids but also hydroxamate- and catecholate-type compounds were found in the liquid medium. Cabala et al. (2009) reported the presence in the rhizosphere of different AM and ECM mycorrhizal plants of metal-bearing aggregates formed during symbiotic action between mycorrhizas and bacteria. These structures enhanced the binding of Zn, Pb and Mn in the rhizosphere. More recently, different studies showed the role of glomalin, a very abundant AM fungal glycoprotein released into the soil where it participates in soil aggregation. Glomalin seems to be involved in heavy metal inactivation in soil (Ferrol et al. 2009; Gamalero et al. 2009). Glomalin extracted from polluted soil or from hyphae irreversibly sequesters metals such as Cu, Cd, Zn and As (Gonzalez-Chavez et al. 2002). Cornejo et al. (2008) showed that a glomalin-related soil protein was more abundant in polluted soils with high concentrations of Cu and Zn. Up to 27 % of the total Cu was bound by this protein, and in a highly polluted soil, with a low pH, up to 90 % of the soil organic carbon was represented by the glomalin-related protein. Similar results were obtained by Vodnik et al. (2008) for the sequestration of Pb and Zn.

15.5.2 Heavy Metal Binding in Fungal Wall

Some of the metals inactivated in mycorrhizal plants are retained by fungal walls. Joner et al. (2000) exposed extraradical mycelium of different *Glomus* spp. isolates to high concentrations of Cd and Zn and measured their capacities to bind these metals. The most tolerant isolate adsorbed more metals than the others. The fungal wall was responsive for 50 % of the metal retained. Orlowska et al. (2008), analysing the elemental distribution in mycorrhizal plants of the Ni-hyperaccumulator *Berkheya coddii*, also reported a high binding capacity of the extraradical mycelium for Zn, Cu and Ni. Using EDXS analyses, with

monoxenic cultures of *G. intraradices*, Gonzalez-Guerrero et al. (2008) showed that Cu, Zn and Cd at toxic concentrations were partly localized in the fungal cell wall. Marques et al. (2007) and Zhang et al. (2009) reported that Zn and Cu were mainly deposited in the cell wall of the root cortex of the mycorrhizal plants, including the AM fungal wall. Several cell wall-binding molecules have been reported, such as glucan, chitin and galactosamine polymers, minor peptides and proteins, all presenting potential binding sites as free carboxyl, amino, hydroxyl, phosphate and mercapto groups (Bellion et al. 2006). Glomalin is also partly located at the AM fungal wall (Purin and Rillig 2008). In ECM, Cd and Zn are predominantly bound in cell wall of mantle hyphae, Hartig net hyphae and cortical cells (Meharg 2003).

15.5.3 Intracellular Heavy Metal Inactivation

After heavy metals have passed through the fungal wall, other avoidance mechanisms, which may be activated, include alteration of heavy metal influx transporter processes and an increase in heavy metal efflux through the cell membrane (Meharg 2003; Ouziad et al. 2005). Many metal protein transporters or metal permeases have been highlighted (Hildebrandt et al. 2007), but their role in cell detoxification is not well defined.

Intracellular compartmentalization strategies to inactivate the absorbed part of heavy metals are relatively well documented. The toxic elements are translocated into fungal vacuoles were they are stored away from the cytosol. According to Gonzalez-Guerrero et al. (2008), the highest metal content is localized in the spores. Ferrol et al. (2009) observed AM fungal mycelium developed in a Cu-enriched medium and showed that when spores appeared in clusters, only one or a few of them contained a high concentration of Cu, thus protecting the rest of the fungal colony. Vesicles of the intraradical mycelium may also serve for the storage of heavy metals (Orlowska et al. 2008).

What are the molecular mechanisms of this compartmentalization? The toxic elements must be bound to other molecules inside the cell to inactive them. Different metal chelators may be involved in this process: organic acids, amino acids, glutathione, phytochelatins (thiol-rich peptides) and metallothioneins (sulphur-rich proteins). Three glomeromycotan metallothioneins have been identified in *Gigaspora* and *Glomus* species (Stommel et al. 2001; Lanfranco et al. 2002; Gonzalez-Guerrero et al. 2007). Metallothioneins have also been found in ECM fungi (Courbot et al. 2004; Bellion et al. 2006, 2007). By contrast, to our knowledge, no specific metal-binding phytochelatin has been clearly identified in AM and ECM fungi. Hegedüs et al. (2007) highlighted the role of glutathione in heavy metal tolerance of ECM fungal isolates of *Paxillus involutus*, from HM-polluted soils. In addition, fungal colonization of roots may directly influence the expression of several plant genes coding for proteins involved in detoxification and plant tolerance to heavy metals, such as heavy metal transporter genes and plant

metallothioneins (Repetto et al. 2003; Rivera-Becerril et al. 2005). More details on this topic are given by Hildebrandt et al. (2007).

Sequencing studies of transcript genomes of ECM fungi in symbiosis with their host plant have been reviewed by Arlt et al. (2009). Experiments have involved specific ECM/plant host models such as *Pisolithus* with *Eucalyptus* or *Quercus*, *Laccaria* with *Pinus*, *Tuber* with *Tilia* and *Paxillus* with *Betula*. However, the authors insist that the screening of patterns of RNA and consequently expressed sequence tags (EST) or identified regulated genes were all non-targeted and not based upon specific hypothesis. It now seems evident that such general transcriptomic approaches and their results need to be connected with the role of ECM fungi in improving plant host tolerance to heavy metals. This could allow the identification of fungal symbiotic genes involved in the metal tolerance mechanisms and expression level variations of these genes, in relation to the presence of the metal.

15.5.4 Response to Oxidative Stress

When toxic metal cations are not inactivated by the described mechanisms, they are generally redox active and can create oxidative stress. They induce high reactive radical hydroxyl and superoxide groups and then the alteration of cellular reactions. Adaptation mechanisms to this stress are not sufficiently understood in mycorrhizal plants, but according to Ferrol et al. (2009), they must include nonenzymatic antioxidant systems such as glutathione and vitamins C, E and B6 and enzymatic systems such as catalases, superoxide dismutases (SOD), thioredoxins and glutaredoxins. A reduction of SOD in plant shoots was found to be related to plant inoculation with AM fungi (Neagoe et al. 2013) and was explained as an effect of a lower oxidative stress in AM fungal inoculated plants. Jacob et al. (2001) highlighted the capacity of ECM fungi to synthesize SOD in reaction to cadmium toxicity. Vallino et al. (2009), studying the ericoid mycorrhizal fungus *Oidiodendron maius*, characterized a new SOD found both in the cell extract and in the growth medium of the fungal culture. They suggested that the presence of this enzyme in the extracellular environment may also protect the plant partner. A few genes involved in oxidative stress homeostasis have been identified in AM fungi: three SOD, ten genes encoding glutathione *S*-transferases, a glutaredoxin, a gene encoding a protein involved in vitamin B6 biosynthesis and a metallothionein (Ferrol et al. 2009). Aloui et al. (2012) quantified a group of isoflavonoids accumulated in the roots of *Medicago truncatula* in reaction to Cd toxicity and reported a strong reduction of three of these compounds in AM fungal inoculated plants, reinforcing the hypothesis that AM colonization buffered the effects of heavy metals in plant roots. In addition, a strong decrease of the transcripts of chalcone reductase, an enzyme involved in isoflavonoid production, was noticed.

15.6 Conclusions

Mycorrhizal fungi are present in all metal-rich soils, and their diversity allows them to adapt to the various concentrations of the different heavy metals present in these soils. Thus, it is now clear that mycorrhizas play an important role in plant tolerance to heavy metals, and this has been highlighted in heavy metal-polluted soils, in ultramafic soils and in mining degraded areas. However, the effects of these symbionts in the alleviation of heavy metal toxicity of plants and on heavy metal accumulation in plant organs are complex and vary with the diversity of physiological and molecular mechanisms involved in these processes and in relation to the diversity of the factors affecting the plant/fungi symbiosis.

This relatively new knowledge has important practical consequences, especially in the fields of phytoextraction, phytoremediation and ecological restoration of mining degraded areas (Khan et al. 2000; Khade and Adholeya 2007; Hildebrandt et al. 2007; Amir and Ducousso 2010). Different concepts and strategies have been proposed for these objectives (Meharg 2003; Audet and Charest 2007; Lebeau et al. 2008; Marques et al. 2008; Amir and Ducousso 2010). The complexity of the processes in plant/soil/microbial systems and the induced variations have to be taken into account. In particular the following factors have to be considered:

- Variations related to fungal isolates: Screening among a collection of fungi for their tolerance to the metals studied and their effects on plant is one of the conditions of the success (Meharg 2003).
- Variation related to plant factors: The plant species and even the plant clone (Sudova et al. 2008) must be screened depending on the objectives (phytoextraction, phytoremediation or ecological restoration). For phytoextraction, plants that accumulate metals are suitable, but generally produce a lower biomass, and a compromise has to be found between accumulation ability and growth rate (Audet and Charest 2007). For ecological restoration, maximal plant diversity is the best (L'Huillier et al. 2010), and mycorrhizal inoculum must be adapted to the plant species.
- Soil characteristics: Experiments with soil (or the substrate) to depollute or to revegetate are necessary, and the possibility of improving the efficiency of the method by amendments or other practices needs to be performed (Ma et al. 2006; Marques et al. 2008; Azcon et al. 2009).

Further studies should focus on the genetic determinism of mycorrhizal effects on plant tolerance to heavy metals and the control of the multivariate aspects of the metal/soil/plant/mycorrhizal fungus system interactions.

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Chapter 16
Arsenic Uptake and Phytoremediation Potential by Arbuscular Mycorrhizal Fungi

Xinhua He and Erik Lilleskov

16.1 Introduction

Arsenic (As) contamination of soils and water is a global problem because of its impacts on ecosystems and human health. Various approaches have been attempted for As remediation, with limited success. Arbuscular mycorrhizal (AM) fungi play vital roles in the uptake of water and essential nutrients, especially phosphorus (P), and hence enhance plant performance and productivity (Smith and Read 2008). As uptake and tolerance to As toxicity in plants are also enhanced by AM fungi (Zhao et al. 2009; Smith et al. 2010; Gonzalez-Chavez et al. 2011). The use of AM fungi has thus been proposed as a potential contributor to enhance plant As uptake and accumulation and to develop plant-based As remediation. Here, we review the problem of As toxicity in terrestrial ecosystems and human health, examine the recent progress in understanding the roles of AM fungi in plant As tolerance and accumulation, and explore the promise and challenges of using AM fungi as phytoremediation approaches to tackle this environmental problem.

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16.2 Arsenic in the Environment and Its Toxicity

Arsenic is an odorless and tasteless semimetal element that occurs naturally in rocks (~3 mg kg^{-1} Earth crust) (Mandal and Suzuki 2002). It can be released into air, water, and soils through natural activities (volcanic action, rock and soil erosion) or agricultural and industrial practices (fertilizers, herbicides, pesticides, mining, semiconductors) (Mandal and Suzuki 2002; Adriano 2001). As accumulation, migration, and toxicity are related to its chemical speciation. Inorganic As species are the more reduced arsenite [H_3AsO_3, As(III)] and more oxidized arsenate [$HAsO_4^{2-}$, As(V)]. Generated from inorganic As via biomethylation, organic As species include mono- and di-methylarsenite [MMA(III) and DMA(III)] and mono- and di-methylarsenate [MMA(V) and DMA(V)] (Cullen and Reimer 1989). Arsenic compounds are the most notorious toxins in human history and linked to many forms of cancer, diarrhea, nausea, stomach pain, vomiting, numbness, partial paralysis, and blindness (Nriagu 2002). The toxicity order of As is as follows: MMA(III) > DMA(III) > As(III) > As(V) > MMA(V) > DMA(V) (Ali et al. 2009; Kim et al. 2009; Ralph 2008).

Arsenic exposure occurs primarily through drinking water and food. The standard for drinking water to prevent chronic effects is ≤0.01 mg As L^{-1} (0.01 ppm) (http://www.who.int/mediacentre/factsheets/fs210/en/). More than 150 million people worldwide get exposed to 0.01–0.05 mg As L^{-1} drinking water, including countries in Southeast Asia, North and South America, and Europe (Bhattacharjee 2007; Kim et al. 2009; Smith et al. 2000). At present, the World Health Organization and most countries have not established legal As limits in food, though the US FDA recommends a "tolerable daily intake" of 0.13 mg As in food (Stone 2008).

16.3 Arsenic Biogeochemistry in Soil and Its Uptake in Plants

Arsenic and P belong to the same V_A chemical group, and they thus display similar chemical properties and geochemical behaviors (Cullen and Reimer 1989; Adriano 2001). However, whereas P is an essential plant nutrient, As can be toxic to crops as well as to primary and secondary plant consumers (Stone 2008; Kim et al. 2009). Various forms of As exist in soils depending on pH and redox status. As(III) dominates in anaerobic substrates, while As(V) dominates in aerobic soils (see Wenzel et al. 2002; Raab et al. 2007; Williams et al. 2007). Typical concentrations of As(III) are 0.01–3.0 μM in contaminated soils, while As(V) concentrations are >2.3 μM in contaminated or <53 nM in uncontaminated soils (Wenzel et al. 2002). Plant roots primarily take up inorganic As(III) and As(V) and are also capable of taking up organic MMA(III) or DMA(III). The toxic limits to most plants are 5–20 mg As kg^{-1} soil (Mendez and Maier 2008), and the common symptoms of As toxicity include reduced root growth, leaf chlorosis, increased sterility, and yield

reduction (Meharg and Hartley-Whitaker 2002; Raab et al. 2007; Smith et al. 2010 and references therein).

The known plant uptake pathways for reduced and oxidized inorganic As are via silicon (Si) and phosphate (PO_4^-) transporters (Pht), respectively. As(III) enters into rice (*Oryza sativa*) roots passively by sharing a Si transport pathway through nodulin 26-like intrinsic proteins (NIPs) (Maurel et al. 2008; Ali et al. 2009; Zhao et al. 2009 and references therein). As(III) uptake was inhibited by glycerol and antimonite (Sb), but not by P (Abedin et al. 2002a, b; Meharg and Jardine 2003). In contrast, As(V) is taken up actively through PO_4^- transporters (e.g., Pht1;1 and Pht1;4) (Shin et al. 2004), which have a lower affinity for As(V) than P (Meharg and Macnair 1990; Meharg and Hartley-Whitaker 2002). The rapid reduction of As (V) to As(III) was demonstrated in tomato (*Lycopersicon esculentum*) and rice roots (Xu et al. 2007).

The uptake competition between As(V) and P was exhibited by excised roots of barley (*Hordeum vulgare*), velvet grass (*Holcus lanatus*), or mouse-ear cress (*Arabidopsis thaliana*) in solution culture (Meharg and Macnair 1990; Meharg and Hartley-Whitaker 2002; Zhao et al. 2009), but not by medic (*Medicago truncatula*) or barley in soil/sand (2:8) media (Christophersen et al. 2009a). The influx of As(III) was generally comparable to that of As(V) under low ($<$50 μM, high-affinity transporter range) but considerably higher under high ($>$100 μM, low-affinity transporter range) concentrations (Meharg and Macnair 1990; Meharg and Jardine 2003).

16.4 Arsenic Transport and Hyperaccumulation in Plants: The Basis for Phytoremediation

Arsenic is primarily accumulated in roots of most plants because its low mobility restricts its root-to-shoot translocation, except in As hyperaccumulators (Raab et al. 2007). Brooks et al. (1977) defined "hyperaccumulators" as plants that could tolerate and accumulate $>$1 mg metal g^{-1} (0.1 %) dry mass. An As hyperaccumulator has greater antioxidant capacity and lower reactive oxygen concentration and thus greater As tolerance than a non-As hyperaccumulator (Srivastava et al. 2005; Singh et al. 2006). After uptake, As(V) is rapidly reduced by As(V) reductases in roots to As(III), which can then be detoxified by complexation with glutathione (GSH) or phytochelatins (PCs) (Raab et al. 2005; Zhao et al. 2009; Zhu and Rosen 2009). As(III) or the complexed As(III) is transported across tonoplasts and sequestered in vacuoles, loaded into xylem, and translocated to and accumulated in shoots (Xu et al. 2007; Su et al. 2008).

High As tolerance and accumulation capacity constitute the basis of exploring plant hyperaccumulators for As phytoremediation. Candidate plants for phytoremediation must tolerate and accumulate high levels of As in their tissues and possess high biomass production potential. At present, several fern species and

Table 16.1 Potential As hyperaccumulator plant species (grouped according to De Koe 1994; Bech et al. 1997; Tu et al. 2002; Baldwin and Butcher 2007; Tripathi et al. 2007; Zhao et al. 2009)

Plant	Species
Ferns	*Pityrogramma calomelanos* (L.) Link (silverback fern), *P. austroamericana* Domin (leatherleaf goldback fern), *Pteris aspericaulis* (tricolor fern), *P. biaurita* (thinleaf brake fern), *P. cretica* var. *albolineata* (table fern), *P. cretica* var. *nervosa* (Cretan brake fern), *P. cretica* cv Mayii (moonlight fern), *P. fauriei* (Faurie's brake fern), *P. longifolia* (longleaf brake fern), *P. multifida* Poir. and *P. multifida* f. *serrulata* (spider brake fern), *P. oshimensis* Hieron. (an Asian fern), *P. quadriaurita* (striped brake fern), *P. ryukyuensis* Tagawa. (an Asian fern), *P. umbrosa* (Australian jungle brake fern), *P. vittata* (Chinese brake or ladder fern)
Grasses and forbs	*Agrostis castellana* (bentgrass or dryland browntop), *A. delicatula* (bentgrass), *Bidens cynapiifolia* (West Indian beggarticks)

a number of grasses and forbs have been identified as As hyperaccumulators (De Koe 1994; Bech et al. 1997; Tu et al. 2002; Baldwin and Butcher 2007; Tripathi et al. 2007; Zhao et al. 2009; see Table 16.1). As(III) generally accounts for 60–90 % of the total As in the shoots of As hyperaccumulator *Pteris* species, and the ratio of shoot-to-root As accumulation (translocation factor (TF)) ranges between 5 and 25 in hyperaccumulators (Tu and Ma 2002; Tu et al. 2002; Zhao et al. 2009; Leung et al. 2010a, b, 2013). This high As accumulation in plants can lead to demonstrable reductions of soil As content via phytoremediation programs (Xie et al. 2009). For instance, *Pteris vittata* (Chinese brake fern) was capable of reducing As from 190 to 140 mg kg^{-1} soil after 2 years growing in an As-contaminated field (Kertulis-Tartar et al. 2006) or from 130 to 10 μg L^{-1} after 4–6 weeks growing in an As-contaminated groundwater (Natarajan et al. 2008).

16.5 Mycorrhizal Symbiosis

The potential for mycorrhizal symbiosis to improve As tolerance and phytoremediation has been only partially explored. About 90 % of higher plants associate with mycorrhizal fungi (Wang and Qiu 2006; Smith and Read 2008; Brundrett 2009). There are about 200 AM fungal species, and all of them belong to the phylum Glomeromycota (Walker et al. 2007a, b; Palenzuela et al. 2008). AM fungi are asexual obligate symbionts, and most of them are widespread and not host specific. In AM associations, fungal hyphae penetrate inside the walls of root cortical cells to form either "little-tree-shaped" structures, called arbuscules, or hyphal coils, both of which serve as the main nutrient exchange sites between fungus and plant.

While aboveground plant structures are easily observed, mycorrhizal fungi and their activities are challenging to characterize. A single gram of soil may contain up to 50 m of AM hyphae, which can extend >9 cm beyond the roots and expand

extensively throughout the soil matrix (Nasim 2005). The small 2–10 μm diameter of mycorrhizal fungal hyphae can efficiently explore soil volume and microsites inaccessible to plant roots. One important function of mycorrhizal fungi is to enhance host plant nutrient acquisition by increasing access to inorganic N and P by hyphae extending beyond depletion zones caused by direct uptake by roots and by access to organic N and P via their extracellular protease and phosphatase activity (Smith and Read 2008).

16.5.1 Roles of Mycorrhizal Fungi in Arsenic Tolerance

There are several hypothesized mechanisms by which mycorrhizal fungi could affect host plant As tolerance (Meharg and Hartley-Whitaker 2002; Zhao et al. 2009; Smith et al. 2010; Gonzalez-Chavez et al. 2011). First, it has been hypothesized that AM fungi increase plant P nutrition and growth and thus alleviate toxic effects of As on plants due to the dilution of As uptake because P shares chemical properties with As (Adriano 2001). Second, As-tolerant fungi could provide added functional benefits over non-tolerant fungi. Numerous AM studies have addressed these hypotheses, with most studies focused on P nutrition effects.

Hypothesis 1: *P Nutrition Effects*. Consistent with the hypothesis that mycorrhizally mediated improved P nutrition enhances As tolerance, plant growth and P nutrition were simultaneously improved under As stress conditions by AM in most studies. For instance, As uptake, As tolerance, and P nutrition in both shoots and roots of maize (*Zea mays*) (Xia et al. 2007; Bai et al. 2008; Wang et al. 2008; Yu et al. 2009, 2010), lettuce (*Lactuca sativa*; Cozzolino et al. 2010), and *Eucalyptus globulus* (Arriagada et al. 2009) were concurrently enhanced by AM fungi. In addition, the activity of peroxidase, superoxide dismutase, and As(V) reductase was suppressed by *Glomus mosseae* (now *Funneliformis mosseae*), indicating that AM colonization could inhibit the reduction of As(V) to As(III) and As toxicity to plants could hence be alleviated (Yu et al. 2009). By contrast, the phytotoxicity of arsenate (AsV, $Na_2HAsO_4{\cdot}7H_2O$) led to an increase in superoxide dismutase, catalase, and peroxidase activities in a 1-month-old pea (*Pisum sativum*) (Garg and Singla 2012). Similarly, P accumulation was significantly higher under all As levels of 10, 50, 100, and 200 mg kg^{-1} soil in a 2-month-old mycorrhizal medic inoculated with *G. mosseae* BEG167 (Xu et al. 2008). Both As and P uptake were higher in 3-month-old *G. mosseae* BEG167-inoculated tomatoes growing in 25, 50, and 75 As kg^{-1} spiked soil, but similar in 150 mg As kg^{-1} spiked soil (Liu et al. 2005a). A hydroponic study with a 1-month-old *Pennisetum clandestinum* Hochst (kikuyu grass) showed that As(V) uptake was competitively inhibited by P uptake because of a higher selectivity of membrane transporters with respect to P rather than As(V) (Panuccio et al. 2012). Smith et al. (2010) and Christophersen et al. (2012) recently detailed mechanisms of direct root and/or mycorrhizal Pi/As (V) uptake pathways, summarizing the physiological basis for the observed P-mediated effects on As accumulation of both AM-responsive and

AM-nonresponsive plants. Thus, there is relatively strong support for this hypothesis.

Hypothesis 2: *Fungal As Tolerance*. Some pure culture studies suggest that the extent of As(V) toxicity to mycorrhizal fungi could vary among fungal taxa, increasing the potential for the selection of appropriate fungi for remediation efforts. There is some evidence that AM fungal populations can develop tolerance to As and that this tolerance results in improved host performance. For instance, fungal isolates of *G. mosseae* and *G. caledonium* associated with velvet grass roots from the As-contaminated site were more As(V) tolerant than those from the non-As-contaminated site (Gonzalez-Chavez et al. 2002). Root high-affinity As(V)/PO_4^- transportation was suppressed by both tolerant and non-tolerant *G. mosseae* in both tolerant and non-tolerant velvet grasses. As(V) uptake in the tolerant velvet grass growing in the As-contaminated site was reduced by inoculating with the tolerant AM isolates. The authors concluded that AM fungi had evolved As (V) tolerance and conferred enhanced As tolerance on velvet grass (Gonzalez-Chavez et al. 2002).

In summary, mycorrhizal fungi have been consistently shown to confer As tolerance on their host plants. The possible mechanism of As tolerance in mycorrhizal plants might be one or a combination of the following. First, AM fungi enhance P nutrition and plant growth, resulting in a higher P/As ratio and a relative As dilution in tissues of mycorrhizal plants (Liu et al. 2005a, b; Ahmed et al. 2006; Chen et al. 2007; Ultra et al. 2007a, b). The corresponding reasons are the induction of HvPht1;8 (*H. vulgare* phosphate transporter) and downregulation of HvPht1;1 and HvPht1;2 (Christophersen et al. 2009b) and both the upregulated and downregulated expressions of up to 130 life proteins, particularly for some glycolytic enzymes including glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and enolase (Bona et al. 2010, 2011). This could provide "protective effects" against As uptake or stress because P shares chemical properties with As. Second, As-tolerant mycorrhizal fungi enhance As(III) exudation to the external media and reduce As(V) uptake at the As-contaminated habitats and thus confer enhanced As tolerance on AM plants (Gonzalez-Chavez et al. 2002), since the induction of GiPT (*Glomus intraradices* high-affinity phosphate transporter) expression correlates with As(V) uptake in the extra-radical mycelium of *G. intraradices* (Gonzalez-Chavez et al. 2011). In addition, under 2 μM As(III), both the Lsi1 and Lsi2 As(III) transporters were significantly decreased by 0.7- and 0.5-fold in mycorrhizal than in non-mycorrhizal 2.5 month-old rice seedlings, leading to a decrease of As(III) uptake per unit of root dry mass (Chen et al. 2012).

16.5.2 Roles of Mycorrhizal Fungi in Arsenic Uptake

The chemical similarity of P and As, combined with the mycorrhizal role in P nutrition, provides the likelihood that mycorrhizal fungi may enhance As uptake. Given that mycorrhizas generally enhance P uptake, it is possible that mycorrhizal

fungi will also increase the uptake of As in their plant hosts. However, depending on mycorrhizal specificity for P vs. As uptake (Li et al. 2011), a variety of outcomes are possible. For example, if mycorrhizal fungi have more specific P uptake mechanisms than their hosts, they may reduce the proportional uptake of As relative to P (Smith et al. 2010).

Arsenic Accumulations in Shoots of Herbaceous Plants. Consistent with an enhanced As uptake via mycorrhizal fungal symbiosis, AM fungi appear to increase As accumulation in their hosts. Compared to the non-AM seedlings, As accumulation (both concentration and content) was increased in shoots and roots of 2- or 3-month-old *G. mosseae*-inoculated maize seedlings growing in 75 and 150 mg As kg^{-1} soil/sand (3:1) media (Wang et al. 2008), in 100 mg As kg^{-1} soil (Yu et al. 2009), and even in 600 mg As kg^{-1} soil/sand (2:1) media (Xia et al. 2007). A mixed inoculum of indigenous AM isolates (*Glomus* spp. and *Acaulospora* spp.) from As-contaminated soils, not the nonindigenous *G. caledonium* 90036 from non-As-contaminated soils, increased As accumulation in shoots of a 2.5-month-old maize in 185 and 290 mg As kg^{-1} soil (Bai et al. 2008). Plant total As accumulations were significantly increased in a 3-month-old *G. mosseae*-inoculated white clover (*T. repens*) and ryegrass (*Lolium perenne*) growing in 600 mg As kg^{-1} soil/sand (1:1) media (Dong et al. 2008). Shoot As and toxicity symptoms were reduced in a 6-week-old *G. aggregatum*-inoculated sunflower (*Helianthus annuus*) growing in 620 mg As kg^{-1} contaminated soil (Ultra et al. 2007a, b). Arsenic accumulation was also significantly increased in a 2-month-old *G. mosseae* BEG167-inoculated medic growing in 200 mg As kg^{-1} soil but was similar between the non-mycorrhizal and mycorrhizal plants under 10, 50, or 100 mg As kg^{-1} soil (Xu et al. 2008). In addition, P accumulation and P/As ratio of both shoots and roots were always higher in all mycorrhizal plants than in their non-mycorrhizal counterparts in almost all these studies, suggesting that AM fungi may have more specific uptake of P relative to As when compared with non-mycorrhizal plants.

Higher As Accumulations in Roots than in Shoots of Herbaceous Plants. Although the increase in As accumulation in crop plants might be of concern from a food chain perspective, interestingly, As accumulation in roots, rather than in shoots, was much more enhanced by mycorrhizal fungi in most studies with herbaceous plants, as 80–90 % of As accumulated in roots of maize, ryegrass, and clover (Dong et al. 2008; Wang et al. 2008). Grown under a range between 100 and 600 mg As kg^{-1} soil/sand media, accumulations of As contents were 10–50 times higher in roots than in shoots in tomato (Liu et al. 2005a), sunflower (Ultra et al. 2007a, b), medic (Xu et al. 2008), white clover (*Trifolium repens*), ryegrass (*L. perenne*) (Dong et al. 2008), and maize (Xia et al. 2007; Wang et al. 2008; Yu et al. 2009). In contrast, P accumulations and P/As ratio were generally higher in shoots than in roots in all these studies. In addition to the food chain implications, the enhancement of As accumulation in roots has implications for mycorrhizal plant utility in bioremediation efforts, as we shall see below. Also of relevance to As accumulation in the food chain, As concentrations in pods were reduced, while P uptake was increased in a 9-week-old nodulated AM (*G. mosseae*) lentil (*Lens*

culinaris cv. Titore) irrigated with 1, 2, 5, and 10 mg As(V) L^{-1} to the sand/terra (1:1) media (Ahmed et al. 2006). Lower As concentration in pods would most likely reduce As toxicity risk in the food chain. Further studies are required to understand if this is a general consequence of mycorrhizal colonization. In addition, the highest As accumulated in maize roots when inoculated with *Acaulospora* spp. or *Glomus* spp. and earthworm (*Eisenia foetida*) (Hua et al. 2009, 2010).

Arsenic Accumulation in Fronds of Ferns. The roles of AM fungi in As uptake and tolerance have also been investigated in the As hyperaccumulation ferns. Similar to studies summarized above, there was often an increase of As accumulation in mycorrhizal ferns (Liu et al. 2009), though there were intraspecific differences in AM fungi on As accumulation in *P. vittata* (Wu et al. 2009). In contrast, mycorrhization led to an increase in the relative proportion of As accumulated in fronds vs. roots. For example, compared to its non-mycorrhizal counterpart, the amounts of As accumulation were about five times higher in fronds, but similar in roots, in an 8-month-old mycorrhizal *P. vittata*, when grown in 100 mg As(V) with 25 or 50 mg P kg^{-1} soil and inoculated with an AM inoculum from an As-contaminated site (Al Agely et al. 2005), and in a 4-month-old *G. mosseae* BEG167-colonized *P. vittata* growing in 300 mg As kg^{-1} soil (Liu et al. 2005b). Arsenic accumulations in fronds and roots were 3.0–3.9 and 2.5–3.6 times higher, respectively, in a 2-month-old mycorrhizal (an indigenous soil inoculum) *P. vittata* than in non-inoculated plants growing in 50 or 100 mg As kg^{-1} soil (Leung et al. 2006). However, As accumulation in *P. vittata* was not affected by 2- or 3-month inoculation with either *G. mosseae*, *G. caledonium*, or *G. intraradices* growing in 106 mg As kg^{-1} soil (Chen et al. 2006). Compared to non-mycorrhizal plants, frond As accumulation was reduced, while similar in roots, in an 8-month-old AM *Pityrogramma calomelanos* (silverback fern) growing in 240 mg As kg^{-1} soil (Jankong and Visoottiviseth 2008). However, a commercial AM inoculum (a mixture of *G. mosseae*, *G. intraradices*, and *G. etunicatum*) was applied to this 8-month-old silverback fern for only 2 months, possibly reducing mycorrhizal effects on the outcome. Soil As concentration was reduced by 24 %, while tissue As accumulation was up to 0.2 % in *P. vittata* growing under a mixed inoculum [indigenous AM fungi (*G. intraradices*, *G. geosporum*, *and G. mosseae*) + nonindigenous *G. mosseae*] and the addition of phosphate rock (Leung et al. 2010a). The contrasting results may be derived from experimentation with different AM isolates, different host plants, or other experimental conditions. Further assessments of mycorrhizal effects on As accumulation are needed, particularly under field conditions. In general, most of these fern studies showed a higher ratio of frond/root As accumulation in the mycorrhizal ferns than in their non-mycorrhizal counterparts, suggesting that As translocation from root to shoot was enhanced by mycorrhizal fungi even in As hyperaccumulation ferns. The mycorrhizal-mediated enhancement of As tolerance and accumulation either in shoots of As hyperaccumulating ferns or in roots of herbaceous annuals and perennials offers potential for screening fungal species for As remediation purpose.

16.6 Potential of Mycorrhizal Fungi in Arsenic Phytoremediation

Phytoremediation is a promising alternative for As remediation from contaminated soils and water since the chemical and physical remediation technologies are quite expensive and limited to on-site applications (Mendez and Maier 2008; Mondal et al. 2006; Tripathi et al. 2007; Wenzel 2009; Garg and Singla 2011). Genetic manipulation of As hyperaccumulating traits could contribute our efforts to As phytoremediation (Zhu and Rosen 2009), though the traits and genes are largely unknown to date. Because aboveground plant parts are easier to harvest, most attention has been given to identify high shoot As accumulators for phytoextraction by aboveground harvesting, while less has been given to high root As accumulators by belowground harvesting. But all shoot As hyperaccumulation ferns require a tropical or subtropical climate and may not grow well in other habitats. As an alternative, if roots could be easily harvested, then root hyperaccumulators could be used for phytoremediation, especially in herbaceous plants with dense root systems in shallow soil profiles, though root removal technique is not available or currently impractical. Further testing of a broad suite of species is needed for screening both shoot and root hyperaccumulators, in addition to those listed in Table 16.1. The potential roles of AM fungi (Gaur and Adholeya 2004; Garg and Singla 2011) and plant-associated bacteria (Khan 2005; Weyens et al. 2009) in heavy metal phytoremediation have been respectively proposed. However, the potential for AM fungi to contribute to As (a semimetal element) tolerance and hyperaccumulation in their host plants is poorly explored, particularly under field conditions.

Can mycorrhizas potentially offer a more cost-effective, environmentally sound, and sustainable pathway to global As phytoremediation? As seen in the previous sections, mycorrhizal fungi can tolerate and perform well in high levels of As under laboratory conditions and contaminated field sites, and they also can facilitate As accumulation in host plant tissues or increase the transfer of As from roots to shoots by indigenous isolates in particular (Orlowska et al. 2012). This indicates that mycorrhizal fungi could confer both As tolerance and accumulation ability on their host plants. A range of 10 and 50 times higher As accumulations in roots than in shoots had been reported for some annuals or perennials, including lentil, maize, medic, ryegrass, sunflower, tomato, and white clover (Liu et al. 2005a, b; Ahmed et al. 2006; Xia et al. 2007; Bai et al. 2008; Dong et al. 2008; Wang et al. 2008; Xu et al. 2008; Yu et al. 2009; Ultra et al. 2007a, b; Garg and Singla 2012), or in shoots than in roots for a dozen ferns (Al Agely et al. 2005; Leung et al. 2006; Chen et al. 2006; Jankong and Visoottiviseth 2008; Zhao et al. 2009). If these phenomena are generally true, the selection of combinations of plant and fungal species with high As tolerance and accumulation ability would tap their potential for As phytoremediation, particularly for both phytoextraction and phytostabilization (Mendez and Maier 2008). At present, no one has identified either a woody As phytoremediation plant or a candidate with both high shoot and high root As accumulation capacity. Thus, the current phytoremediation

strategies are focused on herbaceous shoot hyperaccumulators, and phytostabilization is focused on herbaceous root hyperaccumulators. Furthermore, almost all current As phytoremediation practices are limited to laboratory experiments and a few very small field trials, where plants are introduced into the soil without established mycorrhizal symbioses.

Mycorrhizal diversity is high and mycorrhizal symbiosis develops well with the shoot As hyperaccumulation ferns even on As-contaminated field sites. A field investigation on both As-contaminated and As-uncontaminated fields in Central, Southern, and Southeastern China showed that the As hyperaccumulator *P. vittata* was associated with the fungal genera *Acaulospora*, *Diversispora*, *Glomus*, *Paraglomus*, and *Scutellospora*, with the common species *Glomus brohultii*, *G. geosporum*, *G. microaggregatum*, and *G. mosseae* (Wu et al. 2007). This high mycorrhizal fungal diversity may have significant ecological and physiological contributions to their host plants in contaminated sites. The known root As hyperaccumulation annuals and perennials mentioned above are mycorrhizal (Brundrett 2009; Wang and Qiu 2006). Given that indigenous AM fungi from contaminated soils performed better in both accumulation of As and plant growth (see the above section), these adapted indigenous fungi are a promising tool for As phytoremediation from the contaminated soil, particularly when large-scale on-farm production of mycorrhizal inocula becomes available (Douds et al. 2005; Ijdo et al. 2011). The introduction of As-tolerant mycorrhizal fungi to sites with no, limited, or unadapted mycorrhizal fungi could speed up not only As remediation with the establishment of mycorrhizal symbiosis between plants and fungi but also soil reclamation and vegetation restoration. Therefore, there is great potential to screen and then to integrate fungal isolates that enhance both As tolerance and hyperaccumulation with a shoot or root hyperaccumulation plant. In addition, the combination of mycorrhizal fungi with N_2-fixing microorganisms (*Rhizobia* or *Frankia*), As(V)-reducing bacteria (*Comamonas* sp., *Delftia* sp., *Rhodococcus* sp., and *Streptomyces* sp.), and dual AM and ectomycorrhizal (EM) or the tripartite AM, EM, and N_2-fixing plant (He et al. 2005, 2009; Roy et al. 2007; Yang et al. 2012) would further extend our efforts to identify plants with high As tolerance and accumulation capacity capable of functioning under nutrient-poor conditions.

Hyphae of a single fungal individual can potentially interconnect many plants of the same or different species, and a single plant can form mycorrhizas with many fungi as well. As a consequence, a common mycorrhizal network (CMN) forms within and between plant roots to link plants together (Newman 1988; He and Nara 2007; He et al. 2009). CMNs provide pathways to shuttle nutrients, such as C, N, P, and water, from one plant to another between the same and different plant species (Newman 1988; He and Nara 2007; He et al. 2009). These extensive mycorrhizal mycelia and networks could enhance As uptake and accumulation in shoots and/or roots. The transfer of As from a plant to another via a CMN has evidenced this potential. Plants were grown in two separate chambers separated by 25 μm steel mesh with a 1.0 cm air gap between chambers to restrict root growth but allow hyphal linkages. After 1 week of 0.1 % Na_2HAsO_4 application to leaves of a 50-day-old donor (either a grass of *Bromus hordeaceus*, *B. madritensis*, *Nassella*

pulchra or a forb of *Madia gracilis*, *Sanicula bipinnata*, *Trifolium microcephalum*), AM-mediated transfer of As occurred between grass donors and forb receivers, but not the other direction (Meding and Zasoski 2008). By growing plants with high biomass production but low As uptake capacity together with those having low biomass production but high As uptake capacity, As transfer between mycorrhizal plants via CMN may provide another plant-based phytoremediation strategy.

The current barriers to the adoption of mycorrhizal inoculation reside at several levels. First, there is the need to identify the best candidate fungi for both phytoremediation and phytostabilization. Inoculum sources for mycorrhizal fungi used in phytoremediation should be derived from a similar soil, climate, and geographic region as the phytoremediation site as possible. This will both increase the chances of success and minimize the likelihood of the transfer of unwanted invasive soil organisms with the fungal inoculum (Schwartz et al. 2006). In addition, screening sites with naturally high As or long-term As contamination will provide the highest likelihood for encountering As-tolerant mycorrhizal fungal populations. Given that it is likely that the best strains will be isolated from sites that have naturally high As, in these locations, mycorrhizal fungi native to the site may be sufficient as an inoculum source, greatly simplifying the process of inoculation for phytoremediation. Second, for cases where inoculation is necessary, there are existing biotechnological approaches to producing large quantities of fungal inoculum (Douds et al. 2005; Ijdo et al. 2011), but such approaches are limited at present to very few fungal strains. The magnitude of this limitation will depend on the tractability of otherwise suitable mycorrhizal fungal inocula. It may be that native soil inoculum from sites discussed above could be used when otherwise appropriate (e.g., when conforming to regulations regarding soil transportation). Third, in temperate regions, the barrier to mycorrhizal fungal use for phytoextraction is the lack of appropriate host plants, because most mycorrhizally enhanced As accumulation outside tropical ferns occurs in host roots, which are more challenging to harvest. Up to 1,400 or 1,600 mg As DW kg^{-1} was accumulated in mycorrhizal roots of tomato (Liu et al. 2005a), ryegrass, and clover (Dong et al. 2008) compared to 70 or 80 mg As kg^{-1} DW accumulated in shoots, when growing under 150 or 600 mg As kg^{-1} soil-like media. In addition, annual or perennial bentgrass (*Agrostis castellana* and *A. delicatula*) and West Indian beggarticks (*Bidens cynapiifolia*) could accumulate 1,000–1,800 mg As DW kg^{-1} in roots at As-contaminated mine sites (De Koe 1994; Bech et al. 1997), though their mycorrhizal status had not been reported. Considering that the *Agrostis* and *Bidens* genera have more than 100 or 200 species and almost all tested species are mycorrhizal (Wang and Qiu 2006), it is likely that these species are mycorrhizal. The potential range of plants for phytoremediation could thus greatly be expanded if root-harvesting technologies that are economically and environmentally appropriate could be explored in the near future. Pilot studies are urgently needed to determine whether root As accumulation is common in a magnitude sufficient to make root As harvesting feasible for those herbaceous plants with dense, sufficiently accessible root systems. Identification of such plants and appropriate root-harvesting technologies, such as those widely used for root or tuberous crops, would

greatly expand the potential range of plants for extraction of As-enriched root systems. Furthermore, if these plants can be identified, then the incorporation of mycorrhizal inoculation with appropriate strains should greatly enhance phytoremediation efforts for a broad range of host plants.

16.7 Conclusion

Chronic As exposure through drinking water or food consumption has become a major global environmental problem. Cost-effectively and environmentally sound plant-based As remediation technologies are urgently required. A number of As hyperaccumulation plants have been identified. Mycorrhizal plants display much greater tolerance to As toxicity under high As levels and exhibit enhanced As accumulation even in high As soils. These results demonstrate that mycorrhizas may offer global potential in As phytoremediation. With an appropriate combination of fungal and plant species, mycorrhizal plants with strong As tolerance and As hyperaccumulation capacity could thus be screened, particularly from naturally As-enriched sites, for As phytoextraction or phytostabilization. Biotechnological developments in the important ecological and physiological functions of mycorrhizas relevant to phytotolerance and phytoremediation will enhance the potential of mycorrhizal fungi to contribute to our efforts to curb global As contamination in a more environmentally sound, effective, practical, and sustainable manner, particularly by large-scale application of mycorrhizal inocula through on-farm production (Douds et al. 2005; Ijdo et al. 2011).

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Chapter 17
Arbuscular Mycorrhizal Colonization and Agricultural Land Use History

Irnanda A.F. Djuuna

17.1 Introduction

Most plant species form symbiotic associations with mycorrhizal fungi (Newman and Reddell 1987) and arbuscular mycorrhizal (AM) fungi are widespread in natural and agricultural ecosystems (Brundrett 1991). AM fungi can contribute to plant growth by enhancing water and nutrient uptake, especially phosphorus (P) (Ortas 1996; Jacobson 1997; Watts-Williams et al. 2014). Although AM fungi colonize roots of most plant species (Harley and Harley 1987; Smith and Read 2008), plants differ in their growth response to mycorrhizal colonization. Furthermore, plant species can influence the population of AM fungi (Crush 1978; Hiiesalu et al. 2014). AM fungi may also contribute to soil fertility by enhancing soil structure and protecting crops from root pathogens (Douds and Johnson 2003; Sharma et al. 2013). The soil environment, particularly those factors that control mineral fertility, strongly influences mycorrhizal function (Abbott and Robson 1982; Sikes et al. 2014).

In agricultural fields, the status of AM fungi is influenced by soil conditions and management practices (Jansa et al. 2014). The diversity of AM fungi species can be lower in agricultural systems than in nearby natural fields (Helgason et al. 1998; Sieverding 1991) or forested areas (Boerner et al. 1996). However, factors such as crop and rotation history can also influence the abundance of AM fungi in agricultural soil (Douds and Johnson 2003; Helgason et al. 2014).

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17.2 AM Fungi in Agricultural Systems

The impact of farming practices on AM fungi has been studied extensively (Abbott and Robson 1994; Gavito and Miller 1998; Thompson 1994; Barber et al. 2013). Agricultural practices such as tillage, crop rotation, and use of chemical pesticides and fertilizers (Helgason et al. 2014; Kurle and Pfleger 1994; Ortas et al. 2013) as well as clean fallowing, topsoil removal, fires, and waterlogging (Thompson 1994) have all been shown to influence the abundance of AM fungi, often reducing the level of colonization. Rotation and fertilizer practices are major factors that affect the abundance of AM fungi, especially in Mediterranean agriculture (e.g., Abbott et al. 1995). Difference may not be so marked in soils with high P (e.g., Franke-Snyder et al. 2001). However, geography and other landscape characteristics may override effects of land management on AM fungal communities (Jansa et al. 2014).

Crop rotation is a very important factor in managing nutrient supply. In general, preceding crops can affect the growth and yield of subsequent crops (Karlen et al. 1994; Brito et al. 2012). The choice of crop and crop rotation history can influence the community of AM fungi in agricultural soil because plants differ in their susceptibility to colonization. A study of 27 species of plants demonstrated that AM fungi were present in most legumes whereas Poaceae were poor hosts (Eschen et al. 2013). However, the length of root colonized by AM fungi can be higher in some grasses than in non-grasses grown in the same soil. The Leguminosae can be superior in terms of concentration of fungal hyphae per unit weight or length of root, but in terms of total length of mycorrhizal root available to exploit a given soil volume and in terms of the likely residual population of mycorrhizal propagules, the Gramineae would be superior (Thompson and Wildermuth 1989). Plants with less dense roots (fewer and coarse roots) can have high mycorrhizal colonization (Hetrick 1991) and plants with poorly developed root hairs can be highly dependent on mycorrhizas (Baylis 1970). The growth of highly mycorrhizal-dependent crops like linseed can leave a high level of mycorrhizal inoculum for subsequent crops (Thompson 1994). In addition, other factors that need to be considered when managing crop rotations to obtain maximum benefits from AM fungi include (1) whether AM fungal inoculum in the soil is low after practices such as clean fallowing, (2) whether a nonhost crop has been grown, (3) whether rice has been grown under waterlogged conditions, or (4) whether crops with low mycorrhizal dependency have been grown. If a crop with high dependency is grown for other reasons (e.g., disease control), then a high P fertilizer rate and possibly Zn fertilizer might need to be used to compensate for lower levels of mycorrhizal development if management practices have reduced their infectivity (Thompson 1994).

For soils with naturally high P fertility and high use of P fertilizer, colonization by AM fungi would not be expected to make a contribution to plant growth (Galvez et al. 2001; Kahiluoto et al. 2001). However, this is not always the case as it may depend on the soil type. P applications to field soils may be accompanied by a

decrease in the proportion of root length colonized by AM fungi (Abbott and Robson 1984; Clarke and Mosse 1981; Liu et al. 2000) but this is not always the case (Gryndler et al. 1990). The application of phosphate fertilizer to soil can delay in mycorrhiza formation as well as a decrease in the proportion of the root system colonized (Solaiman and Abbott 2008; De Miranda et al. 1989). In contrast, the addition of P fertilizer to soil with extremely low available phosphorus can increase the colonization, possibly through a direct effect on AM fungi (Bolan et al. 1984). Some studies have reported that farms which use alternative (e.g., low input) practices have higher levels of AM colonization than nearby conventional farms because of a lower available soil P associated with reduced applications of soluble P fertilizer (Mäder et al. 2000; Ryan 1999; Ryan and Ash 1999; Kahiluoto et al. 2012).

Nitrogen fertilizer may affect the infectivity of AM fungi but this is less marked than effects of P (Hodge and Storer 2014). Application of high doses of nitrogen fertilizer can reduce colonization by AM fungi (Hayman 1975; Johnson et al. 2003, 2010). Application of ammonium to soil prevented colonization by indigenous AM fungi and nitrate application resulted in a low (6 %) level of root colonization (Ortas and Rowell 2004). AM fungi can also be involved in the decomposition of complex organic material in soil and increase nitrogen capture by plants (Hodge et al. 2001).

Tillage practices can alter AM fungal populations and species composition, reduce root colonization and P uptake (Kurle and Pfleger 1994; McGonigle and Miller 2000; Brito et al. 2012), and disrupt the hyphal network (Jasper et al. 1989; Evans and Miller 1990). The physical disruption of fungal mycelia may change physicochemical properties and influence soil aggregation (Duchicela et al. 2013). Excessive secondary tillage and traffic increased soil bulk density and decreased root growth, mycorrhizal colonization, and top growth of *Phaseolus vulgaris* (Mulligan et al. 1985). On the other hand, reduced tillage intensity can favor higher colonization by AM fungi (Yocum et al. 1985; Mulligan et al. 1985; Brito et al. 2012). Soils in low-input agricultural systems can have higher populations and more propagules of AM fungi than soils under conventional management (Douds et al. 1993, 1995; Galvez et al. 1995; Kahiluoto et al. 2012). An investigation of a 7-year crop rotation and tillage scheme practice showed root length colonized by AM fungi was up to 60 % higher in plants grown in soils from low-input farming systems than in those grown in conventionally fertilized soils (Mäder et al. 2000). Similarly, AM fungal hyphal density was greater in no-till than in reduced tillage systems and lowest in a conventional tillage system (Kabir et al. 1997).

Fallowing land for an extended period without a crop is common practice in some agricultural systems. However, long fallow periods without plant cover may be detrimental to contributions by AM fungi (Douds and Johnson 2003). In some farming systems, weeds are allowed to grow and fallows are grazed by livestock. In other dry-land agricultural systems, fallows are used to accumulate soil water and nitrate and so are kept weed-free. However, longer fallows can result in reduced numbers of spores of AM fungi and lower levels of root colonization (Thompson 1991). Clean fallowing can reduce inoculum levels and colonization by AM fungi

in the following crop (Black and Tinker 1979; Thompson 1987). The reduction in abundance of AM fungi in soil during periods of fallow can be substantial, and Harinikumar and Bagyaraj (1988) reported a reduction in AM fungi colonization by 40 % associated with a long fallow period.

The application of pesticides to agricultural soils throughout the production cycle may have a range of effects on AM fungi. Some pesticides may be toxic to AM fungi (Abd-Alla et al. 2000; Jalali and Sharma 1993). Methyl bromide can kill AM propagules deep in the soil profile because it is denser than air (Menge 1982). The use of herbicides can have indirect effects on AM fungi by changing the relative abundance of plant species associated with the length of roots of species that differ in mycorrhizal dependency in the soil.

Grazing livestock can influence AM fungi through influences on root growth, changes in soil structure, and removal and return of nutrients (Harrier and Watson 1997; Davinic et al. 2013). Moderate and intense grazing resulted in increased root colonization and changes in AM fungal species composition of tall grass prairie (Eom et al. 2001). However, grazing can alter root biomass and structure, especially when compounded with other management practices such as N application (Yan et al. 2013) which can further influence communities of AM fungi. However, studies of the effect of grazing (e.g., by domestic animals) on AM fungi in agricultural fields have been inconsistent. In some situations, little effect of grazing on AM fungi has been observed (Torres et al. 2011), but grazing has been shown to have a negative effect on AM fungi in other situations (Saravesi et al. 2013). Furthermore, where domestic animal grazing influences soil structure, there are likely to be associated changes in the abundance and diversity of AM fungi in soil.

17.3 A Conceptual Model of AM Fungi in Soil

A conceptual model of factors influencing the status of AM fungi in agricultural soil is presented in Fig. 17.1. The distribution and abundance of AM fungi in soil can be influenced by a range of factors (e.g., climate, soil properties, management practices, and socioeconomic factors related to the farming enterprise). The dominance of particular influences would be site specific and include soil and geography (Jansa et al. 2014).

Field surveys have shown correlations between the distribution of AM fungi and soil pH. The distribution of some AM fungi can be restricted in either acid or alkaline conditions, while others have been found in both types of soil (Abbott and Robson 1991). For example, in a range of agricultural soils in southwestern Australia, *Acaulospora laevis* spores occurred only in more acid soils (pH in 1/5 0.01 M $CaCl_2$ less than 5.3), and *Glomus monosporum* spores occurred only in soil with pH greater than 4.85 (Abbott and Robson 1977). There was no correlation between the abundance of different spore types and soil pH. The level of root colonization was only slightly affected by pH over a range of soils at pH 4.5–7.5

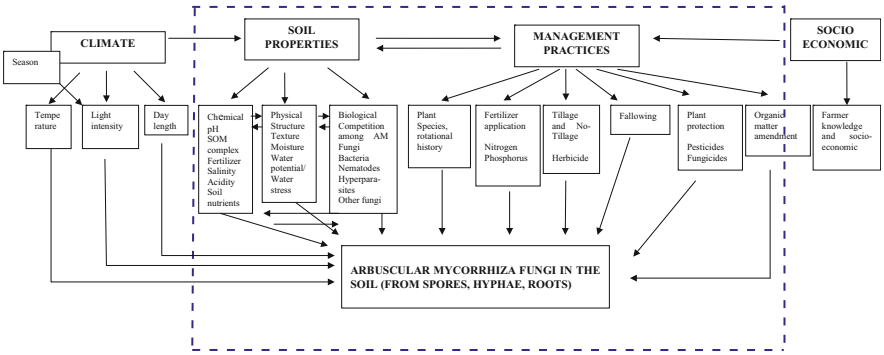

Fig. 17.1 A theoretical model of the factors which could affect the status of AM fungi in agricultural soil

(Wang et al. 1993) or at pH 4.7–7.7 (Porter et al. 1987) but different AM fungi were present at the pH extremes.

Increases in soil salinity in agricultural soils may influence the growth and activity of mycorrhiza fungi. Under saline conditions, AM fungi may have the ability to protect plants from salt stress (Rosendahl and Rosendahl 1991). Salinity can reduce the growth of AM fungi and root colonization in various ecosystems (Juniper and Abbott 1993; McMillen et al. 1998; Carvalho et al. 2003), but this is not always the case (Ruiz-Lozano et al. 1996).

Colonization by AM fungi in pot experiments is commonly reduced by low temperatures (e.g., Baon et al. 1994; Ruotsalainen and Kytöviita 2004) and increased by higher temperatures (e.g., Domisch et al. 2002) when measured as proportion of root length colonized. In the latter case, length of root colonized increased more than did the length of new roots and similar effects could influence the dynamics of mycorrhiza formation under field conditions.

17.4 Conclusion

The infectivity of AM fungi can be influenced by soil factors (chemical, physical, and biological) and agricultural practices, including plant components of agricultural systems. These factors vary across landscapes and geostatistical methods are available for quantifying them. Spatial and temporal variability in infectivity of AM fungi is expected to vary among sites and for different environmental conditions, depending on soil type and soil management. Some soil properties and agricultural practices can enhance the formation of mycorrhizas, but others can apparently be detrimental. Furthermore, as different methods have been used to measure infectivity of AM fungi, this should be considered when interpreting the effects of soil, plant, and environmental factors in these fungi. The conceptual model outlined here

for the development of AM could be used to predict the status of AM fungi in agricultural field even though this is not quantitative.

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Chapter 18
Contribution of Arbuscular Mycorrhizal Fungi to Soil Carbon Sequestration

Zakaria M. Solaiman

18.1 Introduction

An arbuscular mycorrhiza (AM) is a mutually beneficial association between species in the fungal phylum Glomeromycota and higher plants roots. The symbiosis is thought to have contributed to plant invasion of dry land ca 450 Ma ago and the vast majority of terrestrial plants currently form this association (Smith and Read 2008). AM fungi perform various ecological functions in exchange for host photosynthetic carbon (C) that almost always contribute to the fitness of hosts from an individual to community level (Willis et al. 2013). Soil contains more C than the atmosphere and vegetation combined (Averill et al. 2014). Understanding the mechanisms controlling the accumulation and stability of soil C is critical to predicting the Earth's future climate change (Averill et al. 2014).

AM symbioses can contribute to C fluxes between the plants and the atmosphere through different pathways (Fellbaum et al. 2012; Zhu and Miller 2003). A commonly known pathway by which AM fungi sequester C in soil is the transfer of photosynthates from the host plants to the AM fungal intraradical hyphae and subsequently to extraradical hyphae before release to the soil matrix (Bago et al. 2002, 2003; Leake et al. 2004; Parniske 2008; Solaiman and Saito 1997). Although the life span of extraradical hyphae attached to the plant roots is difficult to measure, it is believed to be short. The overall contribution of AM fungi to soil C sequestration may be dependent on the volume of hyphal biomass produced, the turnover time of accumulated hyphal biomass and the role played by these fungi in the stabilisation of soil aggregate formation (Zhu and Miller 2003). The turnover of hyphal cell walls, cytoplasm and extracellular polysaccharides represents a

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relatively labile organic C pool in soils. For example, the Glomeromycota fungi found in grassland soils represent a significant proportion of the fungal biomass pool, and it has been reported that 20–30 % of microbial biomass C come from AM fungi (Miller et al. 1995; Olsson 1999; Leake et al. 2004; Zhu and Miller 2003). The extraradical hyphae in prairie soil have been assessed to be as high as 28 m/cm^3 soil with an annual hyphal turnover of 26 % (Miller et al. 1995; Miller and Kling 2000). However, a higher hyphal turnover rate for AM fungi has been estimated to be 5–6 days (Staddon et al. 2003). The discrepancy between these studies is most likely associated with differences in sampling and methods of measurement. The former study used a topmost and uneven approach to quantify both a short-lived exploratory hyphae and longer-lived main hyphae, whereas the latter study quantified turnover of exploratory hyphae using ^{14}C isotope as a tracer.

A great proportion of C transferred from plants to AM fungi is incorporated into extraradical hyphal biomass. Fungal hyphae consist of recalcitrant compounds that contribute to a slower turnover of soil organic C (Olsson and Johnson 2005). The cell wall of extraradical hyphae is composed primarily of chitin, a carbohydrate that is recalcitrant to decomposition. Therefore, the rapid turnover of live extraradical hyphae could cause hyphal residues to accumulate within the soil matrix (Staddon et al. 2003). Limited information is available so far on the residence time of chitinous cell wall residues in the soil matrix, although some studies show a residence time of 49 ± 19 years for protein/amino acid/chitin-derived pyrolysis products (Gleixner et al. 2002). The typical dry weight of AM hyphae in a grassland soil has been projected to be between 0.03 and 0.5 mg/g and can characterise a large proportion of soil microbial biomass (Miller et al. 1995; Olsson 1999). At a soil depth of 30 cm with bulk density 1.2 g/cm^3 and 50 % C content of dry hyphae, the amount of soil organic C derived directly from AM fungi ranges from 54 to 900 kg/ha (Zhu and Miller 2003).

Studies of prairies and their restoration provide insights into mechanisms controlling the sequestration of C in soils and the generation of stable soil aggregate structure. They reveal the importance of plant traits in association with AM fungi in the physical protection mechanisms that allow for accumulation of detrital (granular) materials into longer-turnover soil carbon pools. For example, the stable soil aggregate structure can develop under restored prairie vegetation even though soils had been cultivated continuously for nearly 150 years (Miller and Jastrow 1992, 2000; Jastrow et al. 1998).

The soil organic C pool is an important component of terrestrial ecosystems and is a crucial regulator of C fluxes between the biosphere and the atmosphere. Mechanisms influencing soil organic carbon (SOC) storage depend mainly on net primary production and the distribution of photosynthates between above- and below-ground structures. Although primary production is a major determinant in the sequestration of C in soils, it is the size and activity of the microbial biomass of the soil that regulate C accumulation via mineralisation and immobilisation of plant and microbially derived residues in the soil. The exact amount of sequestration appears to depend on land management practices, soil factors, climate change and the amount as well as quality of plant and microbial inputs. The sequestration of C

in soils used for agriculture, forestry and land reclamation has been recognised as a potential option to mitigate global change (Batjes 1996; IPCC 1996; Smith et al. 1997; Lal 2003). Recent research suggests that AM fungi might be an important component of the soil organic C pool, in addition to facilitating C sequestration by stabilising soil aggregates along with glomalin formation.

18.2 Role of AM Fungi on Carbon Fluxes Between the Plants and the Atmosphere

The symbiotic association between plant roots and AM fungi is ubiquitous in terrestrial ecosystems (Smith and Read 2008). The role of AM fungi in mediating the ecosystem response to global climate change has been reviewed previously (Zhu and Miller 2003; Rillig et al. 2002; Staddon et al. 2002). In view of the importance of AM fungi in ecosystem processes, these reviews highlight the importance of research addressing the contributions of AM fungi to terrestrial C cycling. Several studies using ^{14}C labelling indicated that photosynthate is transferred from host plants to AM fungi hyphae within hours after labelling (Solaiman and Saito 1997; Johnson et al. 2002). It is also generally accepted that AM fungi receive all their carbohydrate from the host plant and that the association of AM fungi with roots could create a sink (i.e. a demand for carbohydrate) which could result in a 4–20 % drain of carbon from the host plant and could indirectly influence carbon sequestration in soils (Graham 2000). Furthermore, upregulation of photosynthesis by AM fungi is indicated where the amount of fungus in the root system is related directly to net C gain of the host (Miller et al. 2002). Such fungus-mediated effects on plant growth can potentially improve C sequestration by increasing net primary production, especially in nutrient-limited environments (Table 18.1).

18.3 Extraradical Hyphae and Carbon Sequestration in Soils

AM fungi could directly influence soil C sequestration through the growth and turnover of extraradical hyphae in rhizosphere and bulk soil. The decomposition time of extraradical hyphae in soil is relatively short compared to other organic biomass, and it has rarely been estimated. For example, Staddon et al. (2002) used accelerator mass spectrometry microanalysis of ^{14}C to quantify the turnover rate of extraradical hyphae in plants grown in a controlled environment and found that the turnover rate of extraradical hyphae attached to plant roots averaged 5–6 days. The authors indicated that C flow from host plants to AM fungi in soil might quickly be respired back to the atmosphere. More importantly, their findings suggest a rapid pathway for atmospheric C to enter the soil C cycle because AM fungi hyphal cell

Table 18.1 Role of AM fungi in regulating carbon fluxes between the atmosphere, plants and soil

AM fungi functions	Response	Source
Mycorrhizal external mycelium was the dominant pathway through which carbon entered the soil organic matter pool, exceeding the input via leaf litter and fine root turnover	Increase soil carbon sequestration	Godbold et al. (2006)
More carbon sequestration in ectomycorrhizas and ericoid mycorrhizas compared to arbuscular mycorrhizas	The effect of mycorrhizal type on soil carbon	Averill et al. (2014)
Colonisation by AM fungi could increase C transfer from rice to watermelon, while intercropping with watermelon could promote AM fungal colonisation and P uptake by rice	Carbon transfer	Ren et al. (2013), Schulze (2006)
A teleonomic model represents carbon (C), nitrogen (N) and phosphorus (P) substrates with structure in shoot, root and mycorrhiza	Above- and below-ground interactions	Thornley and Parsons (2014)
AM fungal extraradical hyphae form glomalin which is associated with aggregate stability and protection of organic C in soil	Glomalin formation by extraradical hyphae	Wright and Upadhyaya (1996)
1. Carbon acts as an important trigger for fungal N uptake and transport	Carbon availability triggers fungal nitrogen uptake and transport	Fellbaum et al. (2012)
2. The fungus changes its strategy in response to an exogenous supply of carbon		
3. Both plants and fungi reciprocally reward resources to those partners providing more benefit		
AM fungi stimulated under elevated CO_2	Organic carbon decomposition	Cheng et al. (2012)
C and N flow at the soil-root interface is bidirectional with C and N being lost from roots and taken up from the soil simultaneously	Bidirectional transfer of C and N	Jones et al. (2009)
Soil aggregation and C sequestration are tightly correlated with the abundance of AM fungi	Increase C sequestration and stabilisation of soil aggregates	Wilson et al. (2009)

walls are composed of chitin, a carbohydrate that is recalcitrant; the rapid turnover of live extraradical hyphae would still allow for the accumulation of hyphal residues that could remain within the soil matrix for a considerable time. Currently, limited information is available on the residence time of chitinous cell wall residues, particularly in a soil matrix, although recent studies using pyrolysis GC/MS-C-IRMS (gas chromatography-mass spectrometry-combustion interface-isotope ratio mass spectrometry) indicate a residence time of 49 ± 19 years for protein-, amino acid- or chitin-derived pyrolysis products (Gleixner et al. 2002). Hyphal residue accumulation would have been difficult to measure in the short period of time used in this experiment (Staddon et al. 2002). Furthermore, the use of potting medium consisting of sand and attapulgite clay eliminated the involvement of physical protection which is a major mechanism for stabilising hyphal residues

exposed to soil microbial activity and hence for increasing residence time in soil. Moreover, the Staddon et al. (2002) study did not determine whether the hyphae (characterised by rapid turnover) were decomposed completely to CO_2 or remained as residues within the potting medium.

The typical dry weight of extraradical hyphae in soil, 0.03–0.5 mg/g, represents a large proportion of soil microbial biomass (Miller et al. 1995; Olsson 1999). For a soil depth of 30 cm with bulk density of 1.2 g/cm^3 and 50 % carbon content of dry hyphae, the amount of SOC derived directly from AM fungi ranges from 54 to 900 kg/ha. This range in extraradical hyphae indicates that despite the rapid turnover of live hyphae, the amount of carbon retained by extraradical hyphae in the soil is measurable, and the maintenance of a stable hyphal network is functionally important for the sequestration of carbon below ground. The rather high turnover values reported by Staddon et al. (2002), in combination with results demonstrating a rather large stock of extraradical hyphae biomass, suggest that more than one pool of extraradical hyphae exists, probably distinguished by hyphal architecture (Staddon et al. 2002; Friese and Allen 1991). One pool is composed of hyphae with relatively fast turnover (days), probably related to the hyphal architectural type known as exploratory or absorptive hyphae. Another pool with relatively slower turnover (weeks) is composed of the thicker-walled extraradical hyphae with arterial architecture. These observations suggest a need for future research to consider extraradical hyphae turnover and the resultant contribution to carbon sequestration owing to hyphal architecture.

The extent to which terrestrial ecosystems can sequester C to mitigate climate change is unknown. The stimulation of AM fungi by elevated atmospheric CO_2 has been assumed to be a major mechanism facilitating soil C sequestration by increasing C inputs to soil and by protecting organic C from decomposition via aggregation. Cheng et al. (2012) presented evidence from four independent microcosm and field experiments demonstrating that CO_2 enhancement of AM fungi results in considerable soil C losses. Their findings challenge the assumption that AM fungi protect against degradation of organic C in soil and raise questions about the current prediction of terrestrial ecosystem C balance under future climate change scenarios.

18.4 Role of AM Fungi on Plant C Rhizodeposition in Soil

Plant roots exudate a significant proportion of C assimilated by photosynthesis to the soil which is within the range of 5–30 % (Philippot et al. 2013). Rhizodeposition of C may be in the form of root exudates, mucilage, dead root cells and C transfer to mycorrhizal fungi (Jones et al. 2009; Badri and Vivanco 2009). This continuous supply of C compounds by plant roots influences soil microbial community composition and activity, which can directly influence plant growth (Philippot et al. 2013). Plants have traded photosynthates with AM hyphae over millions of years of co-evolution (Redecker 2000; Wang et al. 2010).

Most terrestrial plants form symbiosis with one or more kinds of mycorrhizal fungi, of which 80 % of plant species are being associated with AM fungi (Smith and Smith 2011). Several studies have shown that the presence of a mycorrhizal association significantly increases the total C assimilation by plants (Miller et al. 2002; Grimoldi et al. 2006; Calderon et al. 2012) and can induce an extra C flux of 3–8 % of gross photosynthesis into the soil (Grimoldi et al. 2006). AM fungi are constantly provided with recent plant photoassimilates which are used to build up their large extraradical hyphal network. Turnover of this mycorrhizal hyphae network is thought to be a main and quickest process for C input into the soil organic matter pool, possibly even greater than shoot or root litter inputs (Godbold et al. 2006).

The transfer of photosynthetic C from plants to soil occurs on a rapid timescale ranging from hours for grasses to a few days for trees (Kuzyakov and Gavrichkova 2010). Root exudation starts to peak only 3 h after photosynthesis in wheat (Dilkes et al. 2004). The core pathway for transport of recently assimilated C from the shoots to the roots is the plant phloem (Mencuccini and Hölttä 2010). However, little is known about rigorous translocation patterns of C along this pathway, and even less is known about the involvement of particular root cells in the process of C being released to the soil matrix (Badri and Vivanco 2009). It is thought that the major proportion of root exudate C is lost passively by the large C concentration gradient between root cytoplasm and the apoplast/soil solution (Farrar et al. 2003; Jones et al. 2009). Thus, not all C exudation into soil occurs directly from roots; some C allocation is associated with the volume of AM fungal hyphal network.

AM fungi colonise roots behind the root hair zone along mature root sections of the roots which have an established phloem and an endodermal layer (Smith and Smith 2011). Carbon is transferred to AM fungi via the mycorrhizal intraradical hyphae in the root cortex (Solaiman and Saito 1997). The intraradical hyphae or arbuscules grow within apoplastic areas of the roots cortex but do not enter the plant cytoplasm. Rather, they form a symbiotic interface made of plasma membranes both of fungus and plant, which is separated by an apoplastic compartment (Smith and Smith 2011). Carbon is supposed to be transferred from the plant as glucose and sucrose to the intraradical hyphae through this symbiotic interface, from where it is transported to the extraradical network which extends into the soil matrix (Bago et al. 2002, 2003; Solaiman and Saito 1997). The pathway along which C is translocated from the phloem cells to the mycorrhizal structures in the cortex remains to be explored.

AM fungi increase plant nutrient uptake especially P in exchange for C, but interactions between these fungi and the soil microbial community have received less attention. Some studies have shown that AM hyphae can exude plant-derived C into the adjacent hyphosphere (Cheng et al. 2012; Johansson et al. 2004; Toljander et al. 2007). In addition, mycorrhizal hyphae are able to transport plant root C and release it beyond the rhizosphere, thereby transferring some of the C to the soil microbial community within the soil matrix that is inaccessible to roots (Herman et al. 2012; Nottingham et al. 2013). A recent study demonstrated that AM fungi may contribute to rhizosphere priming where plants have a role on soil organic

matter decomposition (Cheng et al. 2012). Hyphal exudates may be chemically different from root exudates leading to support a different microbial community compared to that of root exudates (Nuccio et al. 2013). The role of C released from hyphae for use by the soil microbial community needs to be elucidated.

18.5 Extraradical Hyphae, Glomalin Exudation and Soil Aggregate Formation

If the turnover values of hyphae reported by Staddon et al. (2002) can be generalised to all extraradical hyphae, it may be necessary to re-evaluate the contributions of AM fungi to soil structure. However, extraradical hyphae may be relatively persistent within soil aggregates, and their influence on soil aggregation might be even more important to the C stock than the influence of the hyphal standing crop alone (Miller and Jastrow 2000). Through their role in soil macroaggregate stabilisation, extraradical hyphae of AM fungi appear to contribute to the formation of aggregates and help to create a mechanism for increasing the residence time of organic biomass within soil macroaggregates. The extraradical hyphae contribute through enmeshment and stabilisation of soil particles within aggregates (Miller and Jastrow 2000; Oades and Waters 1991). The extraradical hyphae are able to ramify through soil pores within macroaggregates.

The contribution of hyphae of AM fungi to carbon cycling occurs in combination with exudates from roots. AM fungi hyphae are responsible for the production of a glycoprotein-like substance, glomalin (Wright and Upadhyaya 1998), which is fairly stable in soils (Steinberg and Rillig 2003). Radiocarbon dating of the operationally defined glomalin extract indicates a residence time in soils of 6–42 years (Rillig et al. 2001), which is longer than the residence time reported for hyphae of AM fungi. In a tropical forest soil, glomalin carbon was shown to represent up to 5 % of total soil carbon, which is much higher than soil microbial biomass carbon (Miller et al. 1995). The close correlation of the amount of glomalin in soil, hyphal length and stability of soil aggregates (Wright and Upadhyaya 1996) is evidence that glomalin could influence soil carbon storage indirectly by stabilising soil aggregates. One of the modes of action of glomalin could be in facilitating the formation of a sticky string bag of hyphae, the primary mode by which AM fungi contribute to soil aggregation (Johnson et al. 2002). However, the relationship between hyphal turnover and glomalin inputs remains largely unknown, and quantification of the relative contribution of glomalin to carbon cycling still needs to be determined.

18.6 Conclusion

AM fungi can contribute C fluxes between the plants and the atmosphere through increased C assimilation in plants. A key AM fungal-mediated process involved in the sequestration of C in soils is the transfer of photosynthate from host plants to AM extraradical hyphae. Although the turnover of extraradical hyphae linked to plant roots is little known, it is understood that the process is rapid. The overall contribution of AM fungi to soil C sequestration could depend significantly on the quantity and quality of hyphae produced, the age and resilience of hyphal residues, the production of glomalin and the role played by AM fungi in the stabilisation of soil aggregates. More detailed investigation is needed to explore the links between C sequestration in soil and nutrient exchange that are associated with AM fungi.

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Chapter 19
Biochar as a Habitat for Arbuscular Mycorrhizal Fungi

Noraini M. Jaafar

19.1 Introduction

Biochar, the pyrolised product from pyrolysis of "waste" organic material, has been widely proposed as a soil ameliorant for improving soil properties (Lehmann 2007; Rondon et al. 2007; Lehmann et al. 2011). However, biochar incorporation into soil can have both positive and negative effects on beneficial soil microorganisms, including arbuscular mycorrhizal (AM) fungi (Warnock et al. 2007). Both direct and indirect effects of biochar may be involved (Lehmann et al. 2011).

Direct and indirect mechanisms underlying interactions between AM fungi and biochar include the possibilities that (1) biochar provides a suitable habitat or shelter for soil microorganisms, protecting them from predators; (2) soil conditions and plant growth can be influenced by mycorrhizas after biochar addition through changes in soil physicochemical properties such as soil pH and water; and (3) AM fungi interactions with soil microorganisms may stimulate production of signalling compounds or alleviate production of detrimental compounds (Warnock et al. 2007). Other mechanisms linking biochar to changes in the abundance or functioning of mycorrhizas include potential interference in plant–fungus signalling and detoxification of allelochemicals on biochar (Warnock et al. 2007, 2010). Investigations of how biochar might affect soil microorganisms have mostly focused on microbial attachment, microbial community shift and enzyme activities (Atkinson et al. 2010; Joseph et al. 2010; Sohi et al. 2010; Lehmann et al. 2011).

Two main areas of research on biochar and soil microorganisms require clarification. First, generalisations about responses to biochar application need to be considered in relation to the specific characteristics of the biochar product used. Second, experimental evidence is required to clarify mechanisms by which biochar

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influences microorganisms in soil. Biochar may also exhibit different interactions over time after its application to soil, but this is not often studied or considered with regard to soil biota (Lehmann et al. 2011). There is a range of factors that could influence the effectiveness of biochar as a soil amendment. Effects of biochar on soil microbial components need to be considered in the context of different biochar and soil backgrounds as well as soil management practices.

As soil microorganisms are sensitive to soil management, knowing the background of soil and biochar is important when managing soils with biochar, especially for determining the amount applied in combination with fertiliser and organic materials for optimal mycorrhizal symbiosis. This review focuses on biochar properties in relation to the factors controlling its variability, function and management leading to how biochar might alter the abundance and activity of soil microorganisms. Mechanisms by which biochar might enhance the contribution of beneficial microorganisms in soil may also depend on other soil management practices. Based on potential similarities between mechanisms underlying interactions between soil microorganisms and biochar, this review focuses on AM fungi (Warnock et al. 2007) as a case study for considering biological influences of biochar on soil microorganisms especially fungi in soil.

19.2 Biochar as a Soil Amendment

There is a general consensus that the incorporation of biochar into soil could be beneficial to soil microorganisms. However, biochars are heterogeneous, with a range in porosity and surface area and pH although they are commonly alkaline. Biological properties of biochar are often overlooked. The beneficial impacts of biochar on soil have been speculated based on observations of the pyrogenic soil containing burned plant and animal materials generally known as Terra Preta soil as well as dark earth soil. AM fungi, used as a biofertiliser, have been considered in combination with biochar for their influence on soil properties such as nutrient retention, availability and uptake by plants (Warnock et al. 2007). However, the value of biochar as a general soil conditioner remains speculative because both positive and negative responses to biochar of soil microbial communities, including AM fungi, have been reported (Atkinson et al. 2010; Blackwell et al. 2010; Joseph et al. 2010; Moskal-del Hoyo et al. 2010; Sohi et al. 2010; Solaiman et al. 2010). Biochars derived from a range of plant and biomass sources have been studied in experiments that include both naturally occurring and inoculated AM fungi. Thus, some of the observed discrepancies in biochar influences on soil biological properties may have resulted from generalisations based on experiments using biochars of different organic origins or for soils with diverse physical and chemical properties (Rillig et al. 2010).

The type and source of biochar is central to estimating the role of biochar as a microbial habitat and its benefit to soil. For example, in Japan, locally available rice husk biochar increased the proportion of AM roots colonised through soil pH

modification and absorption of toxic substances and agrochemicals which inhibit root growth and microbial activity (Ishii and Kadoya 1994). In Australia, locally available *Eucalyptus* biochar had a similar positive effect on the percent mycorrhizal colonisation, possibly related to water uptake (Solaiman et al. 2010). In other cases, there was no effect for woody *Eucalyptus* biochar (e.g. Rondon et al. 2007) or woody *Leucaena* biochar (e.g. Habte and Antal 2010).

Most studies have examined the effects of biochar on mycorrhizal colonisation and sporulation (e.g. Ishii and Kadoya 1994; Matsubara et al. 2002; Elmer and Pignatello 2011), while measurements of phosphorus availability in plant and soil are used as indirect indicators of AM fungal effectiveness (Solaiman et al. 2010; Blackwell et al. 2010). Variability in soil characteristics and mycorrhizal inoculation methods (inoculation or indigenous) can influence the responses. These factors need to be considered in relation to identifying mechanisms involved in how biochar affects hyphae of AM fungi, spore germination and sporulation, enzymatic activities and the carbon/phosphorus interchange with plants.

19.3 Factors Influencing Biochar: Soil–Microbe Interactions

Warnock et al. (2007) highlighted the potential mechanisms of biochar–AM fungi interactions, including the potential of biochar as a habitat for soil microorganisms. However, studies of the potential impact of biochar–AM interaction have generally not focused on the mechanisms involved. The nature or physical characteristics of biochar in providing the habitat and protection for AM fungi are emphasised here because it is one of the main mechanisms that may involve direct biochar–mycorrhizal interactions.

The heterogeneous properties of biochar from various materials and pyrolysis processes influence their ameliorative effects on soil microbial colonisation, growth and benefit to plant and soil (Chan et al. 2007, 2008; Kuzyakov et al 2009; Thies and Rillig 2009; Blackwell et al. 2010; Rillig et al. 2010). Below, biochar factors and their effects are discussed in relation to AM fungi in optimising both biochar and AM symbiotic benefit towards improving soil properties and plant growth.

19.3.1 Sources of Biochar

Generalisations about the practical application of biochar have proven to be difficult due to heterogeneity among biochars and interactions with the soil environment into which biochar is applied. The heterogeneous properties of biochar can result from diversity of the original material used in the pyrolysis process (Blackwell et al. 2010). Furthermore, for practical purposes, an appropriate range of biochar

particle size, amount and methods of application, especially in the field, need to be considered for different biochar sources (Blackwell et al. 2009; Downie et al. 2009).

The heterogeneity in both physical and chemical properties of biochar is associated with feedstock and pyrolysis parameters (Gundale and DeLuca 2006; Downie et al. 2009). Mycorrhizal interactions with biochar have been compared using different types of biochar in a range of soil environments. Most biochars used have been plant derived and include rice husk, pine and other woody materials (Warnock et al. 2007). Experimental comparisons of the effect of the incorporation of biochars of plant and animal origin into soil on AM fungi are limited (Saito 1990; Warnock et al. 2007). Therefore, the effects of biochar heterogeneity arising from various sources of organic materials, pyrolysis temperature or biochar particle size and application rates on AM fungal growth, symbiosis and functions are not well understood.

Biochar creates a microenvironment in the bulk soil upon its application (Thies and Rillig 2009; Ogawa and Okimori 2010; Lehmann et al. 2011). Within the biochar microenvironment, biochar surfaces and pores can be colonised by bacteria, fungi and soil microfauna (Table 19.1).

Previous studies of microbial colonisation on biochar surfaces included laboratory experiments using biochar retrieved from soil (Ascough et al. 2010a, b; Moskal-del Hoyo et al. 2010), but there has been little characterisation of microbial colonisation of the internal structure of biochar compared to the external surface (Table 19.1). Laboratory studies of fungal colonisation of biochar showed fungal colonisation on surfaces and along cracks (Ascough et al. 2010b). As a consequence, there has been little discussion of experimental conditions and methodologies associated with observations of microorganisms in the biochar microenvironment.

Biochar pores may be structurally stacked and they may be altered by the presence of soil particles. It is expected that microorganisms are preferentially attracted to biochar surfaces rather than to pores (Lehmann et al. 2011). Surface features are important for substrate recognition and attachment by soil microorganisms (Lehmann et al. 2011). Biochar surfaces could provide substrates that are important for biological activity (Thies and Rillig 2009). Furthermore, surface attachment can protect microorganisms and increase the opportunity for synergistic interactions between biochar and soil microorganisms. Biochar pH is usually neutral to alkaline and may contain some phosphorus (Gundale and DeLuca 2006; Yamato et al. 2006) which may be available for microbial uptake.

Fungal hyphae, such as those of AM fungi, have potential to dominate biochar surfaces due to their extensive hyphal networks (Lehmann et al. 2011), and differences in hyphal growth forms have been observed on external compared to internal surfaces of charcoal (Ascough et al. 2010b). Although the surface of biochar has been associated with slow degradation by soil microbial and chemical processes, it can become coated with organic material (Joseph et al. 2010; Lehmann et al. 2011) which contributes to a microbial habitat. Some forms of biochar have been shown to retain moisture and adsorb cations (Liang et al. 2006; Blackwell

Table 19.1 Examples of microscopic observations of biochar as a habitat for soil microorganisms

Experiment	Methodology	Observation	Reference
Comparison of fungal colonisation in biochar feedstocks before and after burning (pyrolysis)	Wood and charcoal fragments were manually broken, observed under reflected light microscope followed by SEM observation on transverse (TS), longitudinal tangential (LTS) and longitudinal radial (LRS) sections	Fungal hyphae observed, some fungal infestation and features of decay were preserved after burning	Moskal-del Hoyo et al. (2010)
Saprophytic white rot fungal colonisation (from laboratory trial on media) on biochar blocks	Blocks were lyophilised, split open, observed using SEM	Distinct fungal growth found on charcoal, hyphal penetration through cracks	Ascough et al. (2010b)
Characterisation of microbial life colonising biochar and biochar-amended soils (fresh corn biochar colonised by microorganisms)	SEM method not available	Fresh corn biochar with microorganisms in pores	Jin (2010); Lehmann et al. (2011)
Fungal hyphae colonisation in fresh biochar pores	Method not available	Fungal hyphae found in fresh biochar	Lehmann and Joseph (2009); Lehmann et al. (2011)
Changes in charcoal particle morphology of 100-year-old char	SEM observation on cross sections, inner and outer parts of biochar, EDX spectroscopy	Filamentous fungi-infiltrated charcoal through larger pores and patches of mineral coating was found	Hockaday et al. (2007)
Ecological study of different ages of wood charcoal from forest humus profiles	SEM observation on transverse and longitudinal plane of biochar	Senescent fungal hyphae in biochar	Zackrisson et al. (1996)

et al. 2010; Solaiman et al. 2010), and this may indirectly influence soil microbial activity on biochar surfaces. A greater number of functional groups and oxidised sites on biochar surfaces could further facilitate microbial oxidation (Hockaday et al. 2007). Higher bacterial growth rates in association with biochar (Pietikainen et al. 2000) indicated that attachment and physical protection may be enhanced by the surface chemistry, including hydrophobicity.

Variation in porosity is expected to alter the suitability of biochar as a habitat for soil microorganisms. Pore size and surface characteristics are likely to influence microbial attachment and presumably the ability of the microorganisms to enter and/or penetrate into the biochar (Lehmann et al. 2011). Biochar includes meso-

(<2 μm), micro- (2–50 μm) and macropore (>50 μm) sizes (Downie et al. 2009) which may create microenvironments. Larger biochar pores may offer a new microhabitat to fungi, but no direct experimental evidence of the extent of pore colonisation by either bacteria or fungi is available. Furthermore, connectivity of pore spaces within biochar particles would influence the availability of important resources for microorganisms such as air and water diffusion through biochar, facilitating colonisation by soil microorganisms. Pores with diameters of 1–4 μm and 2–64 μm would be accessible to soil bacteria and fungal hyphae, respectively (Swift et al. 1979), including hyphae of AM fungi (Saito 1990). However, no studies have qualitatively or quantitatively demonstrated preferential colonisation by fungi and bacteria in biochar pores or on surfaces, and if the connectivity of pores within biochar is restricted, this would limit access by hyphae and bacteria.

Chemical and physical changes in biochar can occur after it is incorporated into soil (Downie et al. 2009). Interactions between soil particles, especially clay, and biochar have been found (Joseph et al. 2010). Quantification of changes in biochar after interaction with soil has not been a focus in investigations of the consequences of microbial colonisation of biochar, but it requires knowledge of the characteristics of biochar pores and surfaces of any biochar applied (Lehmann and Joseph 2009). As soil particles become cemented and the surface area covered, soil may enter biochar pores and alter their porosity and surface area. This could either limit or enhance the habitable spaces of biochar to soil microorganisms depending on the nature of the modification and the soil type. Lehmann et al. (2011) discussed various modes of microbial attachment to biochar, but the role of soil particles in influencing microbial attachment has not been clarified. Biochar surfaces could become cemented by soil and soil could enter biochar pores, but it is not known whether this might have either positive or negative effects on microbial colonisation of biochar.

Among sources of feedstock, woody biochar has potential as a habitat because it has higher porosity compared to the other sources of biochar such as chicken manure (Downie et al. 2009). Pores of 2–80 μm diameter are known to occur in wood-derived biochars and may benefit activity of mycorrhizal fungi (Thies and Rillig 2009). Woody biochar from *Pinus radiata* (Anderson et al. 2011) was able to increase fungal and bacterial abundance and promote P-solubilising bacteria. Fungi, especially saprophytic fungi, may extensively colonise biochar particles due to their association in decomposing fibrous organic matter (Ascough et al. 2010b; Moskal-del Hoyo et al. 2010).

If there is a benefit from provision of habitat, biochar could protect AM fungal hyphae and spores or even stimulate hyphal growth. It has been demonstrated that fungal hyphae penetrate pores of inert material such as vermiculite used for preparation of AM fungal inocula (Douds et al. 2005). Similarly, AM fungi were found sporulating inside the cavities of expanded clay and on the surface of clay material particles (Norris et al. 1992). Saito (1990) stated that the high porosity of charcoal is not an effective substrate for saprophytes, but it can favour AM fungi, although the reason for this is not known. Perhaps hyphae of AM fungi extend into charcoal buried in soil and sporulate preferentially in such particles (Ogawa and

Yamabe 1986; Baltruschat 1987). However, there is little qualitative or quantitative evidence of preferential colonisation by fungi and bacteria in biochar pores or on surfaces compared with soil particles. Furthermore, details of experimental techniques and biochar handling regarding microbial colonisation inside or on biochar surfaces are often lacking.

19.3.2 Method of Biochar Application to Soil

The method of placement of biochar in soil (either as a distinct layer (banded) or mixed through the surface layer) may influence the effects of biochar on soil microorganisms. Biochar banded in soils can increase AM fungal colonisation measured as percentage of roots colonised (Blackwell et al. 2010; Solaiman et al. 2010). Banding biochar into a layer in field soil is normal practice compared to surface application due to the wind problems (Blackwell et al. 2009, 2010). Banding of biochar was effective for both AM fungi and plant growth in a field study at several sites (Blackwell et al. 2010) although this was not compared with any other method of biochar placement.

Banding and surface application of biochar are practical for field conditions, whereas mixing biochar with soils, banding and surface application have been used in pot trials (Blackwell et al. 2009). However, no experimental comparison of these methods is available for AM fungi unlike the pot trial on ectomycorrhizal fungi where responses to different methods of biochar application to soil have been investigated (Makoto et al. 2010). Biochar applied in a layer with ectomycorrhizal inoculum promoted larch plant growth when compared with mixing biochar with soil. This was attributed to the frequency of root contact with biochar enabling effective phosphate utilisation.

Banding biochar in the crop root zone ensures biochar placement in contact with roots at the earliest growth stages (Blackwell et al. 2009). Biochar applied in bands also reduces the potential for biochar and topsoil loss caused by wind erosion and surface disturbance. The improvement in precision of sowing and fertilising machinery provides chances for crops to be sown in, or adjacent to, bands of incorporated biochar. In addition, the appropriate time in applying biochar to soil needs to be considered. Rutto and Mizutani (2006) proposed that biochar is best added once mycorrhizal symbiosis is established. This was based on their conclusion that biochar (or the activated charcoal used in their study) could delay mycorrhizal associations through exudate absorption which adversely affects the fungus signalling process, hence the symbiosis establishment.

19.3.3 Amount of Biochar

The need to apply an appropriate amount of biochar to soil is crucial if it is to restore and maintain soil fertility and to any effects on mycorrhizas, crop growth

and nutrition (Ishii and Kadoya 1994; Solaiman et al. 2010). Provision of soil conditions favourable for growth and activities of AM fungi needs to be taken into account when managing mycorrhizas in agricultural soils (Gazey et al. 2004) and this would apply in the presence of biochar. Biochar sourced from vastly different parent materials and pyrolysis conditions may exert different chemical properties including nutrient concentrations, and this needs to be considered when selecting the appropriate level of biochar for soil amendment. The amount may vary among soil types and land use histories which could undermine generalisations about the effects of biochar in soil (Schmidt and Noack 2000).

Several studies have investigated the quantity of biochar applied on soil micro-organisms (e.g. Kolb et al. 2009; Blackwell et al. 2010; Solaiman et al. 2010). The amount of biochar used for agronomic reasons (Blackwell et al. 2010; Solaiman et al. 2010) is likely to change the soil microbial environment, including that of AM fungi (Glaser et al. 2002; Kolb et al. 2009; Cross and Sohi 2011). In terms of soil microbial biomass, Chan et al. (2008) found an increase in soil microbial biomass carbon (MBC) dependent on the type of biochar and N fertiliser addition. MBC at the higher application levels, 25 and 50 t/ha, was significantly greater than that of the unamended control (Chan et al. 2008).

Selection of suitable amounts of biochar for application to soil to enhance colonisation by AM fungi is expected to differ for soil and biochar source (Blackwell et al. 2010; Elmer and Pignatello 2011). Inhibition of growth of AM fungi could result from application of higher than optimum amounts of biochar. The abundance of AM fungi in roots increased when hydrothermal carbonised biochar was added at 20 % w/w, and higher concentrations resulted in reduced mycorrhiza formation. Inoculum dilution at excessive levels of biochar application or an adverse effect on host plants limiting C supply to the AM fungi has been proposed (Rillig et al. 2010). Furthermore, the most appropriate amount of biochar may depend on the fertility of soil and its management, which could include organic matter management and other soil amendments such as fertiliser and lime (Blackwell et al. 2010). Biochar applied in optimal amounts and forms is expected to increase microhabitat availability in topsoils with low clay content (Solaiman et al. 2010). This may deliver mycorrhizal benefits (e.g. improve P acquisition by plants). Degraded soils may require higher amounts of biochar, but this would vary with organic matter or nutrient status (Liang et al. 2006; Chan et al. 2007, 2008; Steiner et al. 2008; Kolb et al. 2009). Most studies involving different amounts of biochar applied to soil show that levels of biochar that are acceptable for one type of soil and plant may not be suitable in another situation (Kolb et al. 2009; Blackwell et al. 2010).

19.3.4 Biochar Particle Size

There have been few studies of the impact of biochar particle size on microbial responses in soil. Different pyrolysis processes and feedstocks (organic origin) create biochar with different chemical, physical and size fractions (Keech et al. 2005; Gundale and DeLuca 2006; Downie et al. 2009; Verheijen et al. 2009).

Some biochars resemble the original cellular structure of the feedstock, in which large fragments correspond with woody plant material (Downie et al. 2009). Biochars also occur as large (>4 mm) through to fine particles (<20 μm) (Glaser et al. 2001, 2002). Commonly, biochar contains a mixture of particle size (Downie et al. 2009) or it is ground after production into smaller fractions (Sohi et al. 2010). Larger particles of biochar may be less practical for agricultural purposes due to their bulky characteristics compared with smaller particle sizes.

The dust portion of biochar has the greatest surface area but may not be the most effective soil amendment due to wind erosion and practicality (Blackwell et al. 2009). Biochar surfaces can gradually oxidise in response to exposure to air, activities of soil microorganisms or roots, and this may increase the cation-exchange capacity (Joseph et al. 2010). Changes to the surface of biochar after exposure to the soil environment may also alter water and nutrient retention properties of the biochar (Joseph et al. 2010). The size of the charcoal pieces amended to soil is not expected to greatly affect nutrient uptake but may alter surface properties which influence microbial attachment (Verheijen et al. 2009). Habte and Antal (2010) found that mycorrhizal colonisation of *Leucaena* roots was reduced when the growth medium was amended with fine (<0.3 mm in diameter) compared to coarse (<2.00 mm) charcoal. Lower levels of colonisation were associated with girdling of stems where the fine charcoal tended to accumulate. The large surface area could also enable greater absorption of toxic compound in soils (Antal and Gronli 2003; Habte and Antal 2010).

The selection of biochar for use in agricultural soils needs to be based on physical characteristics and chemical composition to achieve success in soil amelioration. Theoretically, biochar with higher porosity, a greater density of larger pores or large quantities of smaller particle sizes may benefit soil microorganisms, including mycorrhizal fungi. However, possible subsequent interactions between biochar and soil need to be taken into account. Furthermore, as the amount of biochar applied to soil can influence microbial processes, mixing biochar in the soil may also influence the distribution of microbial microsites in the soil in a different way to banding (Makoto et al. 2010). The application amount and method (mixing or banding) would normally be taken into account when incorporating biochar alone or with other amendments such as fertiliser for optimisation of nutrient capture (Blackwell et al. 2010). This could also change the interactions between biochar and soil microorganisms when organic matter or fertiliser is included.

19.4 Factors Influencing Biochar–Microbe Interactions: Soil Management

It has been suggested that the efficacy of biochar–AM fungal interactions may be reduced in more fertile soils (Lehmann et al. 2011). Incorporating biochar in farming systems that use other soil amendments and practices could be beneficial,

but biochar has a longer residence time in soil compared to other sources of carbon. As a carbon-rich material, biochar is affected by soil processes, but the changes in biochar occur slowly in soil and the effect on soil nutrients is not well understood (Lehmann 2007; Lehmann and Joseph 2009; Joseph et al. 2010). Application of fertiliser and labile organic matter has been used with biochar to optimise the benefits of these soil amendments (Blackwell et al. 2010; Graber et al. 2010).

Dual incorporation of biochar with organic matter is normally associated with the goal of improving soil fertility. As a carbon source, biochar when added to soil could contribute to increasing the organic content in soil due to its recalcitrant nature. Addition of biochar as a nutrient source has been suggested (Rajkovich et al. 2012), and biochar may also contain small amount of volatiles, substrate for microbial degradation and activities, and nutrients (Downie et al. 2009). Transformation of organic matter which contains phytotoxic compounds by pyrolysis could be used as a soil amendment to avoid a detrimental effect on plant and soil properties (Ishii and Kadoya 1994), but most of the nutrients may be lost during pyrolysis. The availability of nutrients from soil organic matter may not necessarily be improved by biochar addition (Dempster et al. 2012a, b).

There is a potential role of biochar in improving the microbial status of soil amended with other forms of organic matter. For example, Zackrisson et al. (1996) suggested that microbial activity played a part in reactivating charcoal by decomposing attached materials to the charcoal and providing nutrient sources for microbial activity. Organic materials and minerals can be bound to biochar particles and it is important to note this when managing biochar and other sources of organic matter (Joseph et al. 2010). The structural nature of biochar could facilitate microbial development and indirectly accelerate adsorption and degradation of phenolic compounds (Keech et al. 2005). However, negative implications for soil microorganisms are also possible in certain cases involving organic substances through their interaction with biochar. In a study by Rutto and Mizutani (2006), application of activated charcoal slightly alleviated the negative detrimental effect of root bark extract but reduced the benefits derived from mycorrhizas for plant growth. The large surface area of materials such as activated charcoal enhances its ability to absorb organic compounds for soil detoxification purposes (Uchimiya et al. 2010).

Some biochars may contribute slightly to soil nutrient status through provision of small amounts of nutrients or impurities (Lehmann and Joseph 2009). This has been shown by Graber et al. (2010) whereby tar and labile compounds trapped in pores after pyrolysis provided substrate for microorganisms. Furthermore, biochar application can alter soil phosphorus availability through modification to carbon, nutrient and pH in soil (Glaser et al. 2002; Matsubara et al. 2002). Charcoal may improve the growth and spread of AM fungi in roots by neutralising soil acidity (Ishii and Kadoya 1994). In contrast, addition of carbonised materials to soil can cause a decline in AM fungal colonisation (Gaur and Adholeya 2000).

The ability of biochar to retain nutrients and heavy metals is dependent on the sorption characteristics of biochars which are controlled by the relative carbonised and non-carbonised fractions and their surface and bulk properties (Uchimiya

et al. 2010, 2011a, b). However, there are concerns about the ability of AM fungi to develop in biochar-amended soil with high levels of phosphorus. Biochar pH is usually neutral to alkaline and may contain some phosphorus (Gundale and DeLuca 2006; Yamato et al. 2006) which may be available for microbial uptake. AM fungal effectiveness is affected by environmental and biological factors including P availability and mycorrhizal inoculum potential (Smith et al. 1992; Maeder et al. 2002). Thus, AM fungal development and function in soil amended with biochar would depend on biochar characteristics and soil nutrient status.

Wood-based biochar had the capacity to absorb measurable quantities of phosphate ions from a soil-free solution (Verheijen et al. 2009). The sorption of phosphorus to biochar may adversely affect how AM fungal hyphae inhabit the microenvironment of biochar. Mycorrhizal development responded positively to biochar at lower amounts of fertiliser applied to an agricultural soil (Blackwell et al. 2010). In this study, percentage in AM fungal colonisation increased when biochar was applied at 3 t/ha when the low level of phosphorus fertiliser was applied compared to the "full" fertiliser application. In contrast, Yamato et al. (2006) observed that colonisation by AM fungi (measured as proportion of root colonised) was highest for bark charcoal application without phosphorus fertiliser application. A large number of studies on the effect of charcoal application on the enhancement of AM fungal colonisation have been conducted (Ogawa and Yamabe 1986; Saito 1990; Ishii and Kadoya 1994; Ezawa et al. 2002; Ogawa and Okimori 2010) when no fertilisers were incorporated into the soil. However, mycorrhizal–biochar interactions would be expected to depend on the phosphorus status of the soil whether or not phosphate fertiliser was applied (Blackwell et al. 2010; Solaiman et al. 2010).

The absence of fertiliser can be compromised by applying higher amount of biochar. Blackwell et al. (2010) observed that biochar when applied at 3 t/ha resulted in greater root colonisation at the nil or low fertiliser rate. The low-level P fertiliser application in conjunction with biochar seems to have provided better conditions for mycorrhizal colonisation than the unfertilised soil or full fertiliser application. Biochar sorption of labile organic C could serve as a mechanism for decreased soil organic matter decomposition and concurrent P mineralisation and could result in decreased P availability as suggested by Kuzyakov et al. (2009).

19.5 Conclusion

Biochar application to soil involves complex interactions with soil and soil management practices. Biochar is heterogeneous in nature, especially in pore and surface structure associated with pyrolysis processes and feedstock source. These physical features were proposed to be associated with the abundance and development of microorganisms, but quantification of biochar pores and the effect of particle size are inconclusive, making it difficult to support the claim of biochar as a significant habitat for soil microorganisms compared to the soil itself. In

summary, soil background characteristics, including pH and P status, may lead to different interactions between soil and biochars. As biochars are normally applied with fertiliser and commonly discussed in terms of a priming effect with labile organic matter addition, further investigations of interactions with soil microorganisms, including AM fungi, are warranted.

Overall, there are significant effects of the type of biochar used, which largely influences the amount of each biochar application to soil that could lead to beneficial effects on AM fungi. Hyphae of AM fungi have mainly been assessed within roots, not in the biochar microenvironment. The optimum amount of biochar application would need to be identified due to potential detrimental effects of higher biochar application levels on soil microorganisms or plant growth. The significance of biochar particle size has rarely been considered in relation to plant benefit or soil changes, but it may influence attachment of soil microorganisms to biochar surfaces. When biochar is applied with organic amendments, the mineralisation of biochar could be enhanced, but a concurrent effect of biochar on organic matter could also be important. On the contrary, although significant interactions between biochar and fertiliser have been shown, the optimal amount of biochar when interacting with fertilisers may vary with biochar type.

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Chapter 20
Application of AM Fungi in Remediation of Saline Soils

Anne Nurbaity

20.1 Introduction

Rehabilitation of saline soil is crucial because large areas of the world have saline soils or are prone to encroaching salinity. Essential to rehabilitation of saline soil is the revegetation of recharge areas (Stirzaker et al. 2002). Management practices are required that improve the quality and productivity of saline soil and the ability of the plant to better withstand salt stress (Al-Karaki 2001; Caravaca et al. 2002a). For sustainable agriculture, solutions to salinity-related problems must acknowledge biological processes as part of rehabilitation. Practices such as organic matter application (Bell and Mann 2004; Caravaca et al. 2002b) and/or microbial inoculation (Aliasgharzadeh et al. 2001) are options for rehabilitation of degraded land. Increased organic matter levels and reactivated microbial activity either as free-living organisms or in association with plant roots are likely to be important for improving soil quality (Caravaca et al. 2002a; Diaz et al. 1994).

Arbuscular mycorrhizal (AM) fungi have been considered as bio-ameliorators of saline soil (Feng et al. 2002) because they may enhance the tolerance of plants to salinity (Al-Karaki 2001; Boyacioglu and Uyanoz 2014; Cantrel and Linderman 2001; Feng et al. 2002; Ruiz-Lozano et al. 1996). The practical use of AM fungi as a form of biological fertiliser and organic matter as low-input technologies for managing soil fertility has been investigated (Gaur and Adholeya 2002; Gryndler et al. 2002). This review examines biological aspects of rehabilitation of saline soil and considers the role of organic matter and AM fungi in saline environments.

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20.2 Effects of Salinity on Plant Growth and Soil Biological Activities

In general, dissolved salts may affect plant growth and soil organisms by direct injury due to specific ion toxicity and/or indirectly via osmotic imbalance effects (Al-Karaki 2001; Ferguson and Grattan 2005). Specific ion toxicities are due to the accumulation of ions such as sodium and/or chloride in the tissue to damaging levels (Al-Karaki 2001; Juniper and Abbott 1993). In plants, these ion accumulations occur as direct foliar accumulation or via root uptake. The damage is visible as foliar chlorosis and necrosis (Ferguson and Grattan 2005). Ionic effects also include interference with essential ions and a lowering of the net rate of photosynthesis (Ruiz-Lozano et al. 1996). Osmotic effects are caused by the total concentration of salt in the soil solution produced by the combination of soil salinity, irrigation water quality and fertilisation (Ferguson and Grattan 2005) and interfere with the ability of the plant to take up water. These effects are associated with the inhibition of cell wall extension and cellular expansion, leading to reduced plant growth (Boughanmi et al. 2003; Ruiz-Lozano et al. 1996).

High salt concentrations can reduce seed germination and initial seedling growth, as well as the growth of established plants (Feng et al. 2000; Esechie et al. 2002; Zedler et al. 2003). Salinity may induce nutrient deficiencies or imbalances in plants due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+} and NO_3^- (Hu and Schmidhalter 2005). Most plants have a threshold salt concentration value above which yields decline. High salt concentration can also have an adverse effect on microbiological processes, including mineralisation, soil enzyme activities and soil respiration (Nelson et al. 1996; Ramirez-Fuentes et al. 2002; Wong et al. 2004). Mineralisation of C and N can be negatively correlated with salinity (Nelson et al. 1996; Pathak and Rao 1997). Other examples of an inhibitory effect of salinity on microbial processes include the inhibition of oxidation of NO_2^- (Ramirez-Fuentes et al. 2002) and inhibition of dehydrogenase activity (Batra and Manna 1997).

High salinity levels in soil can have variable impacts on the abundance and activity of AM fungi (Juniper and Abbott 1993). Some investigations have shown that excessive NaCl levels in soil inhibit mycorrhizal formation and restrict the activity of most mycorrhizal fungi (McMillen et al. 1998; Ruiz-Lozano and Azcon 2000). The formation of mycorrhizas will generally be inhibited in plants if the concentration of salt in the soil exceeds 3.3 mg/g (Gupta and Mukerji 2000).

Salts can reduce spore germination, hyphal growth and colonisation and spore production for AM fungi (Juniper and Abbott 2004). In addition, roots of *Parthenium argentatum* treated with NaCl had fewer arbuscules and vesicles (Pfeiffer and Bloss 1988). Consequently, a delay in spore germination phases due to dissolved salts in the soil solution may inhibit or even stop the growth of hyphae and colonisation of plant roots and, hence, the establishment of the symbiosis (Juniper and Abbott 2006).

20.3 Management and Rehabilitation of Saline Lands

Management of saline lands for agricultural use is influenced by many factors but particularly by water availability (FAO 2006; Qadir et al. 2000). As a consequence, the solution to salinity problems is not simple, and techniques or agricultural systems developed will need to be suited to different locations and conditions. Physical, chemical and biological amelioration processes included in rehabilitation programmes need to be considered simultaneously. Physical amelioration can include hydrological processes such as drainage and leaching, land tillage and planting practices, and chemical amelioration practices can include the use of chemical amendments such as gypsum, sulphur and mineral fertilisers (FAO 2006).

20.3.1 Role of Organic Matter in Saline Environments

The application of organic matter during management of saline land has included manure incorporation, mulching and incorporation of crop residues (FAO 2006). Microbial inocula including mycorrhizal fungi have potential in improving the quality of saline soils (Aliasgharzadeh et al. 2001; Caravaca et al. 2002a). Soil organic matter influences a wide range of physical, chemical and biological properties of soil (Caravaca et al. 2002b). The beneficial effects of organic amendments on soil physical characteristics are decreased bulk density and increased aggregate stability, water holding capacity, saturated hydraulic conductivity, water infiltration rate and some measures of biological activity (Caravaca et al. 2002b, 2003; Celik et al. 2004). Furthermore, polysaccharides and other biopolymers from organic matter (including composts) can improve soil aggregate stability, which in turn improves water holding capacity and porosity of the soil (Caravaca et al. 2003; He et al. 1992).

In saline soil, benefits of organic matter on physical, biological and chemical properties as well as on plant growth have been reported. Generally, the utilisation of organic matter as mulch can reduce evaporation losses and thus decrease or prevent soil salinisation (Barrett-Lennard 2003). Mulching can delay the return of salt by lowering surface evaporation after salt is leached downward by rainfall and low evaporation during winter (Badia 2000). Furthermore, addition of organic matter may change the biological properties of the soil through improvement in the condition of soil for the multiplication of microorganisms and hence their activity such as microbial respiration or enzyme function (Badia 2000; Gryndler et al. 2005; Johnson 1998). Microbiological effects of organic matter include changes in total microorganism abundance, relative distribution of different groups of microorganisms (bacteria, fungi, actinomycetes) and activities such as mineralisation, nitrification and denitrification (He et al. 1992).

Finally, the combination of physical and biological ameliorative processes will lead to chemical changes in saline soil. There is evidence that incorporation of

organic matter into saline soil (Calcaric Regosols) in a semiarid Mediterranean climate decreased salinity levels (Badia 2000). The production of carbon dioxide from respiration of roots and soil organisms, and the production of organic acids by soil organisms and from organic matter decomposition, can influence dissolution of calcite (Ca^{2+}) (Qadir et al. 2005). Chelation of calcium increased the solubility of $CaCO_3$ and prevented Ca precipitation (Avnimelech et al. 1992), Ca^{2+} exchanges with Na^+ in the clay complex, which can reduce soil salinity (Badia 2000; Qadir et al. 2005). The beneficial effect of organic matter amendment to a great extent depends on the nature, maturity and quantity of organic matter applied (Roldan et al. 1996). For example, non-composted organic residues applied at higher application levels have been shown to be more effective than composted residue in stimulating the microbial activity because they are rich in easily biodegradable compounds (Caravaca et al. 2002b; He et al. 1992).

20.3.2 Role of AM Fungi in Saline Environments

Despite the relatively low mycorrhizal affinity of many halophytic plants, fairly large populations of AM fungi have been reported in some saline soils (Aliasgharzadeh et al. 2001). Experiments that have assessed the status of AM fungi in saline soil have shown a wide range of results, consequently, discussion as to whether biotic or abiotic factors have most influenced the ecology of AM fungi in saline environment is of interest (Carvalho et al. 2001; Mohammad et al. 2003a).

Assessments of spatial and temporal distribution of AM fungi in saline soil show that the abundance of AM fungi is inversely correlated with the level of soil salinity. The number of propagules or the infectivity of fungal isolates decreases with increasing salt (Azcon-Aguilar et al. 2003; Carvalho et al. 2001, 2003; Hildebrandt et al. 2001; Landwehr et al. 2002; Sylvia 1986; Wang et al. 2003). However, spore density had a very weak or no correlation with soil salinity (Carvalho et al. 2003; Mohammad et al. 2003a). In central European salt marshes, a high number of *Glomus* spores was found in saline soils (Hildebrandt et al. 2001), and *Glomus* was the dominant genus in other saline soils (Agwa and Abdel-Fattah 2002; Wang et al. 2003).

Communities of AM fungi have shown significant spatial heterogeneity and non-random associations with different hosts (Husband et al. 2002). For example, the presence of mycorrhizas in a salt marsh was more dependent on host plant species than on environmental stresses (Carvalho et al. 2001). It was also found that differences in patterns of activity of mycorrhizal fungi appeared to be linked to differences in phenology of root growth and not edaphic differences among vegetation zones (Johnson-Green et al. 1995). In contrast, Allen et al. (1995) stated that the diversity of mycorrhizal fungi was not associated with the patterns of plant diversity.

Mycorrhizal symbioses have been shown to improve the ability of some plant species to withstand salt stress (Al-Karaki 2001; Ruiz-Lozano et al. 1996). Some

experiments indicated that salt-treated AM plants produced greater shoot and root dry weights than did non-AM controls. For instance, AM fungi promoted growth of *Zea mays* in saline conditions, with the effect increasing as the degree of stress increased (Feng et al. 1998) and elsewhere (Bhoopander and Mukerji 1999; Cantrel and Linderman 2001). Where the growth of both AM and non-AM plants decreased as salinity increased, the decreases were more pronounced in non-mycorrhizal plants (Asghari 2008).

Inoculation with AM fungi under glasshouse conditions increased shoot contents of P and K (e.g. Asghari 2008). However, reports of effects of AM fungi on Na uptake in saline soil have been inconsistent. Sometimes Na content in shoot tissue was higher in mycorrhizal plants (Cantrel and Linderman 2001; Pfeiffer and Bloss 1988). On the contrary, mycorrhizal plants had less Na content in shoots of tomato (Al-Karaki 2001) and barley (Mohammad et al. 2003b) than did non-mycorrhizal plants when grown in a soil with a high level of salinity.

Starch and total carbohydrate concentrations in leaves and roots have been generally shown to be reduced by salt (Ezz and Nawar 1994). Inoculation of plants with AM fungi under saline conditions generally increased the accumulation of leaf and root carbohydrate (Ezz and Nawar 1994), including proline and total free amino acids. In contrast, Aboul-Nasr (1999) found that proline accumulation was considerably less for mycorrhizal plants than for non-mycorrhizal plants (Aboul-Nasr 1999). AM fungi may also influence some plant hormones (Ruiz-Lozano et al. 1996) and improve water uptake (Augé 2001) leading to increased growth and subsequent dilution of toxic ion effects (Al-Karaki 2001; Ruiz-Lozano et al. 1996). Furthermore, AM fungi have been found to affect the activity of some enzymes. Polyphenol oxidase increased with AM fungi inoculation, but peroxidase activity was not affected (Ezz and Nawar 1994; Santos et al. 2001).

20.3.3 Mechanisms of Improved Tolerance of Plants to Salinity

Various mechanisms of salt tolerance by AM fungi have been proposed. These are (a) improved plant mineral nutrition and/or increased leaf sequestration of chlorides (Copeman et al. 1996; Feng et al. 1998; Juniper and Abbott 2003); (b) altered plant water balance such as reduced water stress of the host plants and dilution of toxic ions such as sodium (Na) and chloride (Cl^-) (Gupta and Mukerji 2000; Juniper and Abbott 2003); (c) compartmentation of ions (Jennings and Burke 1990); and (d) osmotic adjustment by production of compatible solutes by the plant (Jennings and Burke 1990; Hampp and Schaeffer 1995).

The mechanisms of salt tolerance by AM fungi include improved plant nutrition, especially P, or enhanced acquisition of low-mobility nutrients (Al-Karaki 2001; Ruiz-Lozano and Azcon 2000). Maintenance of a high K:Na ratio in shoots has been investigated as a mechanism of salt tolerance (Cakmak 2005). The increasing

K concentration in mycorrhizal plants could be an indirect effect associated with better P nutrition in mycorrhizal plants (Poss et al. 1985).

Potassium could alleviate detrimental effects of salt stress because the impairment of K nutrition is a main characteristic of plants under salt stress (Cakmak 2005). At the cellular level, K deficiency might contribute to salt-induced oxidative stress and related cell damage. Accordingly, improving K nutrition of salt-stressed plants could reduce cell damage (Cakmak 2005). Therefore, increased K or decreased Na concentration in mycorrhizal plants are also believed to increase plant salinity tolerance because the internal K:Na ratio increases (Rinaldelli and Mancuso 1996).

Higher water potential of tomato xylem and improved K nutrition associated with AM fungi indicate that mechanisms other than increased P nutrition may be important for mycorrhizal plants grown under saline stress (Poss et al. 1985). Possible mechanisms for the enhancement of salt tolerance by AM fungi are an effect of mycorrhizas on reducing water stress of plants (Al-Karaki 2001; Augé 2001) and dilution of toxic ions such as Na and Cl^- (Al-Karaki 2001; Gupta and Mukerji 2000; Ruiz-Lozano et al. 1996).

Osmotic adjustment is defined as the capacity of the internal concentration of solutes to increase in response to a decrease in external water potential, particularly due to an increase in salinity (Jennings and Burke 1990). This is one of the best known responses of plants to salinity stress, where plants accumulate soluble, low-molecular-mass solutes such as proline and betaine (Ben Khaled et al. 2003; Ruiz-Lozano et al. 1996). Osmotic adjustment by use of compatible solutes assists salinised plants in the maintenance of leaf turgor and other physiological processes such as photosynthesis, transpiration, conductance and water-use efficiency (Augé 2001; Ruiz-Lozano et al. 1996). Correspondingly, one of the mechanisms of adaptation of fungi to high concentrations of salt is osmotic adjustment by the synthesis of compatible solutes such as polyols (glycerol, mannitol, arabitol, sorbitol) and accumulation of proline and betain. These compatible solutes increase in concentration in the cells of many fungi in response to salinity (Jennings and Burke 1990; Hampp and Schaeffer 1995; Naidu 1998).

20.3.4 Interactions Between Organic Matter and AM Fungi

Organic matter is known to have variable effects on AM associations (Soedarjo and Habte 1993). AM fungi may use soil organic C as an energy source (Caravaca et al. 2002b). Apparent preferential associations between AM hyphae and organic-rich microsites have been attributed to the nutrient-rich status of the sites (Gryndler et al. 2005). In contrast, mycorrhizal tissue (particularly hyphae) has been estimated to comprise a significant fraction of soil organic matter or has potential to form a sink or source of C (Treseder and Allen 2000). These examples demonstrate that potential interactions between organic matter and AM fungi are complex.

Interactions between organic matter and AM fungi in nonsaline environments can be either positive (the majority of cases) or negative.

Generally, AM fungal inoculation (with the assumption that these fungi were salt tolerant), in association with organic amendments, increased the abundance of mycorrhizal propagules in soil, leading to potential benefits at later stages of the revegetation process (Palenzuela et al. 2002). Furthermore, investigations of interactions between organic matter and AM fungi may contribute information that is important for the management of plants in soils with low levels of organic matter and nutrients (Gryndler et al. 2002). In the short-term, the use of organic matter and AM fungi together can increase various physical, chemical and biochemical parameters of rhizosphere soil contributing to improved soil quality (Caravaca et al. 2003).

In nonsaline soil, the beneficial effect of the addition of a combination of organic matter and AM fungi is most likely to be due to the reactivation of microbial activity (Caravaca et al. 2002b), including AM fungi. In saline soil, the mechanism of the interaction between organic matter and AM fungi is not simple. As high salinity negatively affects microbial activity (Batra and Manna 1997), an additional step with the aim of lowering the water table and reducing salinity needs to be put into place prior to management of the AM fungi and other soil microorganisms. Incorporation of organic matter (or mulching) could reduce salinity in the top soil by preventing capillary salt rise.

After salt in the soil profile is decreased and because organic matter and AM fungi have combined effects on the improvement of soil physical (increased aggregate stability and porosity) and biochemical (as source of carbon and nutrients or production of enzymes to access nutrient from organic matter) properties, the activity of soil microorganisms is expected to improve. This increased activity of microorganisms may include the reactivation of AM fungi. Consequently, the interaction between organic matter and AM fungi could increase the water balance, thereby enhancing the dilution of toxic ions.

Finally, AM fungi may contribute to nutritional benefits to plant growth in saline soil. The effect of environmental factors such as rainfall and temperature (or season) is an important point that might influence the interaction between organic matter and AM fungi in saline soil. In hot and dry conditions, soil is likely to be saline, but AM fungi may be present even though they are inactive. In wet conditions, the salt concentration is likely to be lower, hence enabling mycorrhizal to function. Consequently, the possible positive interaction between organic matter and AM fungi depends to a great extent on seasonal change and hydrological status of the saline soil.

20.4 Conclusion

Organic matter incorporation can represent a key focus by which to develop longer term ecologically effective strategies to counterbalance degradation processes such as salinisation in agricultural soil. AM fungi have the potential to play an important role in saline soil, but species of these fungi can be affected by salinity to different extents. There are indications that a combined beneficial effect of organic matter and AM fungi in soil would be due to the creation of soil conditions suitable for the growth of hyphae and increased microbial activity overall.

Spatial and seasonal variations of AM fungi infectivity in saline soil are likely to complicate efforts to maximise the benefit of AM fungi in saline soil. Furthermore, there is uncertainty as to whether environmental or other factors such as plant distribution have the most influence on the distribution and infectivity of AM fungi in saline soil. Therefore, assessment of soil salinity and AM fungi across space and time will enable better evaluation of the potential role of AM fungi in rehabilitation of saline soils. It is possible for AM fungi to be present but inactive when the soil is saline (as in summer in a Mediterranean climate) and active after rain has diluted the salt. Thus, inoculation might only be necessary to overcome spatial rather than temporal heterogeneity in the distribution of mycorrhizas in saline soil in this type of environment.

Both organic matter and AM fungi alone can be effective in the amelioration of the effects of salinity on the growth of agricultural plants. However positive synergistic interactions between organic matter and AM fungi mean that AM fungi could be more effective in reducing the effect of salinity in the presence of organic matter.

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Chapter 21
Use of Mycorrhizal Fungi for Forest Plantations and Minesite Rehabilitation

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21.1 Introduction

The integral role of mycorrhizal symbioses in natural and managed ecosystems has been widely recognised. In the recent decades, more attention has been paid to establishing efficient mycorrhizal fungi on plants at the nursery or seedling stage for forest plantations and minesite rehabilitation. The number of people depending on forests for their livelihoods reaches 1.6 billion, including some 300 million living in them (FAO 2012). Forestry plantations have become an increasingly important supply for wood during the era of rapid deforestation of primary habitats. Due to the increasing demand for consumption of plant products, including timber, fuel wood, leaves, twigs, fruits and other non-wood products, forest plantations have been established largely in recent decades. Exotic trees are preferred over native ones because of their shorter rotation, well-studied biology and paucity of pests in new

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habitats. Among the hundreds of commercial trees, species of *Eucalyptus*, *Pinus* and *Acacia* dominate in forestry plantations worldwide (West 2006). On the other hand, exploitation of mineral resources around the world leaves many closed minesites facing difficulties and challenges in land rehabilitation. It has been recognised that mycorrhizal technology can profitably be applied in forest plantations and land restoration as well as agricultural and horticultural crops for better nutrient utilisation and more effective land use. Introduction of appropriate mycorrhizal symbioses to improve the soil and crop productivity permits a satisfactory reduction of chemical fertilisers and pesticide inputs, thus offsetting ecological and environmental concerns. Appropriate symbiotic fungal partners are critical for some plants to become established, grow normally and withstand better in adverse climatic and soil conditions such as high temperatures and infertile, salinity or polluted soils (Behie and Bidochka 2013). Recent studies suggest that mycorrhizal fungi may exhibit some degree of heavy metal tolerance and, as a result, confer heavy metal tolerance in host plants (e.g. Zaefarian et al. 2013). The conceptual background including the biology and ecology of mycorrhizal symbioses is well addressed in the literature and in other chapters of this volume. This chapter discusses developments and insights regarding the potential of mycorrhizal technologies in forest plantations and minesite rehabilitation with particular reference to the ectomycorrhizal (ECM) fungi.

21.2 Constraints in Plantation Establishment

New plantations may need to be established in cleared forest lands, denuded uplands, contaminated areas or minesites either for the production of timber, wood chips and non-wood products or for revegetation and rehabilitation. Climatic characters and soil physical and chemical properties should be taken into account to integrate the plantation and vegetation programmes. The utilisation of local species for minesite revegetation takes advantage of the inherent attributes of fitness conferred on those species by natural selection, and thus the species are assumed to adapt to local climatic, edaphic and ecological processes (Corbett 1999). The mined environment, however, may be hostile to some local species, and the importance of selecting species suited to the 'new' local conditions must be recognised. Impediments limiting the establishment of new plantations include abiotic factors, such as high temperature, infertile soil, alkaline or salinity soil and heavy metal-polluted soil, and biotic constrains, such as risk of soilborne pathogens and lack of plant beneficial microbes (e.g. mycorrhizal fungi, nitrogen-fixing bacteria). Among these, saline soil is one of the most common types of devastated land, with some 932 million hectares in arid and semiarid regions affected by severe salt accumulation (Summer et al. 1998). The diversity of mycorrhizal fungi in alkaline–saline soil is known to be low (Ishida et al. 2009) and is also affected by various abiotic and biotic environmental factors (Chai et al. 2013).

Low mycorrhizal diversity is common in many new commercial plantation sites (Chen et al. 2007). Fast-growing exotic species are preferred for forest plantations. Establishment and nutrient acquisition strategies of those species are likely to be highly dependent on ECM fungi. Several reports claim that plantations of *Pinus* species fail in the absence of co-introduced symbiotic fungi in exotic habitats, and subsequent success in re-establishing plantations with mycorrhizal seedlings suggests that the failure was due to lack of compatible ECM fungi in the soil (Kohout et al. 2011; Liu and Chen 2007). The diversity of ECM fungi is found to be low in exotic plantations, such as in eucalypt plantations in south China (Chen et al. 2007; Dell et al. 2002). Inoculation with appropriate ECM fungi promotes tree survival and growth of eucalypt plantations in exotic lands (e.g. Chen et al. 2000a; Grove and Le Tacon 1993). The introduction of compatible mycorrhizal fungi is recommended to the new plantation sites along with the plant species when mycorrhizal status in the soil is poor.

Large-scale surface mining represents severe ecological disruption at the landscape level. Soil disturbance, associated with stripping and respreading of topsoil during mining, is known to reduce the diversity and propagule levels of ECM fungi (Malajczuk et al. 1994), arbuscular mycorrhizal (AM) fungi (Jasper et al. 1989), ericoid (Hutton et al. 1997) and orchid mycorrhizal fungi (Collins et al. 2007). Plant species in natural woodland communities surrounding the Ranger lease area of northern Australia are dominated by ECM fungi (Corbett 1999; Reddell and Milnes 1992). With a large-scale population survey of glomalean fungi in disturbed and natural habitats in tropical Australia, Brundrett and Ashwath (2013) concluded that the diversity of AM fungi was substantially lower in disturbed sites than in natural habitats. This reduced diversity in young sites may have resulted from limitations in their dispersal mechanisms resulting in delays in fungal introductions, the absence of appropriate host plants or the inability of fungi to adapt to site conditions resulting in establishment failure (Malajczuk et al. 1994). Establishment of symbiotic microorganisms is often recognised as one of critical issues for the success of minesite rehabilitation (Corbett 1999).

21.3 Applications of ECM Fungi

A number of factors associated with the use of mycorrhizal fungi need to be considered in inoculation programmes for the establishment and production of forest plantations, land revegetation and minesite rehabilitation. The criteria for selecting the optimal fungus, the compatibility of the fungus and host, the suitability of the fungus to the site and the ease of inoculum production must be achieved before large-scale application in the field (Rincón et al. 2001). Once compatibility of the plant and fungus is established, the development of suitable methods for inoculum production and application is necessary. This section therefore analyses advances of the use of various kinds of inoculants, mycorrhization and evaluation, and inoculation effectiveness.

21.3.1 Sources of ECM Inoculants

Various types of ECM fungal inoculants have been developed and tested for applications in plantation nurseries and field trials. Sources of ECM inoculants can be categorised generally as (1) natural inoculants in the form of either airborne spores or colonised soil, (2) mycorrhizal seedlings, (3) vegetative inoculants of ECM fungal mycelium and (4) spores. Because the merits may vary among each kind of inoculum, selection of appropriate types of inoculants for particular application case is recommended. Here the advantages and limitations of each kind of inoculants are compared along with particular references for their use in forest nurseries and the field.

The use of natural inoculants is a simple and effective practice for introducing ECM fungi into new plantation sites. The advantages and disadvantages of the use of natural inoculants have been reviewed by Kendrick and Berch (1985). The practice of natural airborne spore inoculum, however, relies largely on the season in which ECM fungi produce fruiting bodies, and therefore this is unsuited to forestry applications when considering restriction of the availability and the low level of inoculum. Obvious disadvantages of the use of soil inoculants include the risk of introducing diseases and unsuitability for large-scale applications due to the difficulties in transportation of heavy bulky soil to new sites (Kendrick and Berch 1985). For example, hemlock (*Tsuga canadensis*) seedlings propagated in hemlock forest soils had good ECM colonisation, and growth increment of outplanted mycorrhizal plants was observed when comparing seedlings raised in sterilised field soil (O'Brien et al. 2011). Using *E. urophylla* seedlings as bait in a bioassay experiment, Chen et al. (2007) determined inoculation potential of ECM fungi in field soils from various locations in south China where eucalypt plantations are being established. Four morphotypes of ECM were identified (Fig. 21.1) including an indigenous *Laccaria* species which also produced basidiomes in one soil. However the poor colonisation suggested low level of ECM fungal inoculants in the field soils.

Transplanting of mycorrhizal seedlings colonised by a known ECM fungus is another approach to introduce compatible fungal partner to its desired host species. This method has been used especially in the establishment of new mushroom orchards. Attempts to grow edible mycorrhizal mushrooms (EMMs) commenced on the Périgord black truffle (*Tuber melanosporum*) in France and in Italy by transplanting truffle-colonised seedlings raised in forest nurseries (Chevalier and Frochot 2000). This technique has been extensively used in establishment of truffière in European countries and also in Asian and Oceanic countries (Chap. 23). Mycorrhizal seedlings of *Pinus yunnanensis* and *P. armandii* colonised by matsutake (*Tricholoma matsutake*) are outplanted into the matsutake-producing forests in southwest China aiming to the increased mushroom production (Chen 2004). Quality control of mycorrhizal seedlings from nurseries is critical to ensure the extent of colonisation of the proposed fungus and to reduce the risk of introduction of pests and pathogens along with the mycorrhizal seedlings.

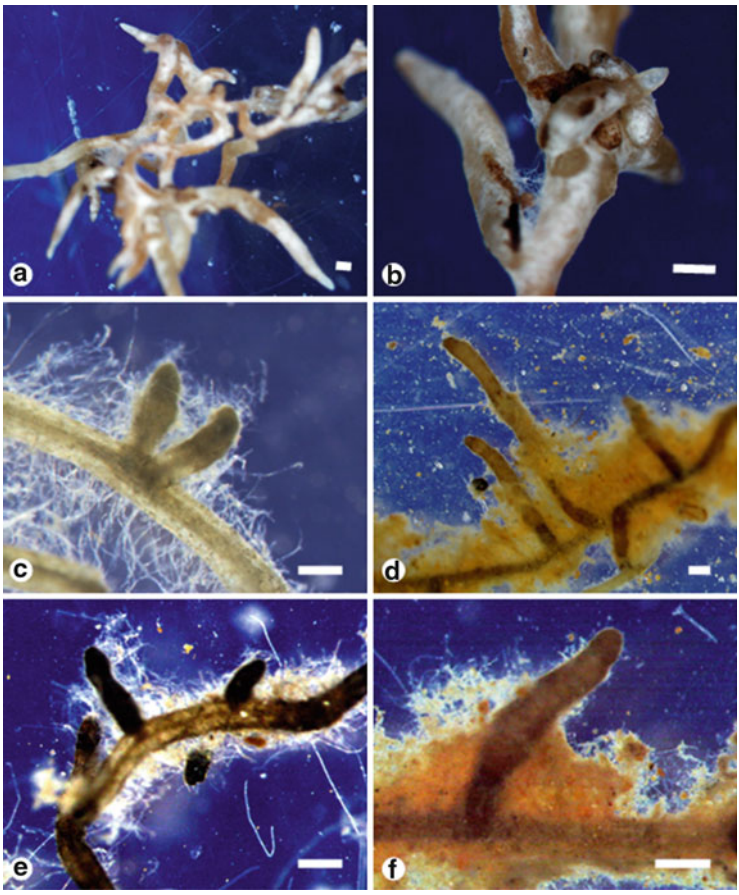

Fig. 21.1 Ectomycorrhizal (ECM) tips on nursery-grown seedlings of *Eucalyptus urophylla* viewed under light microscope. (**a**, **b**) Typical ECMs formed by *Scleroderma*; (**c**) *white* ECM tips formed by *Laccaria* (ECM I); (**d**) *yellowish Pisolithus*-like ECMs (ECM II); (**e**) *jet-black* ECMs formed by an unknown fungus (ECM III); (**f**) *brown* to *dark brown* ECMs formed by an unknown contaminant (ECM IV) (*bar* = 1.0 mm). This figure is extracted from Ying Long Chen's PhD Thesis (2006, Murdoch University, Australia)

Vegetative inoculants of fungal mycelium produced in axenic culture on either solid or liquid medium have been frequently used in plantation nurseries (Brundrett et al. 1996; Kendrick and Berch 1985). The merits of using mycelial inoculants in forests include the facts of known fungal species or isolates involved, the absence of pests and pathogens and the year-round availability. However, cultivation of ECM fungi is expensive because some fungi are difficult to isolate or grow slowly in pure culture. Molina and Palmer (1982) tested a wide range of ECM fungi and found that members of 18 genera, such as *Amanita*, *Boletus*, *Cortinarius*, *Hebeloma*, *Laccaria*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, *Suillus* and *Tricholoma*, are fairly easy to isolate for axenic cultivation. Attempts to produce mycelium inoculants

from a few other ECM fungi (e.g. *Gomphidius*) were unsuccessful, while many are yet to be attempted. Kendrick and Berch (1985) commented that most ECM fungi could be cultivated as long as the nutritional and other growth conditions meet the requirements by the fungus for its development.

Supriyanto (1999) tested the effectiveness of some ECM fungi in alginate beads in promoting the growth of several dipterocarp seedlings. Since ECM fungi are obligately biotrophic in the natural habitat, vegetative inoculants are grown very slowly through the soil before colonising a suitable host root (Kendrick and Berch 1985). The survival, competition with indigenous organisms and persistence in the soil of the introduced vegetative inoculants require further examination. Another drawback is that the storage of mycelial inoculants usually adversely influences its effectiveness, while large quantities of viable inoculants are needed for application on an operational scale. These impediments shadow the use of cultivated ECM fungal mycelium in a wider forestry practice.

Fungal spore inoculants including spores or sclerotia specifically collected for the purpose are commonly used because of the ease of application in plantation nurseries and the availability of large quantities of spores from a few sporocarps (Chen et al. 2007; Dell et al. 2002; Marx and Cordell 1990). It is estimated that the top 5-cm soil in a Douglas fir (*Pseudotsuga menziesii*) stand contained 2,785 kg ha^{-1} dry weight of *Cenococcum geophilum* sclerotia (Fogel and Hunt 1979). Hunt and Trappe (1987) found that in a western Oregon Douglas fir stand, the production of sporocarps of *Hysterangium setchellii* was up to 3,770 ha^{-1}, i.e. 842 g dry weight ha^{-1}. The main disadvantages of spore inoculants are genetic variability, the lack of reliable laboratory methods to determine spore viability and the delay in mycorrhization compared with vegetative inoculants (Brundrett et al. 1996; Chen et al. 2006a). Spores of ECM fungi are generally harder to germinate than are those of saprophytes. Germination is often promoted by the presence of other microbes, growing hyphae of same species or activated charcoal. Coating spores individually on to roots of young seedlings of *P. radiata* enhances germination success to about 30 % in both *Suillus* and *Rhizopogon* (Theodorou and Bowen 1987). Fries (1988) found abietic acid, a diterpene resin acid, in pine roots induced germination of *Suillus* spores. *Descomyces* spores are known to have slow growth, mostly aerial with some submerged hyphae. In contrast, *Pisolithus* and *Scleroderma* spores have moderate to rapid growth, primarily aerial, readily culturable.

Basidiocarps of ECM fungi do not necessarily mature when inoculation programmes are required in the nursery. Therefore, it is often necessary to collect and store spores for a considerable time. Spores of several ECM fungi can tolerate long storage periods. Dry spores of *Pisolithus* could be stored at 5 °C for up to 34 months without significant loss of spore viability (Marx 1976). Castellano and Molina (1989) found that *Rhizopogon* spores could be stored for up to 3 years. *Scleroderma* spores kept at 4 °C for 5 years germinated and formed mycorrhizas on six important plantation tree species and showed effective in producing mycorrhizas as freshly collected spores (Chen et al. 2006a). Torres and Honrubia (1994) examined the viability of basidiospores of 14 species in the genera *Cortinarius*, *Hebeloma*, *Inocybe*, *Laccaria*, *Rhizopogon*, *Russula*, *Suillus* and *Tricholoma* using

nuclear and fluorescein diacetate staining methods. They observed nearly total loss of viability of all fungi stored in refrigerated or frozen slurries after 6 months. However, many successful mycorrhizal experiments have been performed using spore suspensions previously stored at either room temperature or low temperature for up to 10 months (e.g. Chen et al. 2006c; Duñabeitia et al. 2004). Cold treatment enhances spore germination and mycorrhization of *Tuber* (Chen 2002; Chevalier and Frochot 2000).

Spore preparations of *Pisolithus tinctorius* are being produced for commercial use as pellets, sprays or encapsulated on seed (Martin et al. 2003; Marx et al. 1989). Plantation nurseries commonly prefer to handle spore inoculants due to the ease of inoculum production and delivery. Spores of *Pisolithus* and *Scleroderma* collected from the field in south China are being used to effectively inoculate clonal eucalypts in commercial nurseries. Inoculation with a spore rate as low as 10^4 spores seedling^{-1} is appropriate for eucalypt seedlings to form mycorrhizas in containerised nurseries (Chen et al. 2006b). The greatest increases in ECM formation occurred at low to medium spore densities, and there was no inhibition of mycorrhizal development at the highest spore density used (10^8 spores seedling^{-1}). By contrast, Marx (1976) inoculated *P. taeda* seedlings with *Pisolithus tinctorius* basidiospores and found a density of 5.5×10^7 spores per 800 cm^3 soil produced significantly more ECMs than other spore densities tested. Similarly, Torres and Honrubia (1994) observed threshold spore densities when inoculating *P. halepensis* seedlings with basidiospores of *Pisolithus*, *Rhizopogon* or *Suillus*, beyond which high spore densities reduced ECM formation and seedling growth. The recommended dose used for nursery inoculation is usually 10^4–10^8 spores seedling^{-1}. This recommendation has been applied for a range of fungus–host partners: *Abies* (*Scleroderma*) (Parladé et al. 1997), *Afzelia* (*Lactarius*, *Pisolithus*, *Russula*, *Suillus*) (Munyanziza and Kuyper 1995), *Eucalyptus* (*Cortinarius*, *Hydnangium*, *Laccaria*, *Pisolithus*, *Scleroderma*) (Chen et al. 2000a, b; Lu et al. 1998), *Pinus* (*Lactarius*, *Melanogaster*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, *Suillus*) (Duñabeitia et al. 2004; Marx et al. 1989; Ortega et al. 2004; Rincón et al. 2001), *Pseudotsuga* (*Melanogaster*, *Rhizopogon*, *Tuber*) (Parladé et al. 1997) and *Quercus* (*Pisolithus*) (Martin et al. 2003; Marx et al. 1997; Parladé et al. 1997).

Spore inoculants of *Scleroderma* were effective in forming mycorrhizas on eucalypts when a suitable spore density was applied (Chen et al. 2006b). It is not known how many spores germinated in the glasshouse and resulted in mycorrhizal formation with seedlings of commercial plantation species such as *Acacia*, *Eucalyptus* and *Pinus*. Generally, it would be desirable to measure the percentage of spores able to germinate in inoculants being tested. Spores of several *Scleroderma* spp. were taken from sporocarps prior to spore release to test germination in vitro on agar in the presence of eucalypt roots. Where spores germinated, the viability varied from 0.1 to 0.8 % in *Scleroderma* (Chen, unpublished data).

21.3.2 ECM Formation and Evaluation

The development and formation of mycorrhizal associations involves physical, molecular and physiological interactions between the host plant root and the fungal hyphae. Experimental work of Horan and Chilvers (1990) using compatible and incompatible isolates of *Pisolithus tinctorius* and *Paxillus involutus* indicated that specific root exudates may be involved in the ECM formation. It is known that root-released chemical compounds, such as cytokinins and other hormones, promote hyphal branching and growth (Gogala 1991). The presence of fluorescent pseudomonads, so-called mycorrhizal helper bacteria (MHBs), enhances the rate and extent of ECM formation (Garbaye 1994). Some symbiosis-related proteins (i.e. ectomycorrhizins) are found in *E. globulus–P. tinctorius* association (Hilbert and Martin 1988). Hydrophobins have also been strongly implicated in hyphal recognition (Talbot et al. 1993). Smith and Read (2008) concluded that molecular probing with hydrophobin genes or genes for specific membrane transport proteins or host defence responses would be likely to prove valuable, especially using plant–fungus associations with different levels of compatibility. Establishment of functional links between gene expression and the key events of recognition and mycorrhiza synthesis remains challenging.

Horan et al. (1988) developed the paper–sandwich method to investigate synchronous colonisation of lateral roots over a period of days. The cellophane-over-agar method of Malajczuk et al. (1990) enables the same sequence of events to be completed on the primary root within hours. Mycorrhizas synthesised in this way have permitted investigations of fungal and host physiology and interactions, such as rapidity and extent of mycorrhization, host specificity, nutrient uptake and responses to biotic and abiotic stresses.

Hartig net formation is considered as a good indicator of fungus–host compatibility and is correlated with growth responses. A typically compatible fungus–host pair has mantle on the thick-branched roots viewed with eye, well-developed mantle and Hartig net, with elongated epidermal cells or hyphae extending into the cortex (Chen et al. 2006c). Thin roots may or may not have a mantle, often with a wound reaction and no Hartig net indicating incompatible. Partially compatible pair produces branched roots (thicker or not) with or without mantle, in which Hartig net may or may not develop, and limited hyphal penetration between epidermal cells. Superficial ECM has thin roots and thin mantle; although Hartig net is present, generally it is thin due to the lack of expansion of epidermal cells (Chen et al. 2006c). Different fungus–host combinations may have different structure of ECM. Eucalypts tend to have a typical morphology of a well-developed epidermal Hartig net and thick mantle. *Cortinarius* and *Hysterangium* species often form thin mantles and superficial sheathing mycorrhizas on eucalypts indicating host incompatibility (Malajczuk et al. 1987).

Numerous parameters are adopted for quantifying the degree of mycorrhization. Parameters include root length, ECM root tip density (numbers of ECM root tips in a unit soil volume), ramification indices (numbers of ECM root tips per cm root),

frequencies (percentage of ECM root tips over the total root tips), specific root length (cm root g^{-1} root fresh mass), root length density (cm root ml^{-1} soil) and mycorrhizal dependence (dry biomass of mycorrhizal plant over non-mycorrhizal plant).

21.3.3 ECM Fungus–Host Specificity

It is generally accepted that ECM fungi are often host specific in spite of contradictory observations concerning host specificity in some fungal genera (Table 21.1; Cairney and Chambers 1999; Jairus et al. 2011; Zhou and Hyde 2001). Molina and Trappe (1994) recorded three general responses among 20 species of *Rhizopogon* on *Pseudotsuga menziesii*, *Pinus contorta* and *Tsuga heterophylla*: strong specificity to *Pseudotsuga menziesii*, specificity or strongest development on *Pinus contorta* and an intermediate response where ECM formed on two or three of the hosts. The unsuccessful pure culture syntheses make *Eucalyptus–Rhizopogon* association seem unlikely (Chilvers 1973). With few exceptions, field observations and pure culture syntheses confirm the specificity of *Rhizopogon* species for Pinaceae (Molina and Trappe 1994). A few species of *Hysterangium* are widely distributed, but some often display high levels of endemism and discrete host ranges, such as *Quercus*-specific species (Parladé et al. 1997; Rincón et al. 2001). Malajczuk et al. (1987) described the superficial ECM of *Hysterangium inflatum* with *E. diversicolor* as having abundant calcium oxalate crystals on its hyphae although the fungal mantle was only one to five cells deep.

We examined the compatibility of 15 *Scleroderma* collections to form mycorrhizas with seedlings of six plantation trees (*Acacia mangium*, *A. mearnsii*, *E. globulus*, *E. urophylla*, *Pinus elliottii* and *P. radiata*) in a nursery potting mix (Chen et al. 2006c). Observations on mycorrhizal structure confirmed that most collections were able to aggressively colonise eucalypts and pines, while roots of acacias were poorly colonised. The findings demonstrated that the Australian collections were more effective in colonising short roots on eucalypts than the Chinese collections.

Plantation tree species may also perform differently in establishing symbiosis with ECM fungi. Studies showed that *Pinus* spp. had difficulty to establish ECM associations with native fungi in tropical habitats (Walbert et al. 2010). Experimental work also confirms that some *Eucalyptus* species perform better with their co-introduced fungi than with locally available mycobionts, emphasising the importance of a long-term coevolution and enhancement of histological and functional compatibility (Chen et al. 2007; Malajczuk et al. 1984). Compatibility in ECM associations with potential host range deduced from laboratory experiments may not reflect that under field conditions. Thus, the concept of ecological specificity (Molina et al. 1992), embracing all environmental abiotic and biotic factors that affect the ability of plants to form functional ECM with particular fungi, may

Table 21.1 Host range of some key ECM fungal genera

Fungal genus	Host genus
Amanita	*Abies; Allocasuarina; Betula; Carpinus; Castanea; Castanopsis; Casuarina; Eucalyptus; Fagus; Larix; Monotropa; Nothofagus; Picea; Pinus; Platanus; Polygonum; Pseudotsuga; Quercus; Salix; Tilia; Tsuga*
Cantharellus	*Abies; Betula; Carpinus; Castanea; Corylus; Eucalyptus; Fagus; Picea; Picea; Pinus; Populus; Pseudotsuga; Quercus; Shorea; Tsuga*
Cenococcum	*Abies; Acer; Eucalyptus; Juniperus; Larix; Pinus; Pseudotsuga*
Hebeloma	*Alnus; Arbutus; Arctostaphylos; Betula; Castanea; Cistus; Dryas; Larix; Picea; Pinus; Pseudotsuga; Quercus; Tsuga*
Hysterangium	*Arbutus; Arctostaphylos; Larix; Pinus; Pseudotsuga; Tsuga*
Laccaria	*Abies; Betula; Betula; Dipterocarpus; Eucalyptus; Fagus; Larix; Leptospermum; Nothofagus; Picea; Pinus; Pseudotsuga; Quercus; Salix; Tilia; Tsuga*
Lactarius	*Alnus; Arbutus; Arctostaphylos; Betula; Eucalyptus; Fagus; Picea; Pinus; Pseudotsuga; Quercus; Salix; Tsuga*
Paxillus	*Allocasuarina; Alnus; Betula; Castanea; Dryas; Eucalyptus; Fagus; Picea; Pinus; Populus; Pseudotsuga; Quercus; Salix*
Pisolithus	*Abies; Acacia; Afzelia; Allocasuarina; Alnus; Arbutus; Arctostaphylos; Betula; Carya; Castanea; Castanopsis; Casuarina; Eucalyptus; Hopea; Larix; Pinus; Populus; Pseudotsuga; Quercus; Tsuga*
Rhizopogon	*Adenostoma; Pinus; Pseudotsuga; Tsuga*
Scleroderma	*Abies; Acacia; Afzelia; Alnus; Betula; Brachystegia; Carya; Casuarina; Eucalyptus; Hopea; Isoberlinia; Larix; Picea; Pinus; Populus; Pseudotsuga; Quercus; Shorea; Tsuga; Uapaca*
Suillus	*Arbutus; Arctostaphylos; Larix; Larix; Monotropa; Picea; Pinus; Pseudotsuga; Pseudotsuga; Quercus*
Thelephora	*Abies; Acacia; Allocasuarina; Alnus; Arbutus; Arctostaphylos; Betula; Castanea; Castanopsis; Casuarina; Eucalyptus; Fagus; Hudsonia; Larix; Lithocarpus; Picea; Pinus; Pinus; Populus; Pseudotsuga; Quercus; Salix; Tsuga*
Tricholoma	*Abies; Castanopsis; Cedrus; Lithocarpus; Picea; Pinus; Pseudotsuga; Quercus; Tsuga*
Tuber	*Abies; Alnus; Carpinus; Carya; Castanea; Cedrus; Cistus; Corylus; Fagus; Fumana; Helianthemum; Ostrya; Picea; Pinus; Populus; Pseudotsuga; Quercus; Salix; Tilia*

explain why *Suillus* species may exhibit a broader host range under experimental conditions than observed in nature (Dahlberg and Finlay 1999).

Host plant specificity is considered one of the most important factors influencing fungal diversity, particularly for ECM fungi. Nelson (1979) suggested that evolutionary and ecological processes that determine specificity act differently on hosts and their fungal symbionts. Thus, the level of host specificity among mycorrhizal fungi is dynamic and depends not only on symbiotic partners but also on ecological opportunities (Zhou and Hyde 2001). A concern on the invasion of exotic fungi due to host shift has been raised. An Australian fungus *Laccaria fraterna* can colonise European Cistaceae in natural conditions indicating occurrences of host shifts and

invasion of exotic fungal species (Jairus et al. 2011). Host shifting among the Australian and particularly native African ECM fungi in mixed eucalypt plantations in Zambia, south-central Africa, has been identified by analysing rDNA and plastid intron sequences. *Amanita muscaria*, a mycobiont of Pinaceae and Fagaceae, has only recently become invasive in Australia and New Zealand despite two centuries of known introduction history (Orlovich and Cairney 2004). Jairus et al. (2011) recommended that exotic forestry plantations could ideally be established by use of seeds of seedlings pre-inoculated with native ECM fungi, preferably edible mycorrhizal mushrooms (see Chap. 23) as a case in Zambia, to reduce the potential for microbial invasion and encourage utilisation of forestry 'by-products'. Thus, availability and compatibility of native fungal resources must be examined to optimise production of exotic tree plantations.

21.3.4 Effectiveness of ECM Inoculation

Numerous measures can be taken to evaluate effectiveness of ECM inoculation. The positive effect of mycorrhizas on plant growth through increased phosphorus availability is well documented (e.g. Smith and Read 2008). Increased tolerance of saline conditions, uptake of zinc, protection against pathogens and enhanced water uptake are some of the other potential benefits conferred by mycorrhizas. Here we discuss inoculation effectiveness in three general aspects: (1) host response, (2) response to abiotic stresses and (3) response to other biotic organisms.

Commercial plantations have a privilege in the use of fast-growing exotic species such as trees of the genera *Acacia*, *Eucalyptus* and *Pinus* (West 2006). For example, more than 100 species of *Eucalyptus* native to Australia and the surrounding islands are used in plantation trails in tropical to temperate regions around the world (Jairus et al. 2011). Inoculating *Acacia*, *Eucalyptus* and *Pinus* with compatible ECM fungi has been shown to be beneficial in many parts of the world (Chen et al. 2006c; Duponnois et al. 2005; Duñabeitia et al. 2004).

ECM fungi can help improve the establishment and productivity of eucalypt plantations in China (Chen et al. 2000b; Dell et al. 2002). A number of ECM fungi, collected from under *Eucalyptus* in Australia, have been introduced in research trials into eucalypt plantations in south China since the 1990s (Chen et al. 2000b; Dell and Malajczuk 1995). The *Scleroderma* genus is favoured for introduction because it readily colonises eucalypt roots in disturbed habitats. It is easy to collect spores of *Scleroderma* from species that form large epigeous basidiocarps and then to produce spore inoculants for nursery inoculation programmes. This fungal genus has potential for application in commercial plantation forests in the region where mycorrhizal status is poor (Chen et al. 2007). Beneficial *Scleroderma* isolates can vigorously compete with other ECM fungi in the field (Dell et al. 2002; Hall et al. 1994; Martin et al. 2003). In plantations of exotic acacias, eucalypts and pines, these fungi are desirable as inoculants if they are compatible with the host tree and are effective in promoting survival and production in the field (Chen

et al. 2007). Eucalypt seedlings inoculated with cold-stored spores of several *Scleroderma* species were taller than those inoculated with fresh spores (Chen et al. 2006b). Plant growth-promoting bacteria (PGPRs) have been isolated from Western Australian sporocarps of some ECM fungi (B. Dell, pers. comm.). The presence of these bacteria in the cold-stored spore slurry may account for the extra growth stimulation of the host.

Benefits from inoculation with *Scleroderma* fungi were also observed on other *Eucalyptus* species, such as *E. diversicolor*, *E. grandis*, *E. pellita*, *E. tereticornis* and *E. urophylla* (e.g. Reddell and Milnes 1992; Chen et al. 2000a). Stimulations of *Scleroderma* inoculation on the growth of other tree genera were obtained, particularly on *Acacia mangium* and *A. holosericea* (Founoune et al. 2002); *Castanopsis hystrix* (Chen et al. 2001); *Pinus caribaea* in axenic culture (Rangarajan et al. 1990); *P. contorta* in mine spoil sites (Fay et al. 1997); *P. kesiya* in forest and degraded soils (Rao et al. 1996); *Hopea odorata*, *Vatica sumatrana*, *Shorea stenoptera*, *Sh. compressa* and *Sh. pinanga* (Santoso 1991); and *Sh. leprosula* cuttings (Omon 1996). These results, however, are controversial to some other reports where no significant stimulation or even depression of some *Scleroderma* fungi on the growths of several hosts was observed (e.g. Seva et al. 1996; Lu et al. 1998). As soil characteristics, particularly soil nutrient levels, may affect spore germination and mycorrhization, there are challenges in practical applications of ECM fungi in nurseries and the field.

Numerous studies suggest that there is a connection between growth enhancement of host plants following inoculation practices and increased phosphorus accumulations in the host (e.g. Jansa et al. 2011). These studies also claim that enhanced P accumulation appears to relate to the level of ECM colonisation and the surface area of the extrametrical mycelial phase. The pioneering studies of Melin and Nilsson (1950) provided the first experimental evidence of P and N translocation through ECM mycelia. Finlay et al. (1988) examined mycelial uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium by *Pinus sylvestris* plants colonised by four different ECM fungi, *Paxillus involutus*, *Pisolithus tinctorius*, *Rhizopogon roseolus* and *Suillus bovinus*. Absorbed NH_4^+ by the fungi appears to be rapidly incorporated into amino acid precursors within the extrametrical mycelium and is translocated to the host. The extensive rhizomorph network of ECM fungi is largely responsible for enhanced nutrient uptake and seedling growth. Environmental factors often have impacts on mycorrhization and consequently alter the inoculation effectiveness on the host plants. The carbon location from the host plants to the fungi is a cost; therefore, under limiting resources the plant–fungi interaction could change from benefit to cost resulting in a continuum of behaviours from mutualistic to antagonistic (Johnson et al. 1997). However, recent studies of Saner et al. (2011) suggest that a light-constrained environment may not influence seedling growth due to ECM colonisation.

The external mycelium of ECM fungi transports water to the host plant (Duddridge et al. 1980). Radiate pine inoculated with *Rhizopogon roseolus* and *Scleroderma citrinum* performed better particularly in a dry site when compared

with non-mycorrhizal plants (Ortega et al. 2004). Analyses of the internal transcribed spacer (ITS) and large subunit (LSU) regions of the ribosomal DNA revealed that pezizalean ECM fungi associated with pinyon pine (*Pinus edulis*) respond positively to dry conditions in drought-stressed woodlands (Gordon and Gehring 2011). A field experiment of Dixon et al. (1983) showed that black oak (*Quercus velutina*) inoculated with *Pisolithus tinctorius* had higher water potentials and higher soil-to-plant conductance than non-mycorrhizal ones consistently over the growing season. The mycorrhizal roots of *Nothofagus dombeyi* accumulated considerably more N and P during drought and had greater activities of glutamate synthase, glutamine synthetase, glutamate dehydrogenase, nitrate reductase and acid phosphomonoesterase than the non-mycorrhizal ones (Alvarez et al. 2009). Read and Boyd (1986) pointed out that rhizomorphs in *Rhizopogon* play an important role in water uptake and movement in ECM systems. Possible mechanisms of mycorrhizal effects on plant water uptake and drought resistance are discussed in Lehto and Zwiazek (2011).

There is an increased interest in the role of ECM fungi in interactions with soil toxicity such as from heavy metals. ECM symbionts display differential effectiveness in providing resistance to metal toxicity. Field studies have shown that sporocarps of ECM fungi are able to accumulate heavy metals in high concentrations when present on metal-polluted sites (Zaefarian et al. 2013). Barcan et al. (1998) reported the Ni concentrations in *Suillus luteus* growing near the Severonickel plant on the Kola Peninsula were up to 40 times the background level. This fungus was also presented in Zn-contaminated soils and was able to grow at concentrations of 1,000 μg g^{-1} (Colpaert and van Assche 1987). Jones and Hutchinson (1986) suggested that the morphology of *Scleroderma flavidum* mycorrhizas was important in providing Ni tolerance to their host plants. Research on metal tolerance of *Suillus* species indicated that mycobionts with an extensive extramatrical mycelium, a thick mantle and massive carpophores may be more suitable to accomplish a filter function than fungi without these features (Colpaert et al. 1992).

There is evidence that many organisms adapt to high levels for one metal indicating a rather specific biochemical mechanism for metal tolerance. Possible mechanisms for passive binding or metabolic detoxification by the mycobiont, which can lead to metal tolerance, are discussed by various researchers (e.g. Zaefarian et al. 2013). Jourand et al. (2010) reported that Ni-tolerant *Pisolithus albus* isolated from nickel mines in New Caledonia strongly enhanced the growth of the host plant *Eucalyptus globulus* at toxic nickel concentrations. These studies suggest that the use of metal tolerant mycobionts for practical inoculation of nursery plants could be helpful for revegetation of heavy metal-polluted sites.

The study of rehabilitation of the bauxite-mined areas in south-western Western Australia involves a successional process, and the re-establishment of vegetation cover and species composition, soil microbial population size and diversity and soil development have been investigated (e.g. Grant and Loneragan 2001; Ward 2000). ECM fungi are likely to follow successional pathways in rehabilitated bauxite mines (Gardner and Malajczuk 1988). A similar pattern was observed in orchid

mycorrhizal (OM) fungi in rehabilitated bauxite mines when a correlation of OM fungi detection rates with litter measurements and other environmental factors that increase with time in the post-bauxite mining landscape (Collins et al. 2007). They conclude that Jarrah forest OM fungi are expected to re-establish at the rehabilitated sites provided there is continued vegetation development. Meharg (2003) claimed that the exudation of organic acids to alter pollutant availability in the rhizosphere could be the only direct evidence of mycorrhizal adaptation to metal cation pollutants. There may be other mechanism of adaptation, but conclusive evidence of adaptive mechanisms of tolerance needs further exploration.

Most ECM fungi studied exhibit optimal growth at a pH of 5 or 6, and high salinity is less toxic to most ECM fungi than others (Bois et al. 2006; Kernaghan et al. 2002). Some pH-tolerant ECM fungi are identified by in vitro cultivating under alkaline and/or saline conditions (Bois et al. 2006; Kernaghan et al. 2002). Ishida et al. (2009) characterised ECM fungal community in alkaline–saline soil (pH 7.8–9.2) in north-eastern China and identified 11 T-RFLP types from 57 ECM root tips suggesting poor fungal diversity. An uncommon ECM fungus, *Geopora* spp., was dominant in this extreme environment. With respect to low-pH environments, acid-tolerant ECM species have also been observed in the tropics. For example, Kasuya et al. (1990) reported the impact of aluminium on ECM fungi and mycorrhizal formation on *Pinus caribaea* seedlings. Marx and Altman (1979) observed an enhanced survival and growth of pine seedlings inoculated with *Pisolithus tinctorius* on acid coal spoils.

The higher pH of Ranger mine spoil of northern Australia due to high concentrations of magnesium sulphate ($MgSO_4$) is considered a significant problem for rehabilitation (Ashwath et al. 1993). The natural dispersal and re-establishment of ECM fungi on Ranger waste rock dump occur at a very slow rate which may significantly impact on the rate of development and the resilience of the plant community in the area (Malajczuk et al. 1994). The importance of mycorrhizas in the establishment and growth of native vegetation has been recognised. Hinz (1997) believes the growth of the dominant woody species *Eucalyptus tetrodonta* is dependent on an effective association with mycorrhizal fungi. Reddell et al. (1993) found that fungal root colonisation increased with age of rehabilitation and that ECM and fungal fruiting bodies were most indicative of the development of rehabilitated areas. Therefore, future research emphasis should be placed on identifying the factors affecting the establishment of viable mycorrhizal populations on mines with extreme pH.

A number of studies have demonstrated the promoting effect of MHBs on mycorrhizal formation (e.g. Dunstan et al. 1998; Garbaye 1994; Mogge et al. 2000). Frey-Klett et al. (2007) revisited the concept of MHB and discussed three critical functions of practical significance. A range of bacteria associated with ECM and their role in improving the host plants is summarised in Reddy and Satyanarayana (2006). Detrimental effects of rhizosphere microbes on mycelial growth and ECM formation have also been investigated. Bending et al. (2002) found that two bacterial isolates, *Burkholderia* and *Serratia*, from *Pinus sylvestris*–*Suillus luteus* mycorrhizosphere, inhibited ECM formation.

The presence of ECM fungi on the roots of trees has repeatedly been shown to confer some protection against the effects of several important root pathogenic fungi. *Boletus bovinus* helped to protect *Picea abies* from *Fomes annosus* (Stack and Sinclair 1975), and *Pisolithus tinctorius* increased the survival rate of *Pinus taeda* seedlings exposed to *Rhizoctonia solani* (Viljoen et al. 1992). Lei et al. (2005) examined antagonistic interactions between a wide range of ECM fungi and root pathogenic fungi in culture experiment. Strong antagonistic interactions between *Suillus grevillei* and *Boletus* sp. and pathogenic fungi *Fusarium solani* and *Rhizoctonia solani* were confirmed by plate-culture experiments and nursery inoculation experiments (Li et al. 2005). Reddy and Satyanarayana (2006) addressed mycorrhizas may also affect herbivores through alteration of plant growth or foliar chemistry or influencing anti-herbivore defences and/or herbivory tolerance (Gange et al. 2005).

21.3.5 Mycosilviculture

Based on the effects on human health, the fruiting bodies of ECM fungi are edible, medicinal or poisonous including suspected poisonous (Chang 2008). Species known to have toxic fruit bodies should not be introduced to new areas as mycorrhizal partners. For example, the Australian Government refused to allow the importation of cultures of *Amanita phalloides*, which is a good mycorrhizal partner, but its basidiomata contains dangerous levels of ibotenic acid (Trappe 1977). In contrast, EMMs with ecological and economic importance are introduced to exotic mushroom orchards. *Tuber melanosporum*, *T. magnatum*, *Tricholoma matsutake*, *Boletus edulis* and *Cantharellus cibarius* are the most expensive and sought-after edible mushrooms, and their biological and ecological characters have been well studied, and attempts for commercial cultivation are in progress (see Chap. 23). The development of a science-based production of EMMs becomes a new industrial crop referred as mycosilviculture although attempts for the majority of EMMs remain a challenge (Savoie and Largeteau 2011). Understanding the ecology of EMMs and the adapted forest management practices appears to be the means to improve natural mushroom production and introduced new species in forest plantations using mycorrhizal seedlings from nurseries. Application of appropriate mycorrhizal technology enables production of valuable forest mushrooms for human consumption; on the other hand, it also promotes the healthy growth of host plants and other products such as timber, hazelnuts, etc. from mushroom orchards.

21.4 Applications of AM Fungi

21.4.1 Source of AM Inoculum

Unlike ECM fungi, AM fungi cannot be grown in axenic culture, and therefore the sources of AM inoculants are restricted to colonised roots, spores or colonised soil mixed with mycorrhizal root segments, spores and hyphae. These forms of inoculants can be derived from naturally colonised soil or from propagation in a dual culture system with host plant. The root-based hyphal network in soils is the primary inoculum for seedlings that become established on natural grasslands. However, the inoculants of natural soil or colonised roots have some profound disadvantages since they may contain more than one mycorrhizal fungus and may also contain pathogenic organisms, as discussed for ECM above. Spores are perhaps the best inoculants for laboratory experiments because the features diagnostic of individual species are present only in the spores developed primarily on extrametrical hyphae. Natural soil of agricultural crops and forests may contain varying numbers of spores of different AM fungal species. It is estimated that the upper 10 cm of soil in an undisturbed *Acer*-dominated hardwood forest in Michigan contained nearly seven million sporocarps ha^{-1} of AM spores (Kessler and Blank 1972). The dual culture using sterile soil with some kind of quality control is believed a practical approach to produce high level of inoculants for commercial applications. A pot culture of *Glomus versiforme* on Sudan grass (*Sorghum vulgare*) can produce up to 1.8×10^7 spores per month over an extended period (Daniels et al. 1981). Spores from colonised soil near the colonised roots collected from field or pot cultures can be extracted using the traditional wet-sieve method. This approach and the later modified techniques are widely used in extracting spores from soils with modifications.

21.4.2 Evaluation and Selection of AM Fungi

Several properties inherent in all symbiotic systems are also required for evaluation in AM associations, including mycorrhizal dependency, compatibility and specificity. These properties in AM are determined by mycotrophy (plant acquisition of nutrients via a fungus), fungal dependency and mycorrhizal dependency of a plant. Considering that over 200 species of AM fungi form associations with most vascular plant species, the combined response diversity of the fungus–plant symbiosis is likely high. It is generally accepted that AM fungi have no or limited host specificity as they can associate with a wide range of host plants. However, AM fungi are believed to have a certain type of specificity termed ‘functional compatibility’ (Gianinazzi-Pearson 1984) or ‘ecological specificity’ (McGonigle and Fitter 1990) since the extent of colonisation on plant roots may vary among different fungus–host partners.

The extent of AM fungi colonising roots together with propagules produced in soil can be detected using appropriate approaches. The forms of AM fungi occur in the roots are as hyphae, arbuscules and/or vesicles (except for *Gigaspora* and *Scutellospora*) and in the soil as spores, sporocarps and hyphae. Propagules of AM fungi include colonised roots, spores or sporocarps, dead root fragments, other colonised organic materials and networks of hyphae in soil, which are sources of AM inoculum. Techniques have been developed for assessing the level of root colonisation, quantifying spores and determining inoculum potential in the soil (Abbott and Robson 1977). Colonisation characteristics can be assessed using the magnified intersection method (McGonigle et al. 1990). The incidences of some microscopic features of AM root at each intersection between the root and the crosshair can be noted to calculate the percentage incidence of each structure over total colonised intersections. Total proportion of root length that was colonised was based on the presence of any mycorrhizal structure. AM features which can be measured using the McGonigle's method include intraradical hyphae, arbuscules, intraradical spores (thick-walled structures, often occluded by a septum or plug, typical of those found in *G. intraradices*), hyphal coils, vesicles (thin-walled sac-like structures lacking occlusion, typical of fungi in the genus *Acaulospora*), entry points and external hyphae (Tibbett et al. 2008).

Bioassays using bait plants grown in intact soil cores provide a better estimate of mycorrhizal inoculum potential than assays using mixed soil or methods for counting propagules such as spores, root fragments, other colonised organic materials and networks of hyphae in soil (see Djuuna et al. 2009). Using a bioassay with clover (bait for AM fungi) and *Eucalyptus globulus* (for ECM), Chen et al. (1999) assessed inoculum potential of both types of mycobionts in established eucalypt plantations of varying ages in Western Australia. Brundrett and Ashwath (2013) compared the results of bioassay, spore survey and culturing experiments using the same soils collected from both natural and disturbed habitats and found differences in the propagule strategies of AM fungi for survival and spread within tropical Australian soils.

The growth and branching of AM fungal hyphae are induced by root factors exuded by host plants and are followed by the formation of an appressorium leading to the hyphal penetration in the root system (Ramos et al. 2008). These root signalling factors seem to be specifically synthesised by host plants, as exudates from non-host plants are not able to promote either hyphal differentiation or appressorium formation (Giovannetti et al. 1996).

To assist evaluation and selecting AM fungi, fungal effect can be measured in several ways. Dry weight production and mycorrhizal dependence are the two most widely used expressions for evaluating AM effect on host plants (e.g. Kendrick and Berch 1985). Fungal influences on plant physiology such as mineral nutrition particularly phosphorus, plant performance and plant protection are important components in assessing fungal efficiency.

21.4.3 Effectiveness of AM Inoculation

The use of AM inoculants in forest nurseries is far less than the use in the agriculture and is also not as often as the use of the ECM fungi in the plantation practices. This may be due to our understanding of relatively less predominance of AM symbioses in commercial plantation species and variable effects on plant growth (Smith and Smith 2011). Liu and Luo (1988) inoculated *Prunus pseudocerasus* with *G. mosseae* and *G. versiforme* and demonstrated substantial increase of the acquisition on the growth, mineral nutrition and water of host plant. Application of AM inoculants in China for some woody plants is reviewed in Zhang (1995) including species of *Abrus*, *Calamus*, *Casuarina*, *Citrus*, *Dimocarpus* and *Malus*. Inoculation of *Acacia mangium* with AM fungi was less convincing probably due to the presence of native efficient strains in the soil (De la Cruz and Yantasath 1993).

Occurrence of mycorrhizal symbionts is widespread in acid soils in the tropics indicating that mycorrhizal functions and selection of acid-tolerant fungal strains may be important for both trees and crops (Haselwandter and Bowen 1996). Some AM fungi have the capacity to reduce the absorption of toxic metals by plants (e.g. Amir et al. 2008; Bi et al. 2005). Gildon and Tinker (1983) found a heavy metal tolerant strain of the AM fungus *G. mosseae*, collected on a heavily zinc- and cadmium-contaminated site. Mycorrhizal seedlings of *Betula* performed better when exposed to the toxic metals Cu and Ni compared to non-mycorrhizal ones. Furthermore, heavy metal-induced genes encoding glutathione S-transferases in *G. intraradices* are identified (Waschke et al. 2006). However, incidences of AM fungi conferring to toxic metals in plantation species are less well addressed.

There are few reports of resistance of AM to pathogens in woody plants. Tang and Chen (1995) found that *G. mosseae* helped to protect *Populus* seedlings from a canker fungus (*Dothiorella gregaria*) by promoting acquisition of water and P and inducing peroxidise and polyphenoloxidase activities in host. Induction of defence responses in pre-inoculated plants with *G. mosseae* was much higher and quicker than that in non-mycorrhizal plants upon colonisation of *Rhizoctonia solani* (Song et al. 2011). This indicates that induction of accumulation of DIMBOA, an important phytoalexin in corn, and systemic defence responses by AM fungus, plays a vital role in enhanced disease resistance of mycorrhizal plants against sheath blight. However, the effectiveness of AM fungi in biocontrol is dependent on the AM fungus involved, as well as the substrate and host plant.

AM fungi may also have interactions with plant growth-promoting rhizosphere (PGPR) organisms. The concept and role of PGPR plant growth and protection is well documented (e.g. Whipps 2001). The presence of a biocontrol PGPR *Trichoderma harzianum* suppressed hyphal length of *G. intraradices* but no effect on hyphal biomass (Green et al. 1999). Another biocontrol agent *Gliocladium virens* had no detrimental effect on *G. etunicatum* and *G. mosseae* (Paulitz and Linderman 1991).

The effect of AM inoculation may vary since many factors can influence the occurrence of AM (Abbott and Robson 1991). The interplay between environmental factors (phosphorus, pH, nitrogen, water and temperature) and the host–fungus relationship is discussed in Smith and Smith (2011). Abbott and Robson (1982) stressed the importance of knowing the response curve of mycorrhizal and non-mycorrhizal plants to P application when evaluations of the impact of AM fungi on the growth.

21.5 Dual Inoculation

21.5.1 Dual AM and ECM

A few tree genera are ecologically interesting because they can form dual associations with both AM and ECM fungi (Lodge 2000). Plants reported to have dual AM/ECM associations belong to the genera *Casuarina*, *Allocasuarina* (Casuarinaceae), *Eucalyptus*, *Melaleuca* (Myrtaceae) and *Acacia* and *Leucaena* (Mimosaceae) from Australia (Brundrett et al. 1996; Chen et al. 2000a; Saravesi et al. 2011) and *Alnus*, *Populus*, *Salix* and *Uapaca* from Northern Hemisphere (Lodge and Wentworth 1990; Moyersoen and Fitter 1999; Saravesi et al. 2011; Zhao 1995). These genera include some major species used in commercial plantation forestry. Despite the ecological importance of the tripartite associations involving plant, AM and ECM fungi, only a few studies explored the relative benefits from each fungal type to the host plant and interactions between ECM and AM fungi colonising the same root systems (Chen et al. 1998, 2000a; Kariman et al. 2012; Lodge and Wentworth 1990).

The existence of dual association in the same root systems of *Eucalyptus* species has been confirmed both in plantation soils and under controlled conditions (Chen et al. 1998, 2000a; Lodge 2000; Oliveira et al. 1997). Jones et al. (1998) compared the growth response, phosphorus uptake efficiency and external hyphal production of AM and ECM fungi in *Eucalyptus coccifera*. Seedlings of *Eucalyptus urophylla* colonised by both AM and ECM fungi enhanced plant growth, root activity and acquisition of nutrients, amino acids and polysaccharides in root exudates when compared with non-mycorrhizal plants or plants colonised by one type of fungus (Chen et al. 1998). Chen et al. (2000a) established an experimental model to study dual colonisation in *Eucalyptus* and investigated the relative benefits of each type of fungi provided to two tree species and demonstrated several different mechanisms involved in successional replacement. Succession within a root system from predominantly AM to dominance by ECM has previously been reported for *Eucalyptus*, *Populus* and *Salix* in both field observations (Gardner and Malajczuk 1988; Lodge and Wentworth 1990) and glasshouse experiments (Chen et al. 1998; Dos Santos et al. 2001).

Proposed mechanisms to explain successional replacement in tripartite associations include mechanical barriers posed by the ECM sheath, chemicals of fungal or host origin, competition for root carbohydrates and effects on rhizosphere communities (Chen et al. 2000a; Lodge and Wentworth 1990). ECM fungi may have a greater impact on colonisation by AM fungi by causing their host to reduce production of fine roots, thereby limiting the availability of new roots to the fungus. These studies indicate that ECM associations are usually more important that AM associations for *Eucalyptus* species. However, there is evidence that AM associations can provide benefits to eucalypts, especially during seedling establishment despite some ambiguous reports on AM efficiency. There are also cases where the benefits provided by AM and ECM together can exceed those provided by either one alone (Chen et al. 1998, 2000a). The importance of AM associations of eucalypts is likely to be greater in disturbed habitats or exotic locations where there are few eucalypt compatible ECM fungi (Dell et al. 2002), since AM fungi generally exhibit little host specificity (see Sect. ECM section above 21.3.3). Variation of inoculation efficiency between tree species suggests that careful matching of host and fungal species (and genotype) is needed to obtain the best results.

Osonubi et al. (1991) examined effects of ECM and AM fungi on drought tolerance of four leguminous species (*Acacia auriculiformis*, *Albizia lebbeck*, *Gliricidia sepium* and *Leucaena leucocephala*). Under well-watered conditions, there were significant differences between species in development of both ECM and AM associations. They found that imposition of drought stress after colonisation had become established, showing significant reduction of ECM colonisation in *Gliricidia* only. Growth simulation and drought tolerance were observed for all tree species inoculated with ECM and/or AM fungi.

Gange et al. (2005) examined AM and ECM fungi and the interactions between them, on foliar-feeding insect attack of *Eucalyptus urophylla*. Both fungal types affected levels of damage by insect herbivores. Most importantly, herbivory by the pest insects *Anomala cupripes* (Coleoptera) and *Strepsicrates* spp. (Lepidoptera) was decreased by ECM. It is suggested that mycorrhizal effects on eucalypt insects may be determined by carbon allocation within the plant (Gange et al. 2005). This study that has enhanced our understanding of how these different fungi affect insect performance may help in unravelling the complex and little understood phenomenon of dual mycorrhizal plants. A study by Gehring and Whitham (2002) reported that AM colonisation of hybrid cottonwood trees (*Populus angustifolia* × *P. fremontii*) reduced populations of a specialist aphid, *Chaitophorus populicola*, whereas ECM colonisation enhanced aphid numbers. Future studies of mycorrhizal effects on plant growth should include a consideration of the insect herbivores present. These fungi clearly have the potential to influence insect herbivore attack rates, and experiments need to be performed in which fungal species and soil conditions are varied, to determine which, if any, mycorrhizal combinations could be used to reduce potential pest insect levels.

21.5.2 Dual Mycorrhizal Fungi and Nodule-Forming Organisms

Some species in Mimosaceae and Casuarinaceae forming dual AM/ECM associations also have nitrogen-fixing root nodule symbioses. There are two different types of nitrogen-fixing symbioses: the legume–rhizobia symbioses that form between *c*. 80 % of all legumes and rhizobia of the genera *Rhizobium* and *Bradyrhizobium* and actinorhizal symbioses that form between actinorhizal plants and *Frankia* (Katharina et al. 2011). Mycorrhizal and rhizobial/*Frankia* symbioses often act synergistically on colonisation rate, nitrogen-fixing efficiency, mineral nutrition and plant growth (Amora-Lazcano et al. 1998). The mycorrhizal fungi associated with legumes or actinorhizal plants are an essential link for adequate phosphorus nutrition, leading to enhanced nitrogenase activity that in turn promotes root and mycorrhizal growth (Reddy and Satyanarayana 2006).

Dual inoculation with AM fungi and rhizobia enhanced survival and growth of *Centrolobium tomentosum* plantations in the field, and AM fungi seemed to favour the nodule occupation by rhizobia strains as compared to the non-mycorrhizal plants (Marques et al. 2001). Cao et al. (2005) stimulated the growth of *Acacia* and *Leucaena* by dual inoculation with two *Glomus* species and *Rhizobium*. Nodulation, mycorrhizal colonisation, dry weight and nitrogen and phosphorus content of *Leucaena leucocephala* seedlings were improved by dual inoculation with *G. fasciculatum* and *Rhizobium* compared to single inoculation with either organism (Manjunath et al. 1984). This study also showed that inoculation with *Glomus* only improved nodulation by native rhizobia grown in a phosphorus-deficient unsterile soil, and the *Rhizobium*-only treatment improved colonisation of roots by native AM fungi. Diem and Gauthier (1982) demonstrated that mycorrhization of *Casuarina equisetifolia* saplings with *G. mosseae* improved plant growth, *Frankia* nodulation and nitrogen fixation. *Acacia* species are spontaneously associated to the three symbionts in their native soils (Warcup 1980), while in exotic area, local colonisation of mycorrhizal fungi seemed inefficient due to the lack of compatible mycobionts. A pot experiment showed *Medicago sativa* plants co-inoculated with *G. mosseae* and *Rhizobium* greatly increased the survival rate and nutrient uptake in coal mine substrates (Wu et al. 2009). There are fewer studies reported the effect of ECM than AM on nitrogen-fixing plants. Duponnois et al. (2002) observed the positive effect of the controlled dual ECM and rhizobial symbioses on the growth of *Acacia mangium* provenances, the indigenous symbiotic microflora and the structure of plant parasitic nematode communities. However inoculation with *Hebeloma crustuliniforme* alone or in combination with *Frankia* had no effect on the growth and root nodulation of *Alnus crispa* due to the failure of mycorrhization which may suggest incompatibility of the fungus (Quoreshi et al. 2007). The three types of symbioses have been shown to coexist on the same root system, but their functional relevance remains unclear.

21.6 Conclusions

Mycorrhizal colonisation can help establishment of plantations, particularly in eroded, degraded or heavy metal-contaminated areas. In novel habitats, mycorrhizal fungi may transform soil carbon cycling (Chapela et al. 2001), affect mineral nutrient dynamics (Phillips and Fahey 2006) and alter surrounding vegetation (Richardson and Rejmánek 2004). Appropriate mycorrhizal fungi incorporated in forest nurseries for raising mycorrhizal seedlings and transfer of seedlings to the field is a practical inoculation technique currently suitable in plantation crops and trees. Experience of the use of inoculated seedlings has indicated that responses to mycorrhizal inoculation are often greatest under the most extreme conditions, particularly those involving exposure to infertile soils, drought, metal contamination or pathogens (Smith and Read 2008).

Field surveys for the mycorrhizal community associated with the given tree species, combined with estimations of the extent of mycorrhizal colonisation on the roots and propagules such as basidiomata of agarics and spores of AM fungi, can assist in defining the range of fungal symbionts available for the tree. However, this should not rule out the possibility of introducing new and efficient fungal partners to the area. The low diversity of fungi currently being used in Australasian eucalypt plantations may give minimal benefit to tree production because the fungi may not necessarily be well suited to the local site characteristics (climate, soil type, host plants, etc.). In the long term, maintenance of soil structure, fertility and general ecosystem stability in the face of environmental changes and disturbances may be enhanced by the presence of a broader diversity of fungi.

Inoculation of seedlings with mycorrhizal fungi should aim to ensure that seedlings have extensive colonisation at the time of transplanting from the nursery to the field. There are still relatively few examples in the use of AM inoculants under forest field conditions. Thus, more precise experimental work with thoughtful design should be carried out to overcome potential constraints limiting the development and function of introduced symbionts. A mixed inoculum containing fungi with differing ecological strategies might give more consistent and permanent results in promoting plant growth. The additive beneficial effects from insuring simultaneous colonisation by multiple types of symbionts could be useful for the establishment of commercial timber species in adverse sites. Cultural practices may have to be modified to produce conditions which are optimal for the development of symbioses in the nursery. As nutrient supply and composition can influence hyphal development in the nursery, application of fertilisers at appropriate regimes is essential for optimising the potential benefits of inoculation programmes. The practical application of mycorrhizal fungi may be integrated in the disease management by producing mycorrhizal seedlings, so as to prevent primary and secondary colonisation by pathogenic fungi, herbivore insects and other harmful organisms. Further research on optimising mycorrhizal inoculants and seedlings in forest nurseries is required to maximise efficiency and productivity of fungal inoculation. There is also a need for long-term field studies to monitor the

performance and persistence of introduced fungi in the plantation and revegetation sites and their impacts on native microflora.

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Chapter 22
Use of Arbuscular Mycorrhizal Fungi for Reforestation of Degraded Tropical Forests

Keitaro Tawaraya and Maman Turjaman

22.1 Introduction

Tropical forests are important for their diverse bioresources as well as the significance of the carbon pool. Tropical forests are disappearing at the rate of 13.5 million hectares (ha) each year, largely due to logging, burning and clearing for agricultural land, and shifting cultivation (Kobayashi 2004). Timber harvesting has resulted in the transformation of more than five million ha of tropical forest annually into over-logged, poorly managed, and degraded forests. Degraded tropical forests require wide-scale rehabilitation and it is not easy to rehabilitate degraded tropical forests because a major obstacle in the rehabilitation of tropical forests is slow tree growth and high mortality of seedlings in the nursery. It is also necessary to understand the physical, chemical, and biological factors of forest soils, in order to remediate degraded tropical forests. Among these properties, biological properties are least well known. Arbuscular mycorrhizal (AM) fungi affect the maintenance of vegetation in various ecosystems and may play an important role in tropical forests. Most tropical tree species form arbuscular mycorrhizas.

The diversity of AM fungi and the breadth of their associations with plant species in natural environments are crucial to understanding the ecological role of AM fungi in plant coexistence. AM fungal community structures differ significantly between host species and have been reported to increase the growth and survival rate of some tropical tree seedlings (Wubet et al. 2009). Phosphorus (P) limits the productivity of trees in many forests and plantations especially in

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highly weathered, acidic, or calcareous profiles in the world. Most trees form mycorrhizal associations which are prevalent in the organic and mineral soil horizons. Mycorrhizal tree roots have a greater capacity to take up phosphate (Pi) from the soil solution than non-mycorrhizal roots (Plassard and Dell 2010). Rehabilitation of degraded tropical forests following inoculation of AM fungi has potential to restore important ecosystem functions. The purpose of this chapter is to review the effect of inoculation of AM fungi on growth of native tree species from tropical forests.

22.2 Degraded Tropical Forest and Reforestation

The total world's forests cover nearly 3.9 billion ha or nearly 30 % of the world's land area (FAO 2001; Fenning and Gershenzon 2002). The number of tropical forests has been declining owing to illegal logging, fire, conversion into agricultural lands, rubber tree and palm oil plantation, and use of the forest plantation estate as pulp trees. Degraded forests are considered to be low-value resources because they are characterized by the vegetation such as ferns, sedges, and scrub. However, it is not easy to rehabilitate this ecosystem in a short term, because it is necessary to select and produce high-quality tree seedling species that have high survival rates during the rehabilitation process.

Tropical forests contribute considerably in sustaining global biodiversity (Laurence 1999). They are homes to indigenous people, pharmacopeias of natural products, and providers of vital ecosystem services, such as flood amelioration and soil conservation. At regional and global scales, tropical forests also have a major influence on climate and carbon storage. Tropical forestlands have been disappearing at the rate of 13.5 million ha each year. Furthermore, timber harvesting has resulted in the transformation of more than five million ha of tropical forest annually into logged-over, poorly managed, and degraded forests.

One of the most serious world problems affecting tropical rain forest is desertification. This is a complex and dynamic process which is claiming several 100 million ha annually. Tropical forests are particularly affected, resulting in a rapid reduction in area. Human activities can cause or accelerate desertification and the loss of most plant species as well as their associated symbioses. The reduction and degradation caused by anthropological activities affect not only the sustainable production of timber but also the global environment. Accurate scientific information will enable managers to devise silvicultural systems to enhance soil properties and forest resources important for sustainable production and for minimizing deleterious impacts of harvesting and short-rotation plantation. Degraded tropical forested lands require wide-scale rehabilitation and it is necessary to improve the biological diversity of tropical forestlands and to enhance the commercial value of timber.

The rapid production of forest planting stock seedlings of high quality in nurseries is important for replenishing degraded tropical forestlands. Moreover,

many soils of tropical forests are nutrient poor (Hattenschwiler et al. 2011). Soil nutrient availability is one of the limiting factors for the early growth of transplanted seedlings in degraded tropical forestlands. Degraded tropical forestlands are recognized as low-value forest resources without successful natural regeneration that are dominated by grasslands including fern, sedges, or scrub. Nowadays, reforestation programs have to prepare millions of seedling stocks annually. The use of vigorous seedlings in reforestation programs is important. However, seedling stocks of tropical forest species are usually weak, often N and P deficient, and have high mortality rates after transplanting in the field. Phosphorus was the most limiting nutrient for plant growth of four woody legume species (Moreira et al. 2010). Ultimately, rehabilitation can increase the area of forest as well as contribute to conservation of the remaining primary forests and environmental quality.

22.3 Ecology of Arbuscular Mycorrhizal Fungi in Tropical Forests

Tropical rain forest soils often have high P adsorption because of their strong affinity to P to form iron and aluminum oxides and hydroxides, whereas in neutral and alkaline soils, P is adsorbed on the surface of Ca and Mg carbonates (Holford 1997; Whitmore 1989). Soil P concentration of tropical soil is very low (Table 22.1). In most experiment with tropical rain forest plant species, the influence of AM fungi on P nutrition has been evaluated by measuring the growth response of inoculated and non-inoculated plants cultivated in soils with controlled levels of P (Janos 1980). Moyersoen et al. (1998) reported that AM colonization of the tropical tree *Oubanguia alata* (Scytopetalaceae) was positively correlated with increased P uptake despite low P availability in Korup National Park rain forest, Cameroon.

Early studies focused primarily on mycorrhizas of the temperate forests, but attention turned toward mycorrhizas of the tropical rain forests (Torti et al. 1997). In contrast to the temperate zone, where mycorrhizal associations of trees tend to be formed by ectomycorrhizal fungi, the majority of tropical tree species surveyed thus far are formed by AM fungi (Janos 1980). Notable exceptions of tropical trees forming ectomycorrhizas occur in the families Myrtaceae, Caesalpiniaceae, Euphorbiaceae, Fagaceae, and Dipterocarpaceae (Munyanziza et al. 1997). The highest number of species and spores of AM fungi was observed during the dry season, with a marked decrease during the rainy season in a tropical rain forest in Veracruz, Mexico (Guadarrama and Alvarez-Sanchez 1999). Moyersoen et al. (2001) reported that AM colonization was about 40 % in tree species in heath forests and mixed Dipterocarpaceae forest in Brunei. Tawaraya et al. (2003) showed that 17 of 22 tree species in a tropical peat swamp forest in Kalimantan, Indonesia, had mycorrhizas formed by AM fungi. Of the 142 species of trees and

Table 22.1 Arbuscular mycorrhizal colonization, mycorrhizal dependency (MD) of different tree species grown in tropical forests, and soil phosphorus concentration

Family	Species	Growth period (d)	Fungal species	Colonization (%)	MD (%)	Soil P (mg P/kg)	References
Anacardiaceae	*Lithraea molleoides*	90	*Glomus etunicatum*	27	97	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Anacardiaceae	*Anacardium occidentale*	90	*Glomus aggregatum*	71	23	6.6 (Bray-1)	Bá et al. (2000)
Anacardiaceae	*Schinus terebinthifolius*	120	Gigaspora margarita, Glomus etunicatum	20	92	2 (Olsen)	Siqueira et al. (1998)
Anacardiaceae	*Sclerocarya birrea*	90	*Glomus aggregatum*	75	17	6.6 (Bray-1)	Bá et al. (2000)
					57		
Apocynaceae	*Aspidosperma parvifolium*	180	*Glomus etunicatum*	–	68	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Apocynaceae	*Dyera polyphylla*	202	*Glomus clarum*	39	61	4.8	Turjaman et al. (2006)
Apocynaceae	*Dyera polyphylla*	202	*Glomus decipiens*	22	62	4.8	Turjaman et al. (2006)
Apocynaceae	*Stemmadenia donnell-smithii*	180	Mixture* 1	10	55	N.D.	Guadarrama et al. (2004)
					62		
Araucariaceae	*Araucaria angustifolia*	686	*Glomus clarum*	81	71	39	Zandavalli et al. (2004)
Bignoniaceae	*Jacaranda mimosaefolia*	120	*Gigaspora margarita*, *Glomus etunicatum*	77	95	2 (Olsen)	Siqueira et al. (1998)
Bignoniaceae	*Stenolobium stans*	120	*Gigaspora margarita*, *Glomus etunicatum*	72	85	2 (Olsen)	Siqueira et al. (1998)
Bignoniaceae	*Tabebuia serratifolia*	129	*Glomus etunicatum*	72	89	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)

Bignoniaceae	*Tabebuia impetiginosa*	84	*Glomus etunicatum*	41	58	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
					82		
Boraginaceae	*Cordia trichotoma*	90	*Glomus etunicatum*	–	98	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Caesalpiniaceae	*Caesalpinia ferrea*	97	*Glomus etunicatum*	30	76	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Caesalpiniaceae	*Copaifera langsdorffii*	262	*Glomus etunicatum*	–	50	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Caesalpiniaceae	*Senna macranthera*	120	*Glomus etunicatum*	20	87	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Caesalpiniaceae	*Senna spectabilis*	90	*Glomus etunicatum*	63	95	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Caesalpiniaceae	*Dicorynia guianensis*	350	Indigenous	62	52	N.D.	Bereau et al. (2000)
Caesalpiniaceae	*Dicorynia guianensis*	281	Indigenous	60–95	71	21	de Grandcourt et al. (2004)
Caesalpiniaceae	*Eperua falcata*	281	Indigenous	45–75	22	21	de Grandcourt et al. (2004)
Caesalpinoideae	*Bauhinia sp.*	120	*Gigaspora margarita*, *Glomus etunicatum*	9	6	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Caesalpinia ferrea*	120	*Gigaspora margarita*, *Glomus etunicatum*	30	75	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Caesalpinia peltophoroides*	120	*Gigaspora margarita*, *Glomus etunicatum*	33	48	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Cassia grandis*	120	*Gigaspora margarita*, *Glomus etunicatum*	63	71	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Copaifera langsdorffii*	120	*Gigaspora margarita*, *Glomus etunicatum*	–	−4	2 (Olsen)	Siqueira et al. (1998)

(continued)

Table 22.1 (continued)

Family	Species	Growth period (d)	Fungal species	Colonization (%)	MD (%)	Soil P (mg P/kg)	References
Caesalpinoideae	*Peltophorum dubium*	120	*Gigaspora margarita*, *Glomus etunicatum*	18	61	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Schizolobium parahyba*	120	*Gigaspora margarita*, *Glomus etunicatum*	8	−25	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Senna macranthera*	120	*Gigaspora margarita*, *Glomus etunicatum*	81	84	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Senna multijuga*	120	*Gigaspora margarita*, *Glomus etunicatum*	–	38	2 (Olsen)	Siqueira et al. (1998)
Caesalpinoideae	*Senna spectabilis*	120	*Gigaspora margarita*, *Glomus etunicatum*	17	85	2 (Olsen)	Siqueira et al. (1998)
					52		
Casuarinaceae	*Casuarina equisetifolia*	144	*Glomus geosporum*	45	55	0.34 (Olsen)	Muthukumar and Udaiyan (2010)
Cecropiaceae	*Cecropia pachystachya*	98	*Glomus etunicatum*	62	100	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Clusiaceae	*Clusia minor*	420	*Scutellospora fulgida*	100	99	3.05	Cáceres and Cuenca (2006)
Clusiaceae	*Clusia minor*	420	*Scutellospora fulgida*	75	−42	39	Cáceres and Cuenca (2006)
Clusiaceae	*Clusia multiflora*	180	*Scutellospora fulgida*	98	71	3.05	Cáceres and Cuenca (2006)
Clusiaceae	*Clusia multiflora*	180	*Scutellospora fulgida*	92	−21	39	Cáceres and Cuenca (2006)
					27		
Euphorbiaceae	*Croton floribundus*	101	*Glomus etunicatum*	48	92	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)

Euphorbiaceae	*Macaranga denticulata*	120	Indigenous	70	42	4.10 (Bray II)	Youpensuk et al. (2004)
					67		
Fabaceae	*Dalbergia sissoo*	111	*G. albida*, *G. intraradices*, *A. scrobiculata*	70	61	14.9 (Olsen)	Bisht et al. (2009)
Fabaceae	*Dalbergia sissoo*	111	*G. albida*, *G. intraradices*, *A. scrobiculata*	30	59	6.3 (Olsen)	Bisht et al. (2009)
Fabaceae	*Dialium guineensis*	90	*Glomus aggregatum*	50	45	6.6 (Bray-1)	Bá et al. (2000)
Fabaceae	*Leucaena diversifolia*	45	*G. aggregatum*	73	73	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Leucaena leucocephala*	45	*G. aggregatum*	76	79	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Leucaena leucocephala*	56	Mixture	98	77	4.22 (Bray II)	Saif (1987)
Fabaceae	*Leucaena retusa*	45	*G. aggregatum*	56	35	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Leucaena trichodes*	45	*G. aggregatum*	58	70	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Sesbania pubescens*	120	*G. aggregatum*	83	−69	4.8 (Olsen)	Duponnois et al. (2001)
Fabaceae	*Sesbania formosa*	45	*G. aggregatum*	75	24	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Sesbania grandiflora*	120	*G. aggregatum*	90	−4	4.8 (Olsen)	Duponnois et al. (2001)
Fabaceae	*Sesbania grandiflora*	45	*G. aggregatum*	80	35	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Sesbanica nubica*	120	*G. aggregatum*	97	−32	4.8 (Olsen)	Duponnois et al. (2001)
Fabaceae	*Sesbania pachycarpa*	45	*G. aggregatum*	54	17	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Sesbania paludosa*	120	*G. aggregatum*	79	59	4.8 (Olsen)	Duponnois et al. (2001)

(continued)

Table 22.1 (continued)

Family	Species	Growth period (d)	Fungal species	Colonization (%)	MD (%)	Soil P (mg P/kg)	References
Fabaceae	*Sesbania sesban*	45	*G. aggregatum*	70	14	0.02 mg/L	Manjunath and Habte (1991)
Fabaceae	*Tamarindus indica*	90	*Glomus aggregatum*	88	53	6.6 (Bray-1)	Bá et al. (2000)
					35		
Faboideae	*Platycyamus regnellii*	120	*Gigaspora margarita*, *Glomus etunicatum*	–	20	2 (Olsen)	Siqueira et al. (1998)
Faboideae	*Tipuana tipu*	120	*Gigaspora margarita*, *Glomus etunicatum*	75	74	2 (Olsen)	Siqueira et al. (1998)
					47		
Guttiferae	*Calophyllum hosei*	270	*Glomus aggregatum*	18	60	0.17	Turjaman et al. (2008)
Guttiferae	*Calophyllum hosei*	270	*Glomus clarum*	19	57	0.17	Turjaman et al. (2008)
Guttiferae	*Ploiarium alternifolium*	270	*Glomus aggregatum*	32	51	0.17	Turjaman et al. (2008)
Guttiferae	*Ploiarium alternifolium*	270	*Glomus clarum*	27	56	0.17	Turjaman et al. (2008)
					56		
Malvaceae	*Adansonia digitata*	90	*Glomus aggregatum*	63	8	6.6 (Bray-1)	Bá et al. (2000)
Melastomaceae	*Tibouchina granulosa*	144	*Glomus etunicatum*	20	100	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Meliaceae	*Azadirachta indica*	120	*Glomus geosporum*	25	8	N.D.	Muthukumar et al. (2001)
Meliaceae	*Azadirachta indica*	120	*Glomus intraradices*	28	20	N.D.	Muthukumar et al. (2001)
Meliaceae	*Azadirachta indica*	120	*G. geosporum*, *G. intraradices*	44	26	N.D.	Muthukumar et al. (2001)

Meliaceae	*Cedrela fissilis*	96	*Glomus etunicatum*	70	95	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Meliaceae	*Cedrela fissilis*	120	*Gigaspora margarita*, *Glomus etunicatum*	34	41	2 (Olsen)	Siqueira et al. (1998)
					38		
Mimosoideae	*Albizia lebbeck*	120	*Gigaspora margarita*, *Glomus etunicatum*	55	62	2 (Olsen)	Siqueira et al. (1998)
Mimosoideae	*Anadenanthera falcata*	120	*Gigaspora margarita*, *Glomus etunicatum*	11	81	2 (Olsen)	Siqueira et al. (1998)
Mimosoideae	*Leucaena leucocephala*	136	*Glomus etunicatum*	17	92	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Mimosoideae	*Parkia biglobosa*	90	*Glomus aggregatum*	68	32	6.6 (Bray-1)	Bá et al. (2000)
					67		
Myrsinaceae	*Myrsine umbellata*	111	*Glomus etunicatum*	52	98	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Myrtaceae	*Syzygium jambolanum*	120	*Gigaspora margarita*, *Glomus etunicatum*	50	91	2 (Olsen)	Siqueira et al. (1998)
Rhamnaceae	*Hovenia dulcis*	120	*Gigaspora margarita*, *Glomus etunicatum*	47	63	2 (Olsen)	Siqueira et al. (1998)
Rhamnaceae	*Zizyphus mauritiana*	90	*Glomus aggregatum*	98	78	6.6 (Bray-1)	Bá et al. (2000)
					71		
Rubiaceae	*Coffea arabica*	150	*Glomus clarum*	12	−15	3 (Olsen)	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Glomus clarum*	20	61	13	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Glomus clarum*	22	46	27	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Glomus clarum*	26	19	42	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Acaulospora mellea*	26	−31	3	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Acaulospora mellea*	50	55	13	Vaast et al. (1996)

(continued)

Table 22.1 (continued)

Family	Species	Growth period (d)	Fungal species	Colonization (%)	MD (%)	Soil P (mg P/kg)	References
Rubiaceae	*Coffea arabica*	150	*Acaulospora mellea*	32	16	27	Vaast et al. (1996)
Rubiaceae	*Coffea arabica*	150	*Acaulospora mellea*	22	−18	42	Vaast et al. (1996)
					17		
Sapindaceae	*Sapindus saponaria*	116	*Glomus etunicatum*	24	36	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Sapindaceae	*Aphania senegalensis*	90	*Glomus aggregatum*	40	21	6.6 (Bray-1)	Bá et al. (2000)
Sapindaceae	*Sapindus saponaria*	120	*Gigaspora margarita*, *Glomus etunicatum*	41	66	2 (Olsen)	Siqueira et al. (1998)
					41		
Thymelaeaceae	*Aquilaria filaria*	202	*Glomus clarum*	93	53	4.8	Turjaman et al. (2006)
Thymelaeaceae	*Aquilaria filaria*	202	*Glomus decipiens*	87	48	4.8	Turjaman et al. (2006)
					51		
Tiliaceae	*Heliocarpus appendiculatus*	180	Mixture*1	83	−47	N.D.	Guadarrama et al. (2004)
Tiliaceae	*Luehea grandiflora*	120	*Gigaspora margarita*, *Glomus etunicatum*	60	93	2 (Olsen)	Siqueira et al. (1998)
					23		
Ulmaceae	*Luehea grandiflora*	93	*Glomus etunicatum*	21	98	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Ulmaceae	*Trema micrantha*	70	*Glomus etunicatum*	32	98	1 (Mehlich I)	Siqueira and Saggin-Junior (2001)
Ulmaceae	*Trema micrantha*	120	*Gigaspora margarita*, *Glomus etunicatum*	64	92	2 (Olsen)	Siqueira et al. (1998)

					96		
Zygophyllaceae	*Balanites aegyptiaca*	90	*Glomus aggregatum*	52	0	6.6 (Bray-1)	Bá et al. (2000)
Average (all species)					50		

Average of MD of each family was also shown
Mixture*1: Sclerocystis, Acaulospora and Gigaspora

liana surveyed in Guyana, 137 were exclusively formed by AM fungi (McGuire et al. 2008). A light microscopy investigation showed arbuscular mycorrhizas in 112 tree species from 53 families on mineral as well as organic soils in Ecuador (Kottke et al. 2004). In a related study, a segment of fungal 18S rDNA was sequenced from the mycorrhizas of *Cedrela montana*, *Heliocarpus americanus*, *Juglans neotropica*, and *Tabebuia chrysantha* in reforestation plots from degraded pastures in Ecuador and observed distinct species-rich AM communities (Haug et al. 2010). Dual ectomycorrhizal and AM colonization was observed in 4 of 14 ectomycorrhizal tree species belonging to Caesalpiniaceae and Uapacaceae from rain forest in Cameroon (Moyersoen and Fitter 1999). In total, 193 glomeromycotan sequences were analyzed, 130 of them previously unpublished.

Spores of AM fungi have been isolated from soils of tropical forests and their population and richness were affected by environmental conditions. Spore density and richness based on soil cores were higher in the dry season than in the rainy season in a tropical sclerophyllous shrubland in the Venezuelan Guayana (Cuenca and Lovera 2010). Spore numbers of AM fungi were higher in young secondary forest and pastures and lower in pristine forest in the Amazon region (Sturmer et al. 2009), and AM fungal diversity was high in dry tropical Afromontane forests of Ethiopia (Wubet et al. 2009). AM fungal spores in soil decreased from an early plant succession to mature tropical forest in a Brazilian study (Zangaro et al. 2008). AM fungal types that were dominant in the newly germinated seedlings were almost entirely replaced by previously rare types in the surviving seedlings the following years (Husband et al. 2002a). As the seedlings matured in a tropical forest in the Republic of Panama, the fungal diversity decreased and there was a significant shift (Husband et al. 2002b). Based on spore morphology, 29 species of AM fungi were found in the rhizosphere of *Macaranga denticulata* (Youpensuk et al. 2004).

22.4 Inoculation of Tropical Tree Species with AM Fungi

AM fungi have been reported to increase growth of some tropical trees (Table 22.1). AM fungi increased seedling growth of 23 of 28 species from a lowland tropical rain forest in Costa Rica under nursery conditions (Janos 1980). AM colonization of the tropical tree *Oubanguia alata* (Scytopetalaceae) was positively correlated with increased P uptake despite low P availability in a study in Cameroon (Moyersoen et al. 1998). AM fungi improved growth of the Brazilian pine *Araucaria angustifolia* (Araucariaceae) (Zandavalli et al. 2004). There are also reports of improved growth of non-timber forest product tree species following AM fungal inoculation in tropical forests. For example, Muthukumar et al. (2001) reported that inoculation of *Azadirachta indica* (Meliaceae) with AM fungi improved seedling growth. Furthermore, the inoculation of AM fungi with phosphate-solubilizing and nitrogen-fixing bacteria increased the growth of *A. indica*. Conversely, *A. excelsa*

inoculated with AM fungi (without fertilizer) grew more slowly than did the uninoculated plants (Huat et al. 2002). Kashyap et al. (2004) showed that inoculation of *Morus alba* (Moraceae) with both AM fungi and *Azotobacter* increased the survival percentage of saplings.

Clusia minor and *Clusia multiflora* inoculated with *Scutellospora fulgida* in acidic soil had greater shoot and root biomass, leaf area, and height in comparison to the biomass of P-fertilized plants and non-mycorrhizal plants (Cáceres and Cuenca 2006). Inoculation with the AM fungus *Glomus geosporum* improved the growth, nutrient acquisition, and seedling quality of *Casuarina equisetifolia* seedlings under nursery conditions (Muthukumar and Udaiyan 2010). Seedlings of *Araucaria angustifolia* inoculated with *Glomus clarum* had higher shoot biomass; leaf concentrations of P, K, Na, and Cu; and lower concentrations of Ca, Mg, Fe, Mn, and B than controls (Zandavalli et al. 2004). Inoculation with soil-containing AM fungi increased shoot growth nutrient contents when P was limiting but N was applied (Youpensuk et al. 2004). Inoculation with AM fungi *Glomus clarum* and *Gigaspora decipiens* increased shoot N and P uptake of non-timber forest product species *Dyera polyphylla* and *Aquilaria filaria* under greenhouse conditions, indicating that AM fungi can reduce the application of chemical fertilizer (Turjaman et al. 2006). Other studies have used mycorrhizal roots from individual tree species or from a mixture of the four trap species with resulting improvement in growth of 6-month-old *Cedrela montana* and *Heliocarpus americanus* (Urgiles et al. 2009). This latter technique is much easier to handle and has lower costs than spore production for tropical countries with limited facilities for storage of inoculum.

AM fungi increased the growth of *Acacia nilotica* and *Leucaena leucocephala* (Leguminosae) 12 weeks after transplantation under greenhouse conditions (Michelsen and Rosendahl 1990), and similar observations were made for three multipurpose fruit-tree species: *Parkia biglobosa*, *Tamarindus indica*, and *Ziziphus mauritiana* 2 months after inoculation (Guissou et al. 1998). The AM fungus *Glomus aggregatum* stimulated plant growth of 17 leguminous plants (Duponnois et al. 2001), and *Glomus macrocarpum* increased the growth of two species: *Sesbania aegyptiaca* and *S. grandiflora* (Giri et al. 2004). Some studies have successfully used mixed inocula of AM fungi including two (Bá et al. 2000), three (Adjoud et al. 1996), and nine species (Rajan et al. 2000).

Mycorrhizal dependency was calculated to compare the degree of plant growth change associated with AM colonization of 76 species, 25 families (Table 22.1). The average mycorrhizal dependency value of all the plants was 50 % (−69 Min. and 100 Max.). Mycorrhizal dependency was also different among families. It was higher in Ulmaceae and Bignoniaceae. Guissou et al. (1998) reported that mycorrhizal dependency of *Parkia biglobosa* and *Tamarindus indica* was similar, reaching no more than 36 %, while *Ziziphus mauritiana* showed higher mycorrhizal dependency values, reaching up to 78 %. A similar effectiveness of AM fungi for different plant species was also reported by Adjoud et al. (1996). Mycorrhizal dependency is frequently related to the morphological properties of the root of different plant species, and also root systems with only a few, short root hairs are indicative of a high mycorrhizal dependency of the plant species concerned (Baylis

1970). Responses of 12 native woody species to the inoculation of AM fungi were related to root morphological plasticity of the plant (Zangaro et al. 2007).

The survival rate of seedling stocks in the field is vital to reforestation. In one study, the survival rates of AM-inoculated cuttings of *Ploiarium alternifolium* and *Calophyllum hosei* were 100 % after 6 months (Turjaman et al. 2008). These values were higher than the survival rates of two tropical tree species from Panama inoculated with AM fungi, which were *Ochroma pyramidale* (97 %) and *Luehea seemannii* (52 %), respectively (Kiers et al. 2000). Inoculation with AM fungi can reduce the cost of seedling production for reforesting vast areas of disturbed tropical forests. Despite extensive studies of inoculation of tree species under controlled conditions, there are few reports about the effect of AM fungal inoculation on growth of tropical tree species under field conditions. Recently, Graham et al. (2013) showed that inoculation of *Glomus clarum* and *Gigaspora decipiens* increased N and P content of *Dyera polyphylla* under tropical peat swamp forest in Central Kalimantan, Indonesia.

22.5 Conclusion

Colonization of roots by AM fungi can improve growth of many tree species that occur in tropical forests. Survival rate of seedlings is a key measure of success in reforestation and afforestation. Survival rates of inoculated seedlings can be higher than those of non-inoculated seedlings. Inoculation with AM fungi at the nursery stage is a useful technique to include in large-scale reforestation programs. However, mycorrhizal dependency differs among plant species and with species of AM fungi. Therefore, selection of appropriate combination of plant species and fungal species is also important for reforestation programs.

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Chapter 23
Recent Advances in Cultivation of Edible Mycorrhizal Mushrooms

Yun Wang and Ying Long Chen

23.1 Introduction

Edible mushrooms are becoming more popular and important food on our table. There are hundreds of edible mushrooms available in the markets, of which most are saprophytic fungi, such as button mushroom (*Agaricus bisporus*), shiitake mushroom (*Lentinus edodes*) and oyster mushroom (*Pleurotus ostreatus*). However, the most expensive and sought-after edible mushrooms belong to the mycorrhizal group, including *Tuber melanosporum* Vitt., *T. magnatum* Pico & Vitt., *T. aestivum* Vitt. (*T. uncinatum*), the *T. indicum* complex, *Tricholoma matsutake* (S. Ito et Imai) Sing., *Boletus edulis* Bull: Fr. sensu lato, *Cantharellus cibarius* Fr., *Amanita caesarea* (Scop.: Fr.) Pers: Schw., *Lyophyllum shimeji* (Kawam.) Hongo, *Lactarius sanguifluus* (Paul) Fr. and *L. deliciosus* (L. Fr.) Gray (Hall et al. 1998a; Wang and Hall 2004; Hall and Zambonelli 2012). Edible mycorrhizal mushrooms (EMMs) comprise a specific group of fungal species belonging to either the Basidiomycetes or Ascomycetes, which form symbiotic associations with their host plants (Smith and Read 2008; Hall and Zambonelli 2012). EMMs are not only gourmet food but they are also a source of livelihood in many countries (Boa 2001; Molina 1998; Román and Boa 2006; Wang and Hall 2004). *Tricholoma matsutake* in Japan and truffles such as *Tuber melanosporum* and *T. magnatum* in

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Fig. 23.1 Cultivation of Basidiomycete edible ectomycorrhizal mushrooms: Tricholoma, Lactarius, and Rhizopogon. (**a**) A productive plantation of *Tuber melanosporum* at Charente, France, 2011; (**b**) A plantation of *Tuber melanosporum* at West Australia (Photo provided by Nick Malajczuk); (**c**) A truffle plantation at Guizhou Province, China with production of *Tuber melanosporum* and *T. indicum* since 2008; (**d**) A big ascocarp produced from a plantation of *Tuber borchii* at Cantherbury, New Zealand, 2010; (**e**) A small trial of cultivation of *Rhizopogon roseollus* at Cantherbury, New Zealand, 2011; (**f**) Mushrooms produced from an experimental plantation of 4-year old *Pinus radiate* with *Lactarius deliciosus* at Cantherbury, New Zealand, 2011

France and Italy are also an important part of the culture (Chen 2004a; Hall et al. 2007; Ogawa 1978; Renowden 2005; Sourzat 2009; Trappe 1990).

Unlike saprophytic edible mushrooms, the market of EMMs is supplied almost solely from what can be harvested from natural forests. Unfortunately, harvests of many wild forest mushrooms have declined over the past century, due to worldwide environmental changes caused by various natural and social factors (Wang and Hall 2004; Wang and Liu 2011). The falls in the availability of EMMs and increased demand have encouraged scientific research into developing technologies for the cultivation of EMMs and for sustainable mushroom production in forests. A few species of truffles have been produced in commercial quantities, although methods have been developed for many years. Despite numerous scientific publications and the establishment of thousands of hectares of plantations, the downward trend in EMM production continues (Bencivenga et al. 2009; Hall et al. 2003; Hall and Zambonelli 2012; Hosford et al. 1997; Pilz et al. 2003; Reyna et al. 2002; Sourzat 2009; Wang and Liu 2011). Many of the most expensive mycorrhizal mushrooms, including *Tuber magnatum* and *Tricholoma matsutake*, have defied cultivation.

In recent decades, cultivation of EMMs has made good progress. More new EMM species can be cultivated and more plantations of EMMs have been established in different countries (Fig. 23.1) (Savoie and Largeteau 2011; Hall and Zambonelli 2012). In particular, research on genetics and sexuality of truffles has made good progress (Rubini et al. 2010). The genome of *Tuber melanosporum* has recently been sequenced which provides better understanding of truffle fructification (Martin et al. 2010). Furthermore, advanced molecular tools have been developed and are being used to identify truffle species and their mycorrhizal symbioses and microorganism compositions in truffières. Surely, this new achievement would make cultivation of truffles and other EMMs more successful.

23.2 Cultivation Progress

23.2.1 Hypogeous EMMs

23.2.1.1 Truffles

Truffles are the fungi in the genus *Tuber* (Ascomycetes), which form below-ground ascocarps. Some truffle species are highly prized culinary commodities. Eight *Tuber* species, namely, *T. melanosporum*, *T. magnatum*, *T. aestivum* (=*T. uncinatum*), *T. indicum* species complex, *T. macrosporum*, *T. mesentericum*, *T. borchii* and *T. brumale*, are sold on international, mainly European markets and *T. gibbosum* and *T. oregonense* on North American markets. Desert truffles, species in the genera *Terfezia* and *Tirmania*, are popular delicacies in Arabic countries. These commercial truffle mushrooms are mainly harvested from natural forests. Only *Tuber melanosporum* and *T. aestivum* (=*T. uncinatum*) have been cultivated on large commercial scales together with a small proportion of production of

cultivated species of *Tuber borchii*, *T. brumale*, the *T. indicum* complex and the desert truffles (Bencivenga et al. 2009; Chevalier 2009; Hall and Zambonelli 2012; Sourzat 2009; Wang and Hall 2004; Wang and Liu 2011).

Attempts to grow truffles began in the eighteenth century in France and in Italy (Bencivenga et al. 2009; Chevalier 1998; Pierre 2009). The seedlings were raised around black truffle (*T. melanosporum*) trees and transplanted to new areas. However, it was not until the early 1970s that methods of producing truffle mycorrhised seedlings of suitable host plants with truffle spores were developed and in 1978 that truffières yielded first truffles (Chevalier 1998; Bencivenga et al. 2009; Olivier 2000; Sourzat 2009). Since then the technique has been extensively used and hundreds of truffières have been established in European countries. The largest black truffle plantation established in Spain covers 600 ha. The production of truffles from truffières varies: on average, 2–50 kg ha^{-1} without irrigation and up to 150 kg ha^{-1} with irrigation (Chevalier 1998; Reyna et al. 2002; Wang and Hall 2002; Zambonelli, pers. comm.). However, in France, a truffière is considered successful if 10 years after planting, 50 % of the trees produce truffles with yields reaching 15–20 kg ha^{-1} (Chevalier 1998). Similar technology has been used to successfully grow other truffles species, e.g. *T. aestivum* (Chevalier and Frochot 1997; Chevalier and Frochot 1990; Wehrlen et al. 2009) in France, Italy (Bencivenga et al. 2009) and Sweden (Wedén 2004), *T. borchii* (Zambonelli et al. 2002) in Italy and New Zealand (Guerin-Laguette et al. 2009), and desert truffles, such as *Terfezia claveryi*, in Spain (Honrubia, pers. comm.).

Cultivation of truffles has also been successful in several countries outside Europe. In the 1980s, a few Périgord black truffle truffières were established in the USA using inoculated truffle seedlings imported from France or produced by the US company, Agri-Truffle (Picart 1980). In 1991, Northern California and North Carolina welcomed their first harvest of Périgord black truffles (Garland 1999). Many plantations have been established across the USA since then, but less than ten of those plantations have began production with annual production of up to 40 kg. Other species including *T. aestivum* and *T. borchii* were introduced into the USA for cultivation, but there is no report on their production. Attempts on cultivation of several native edible species, including *T. gibbosum*, *T. oregonense*, *T. lyonii* and *Leucangium carthusianum*, have been unsuccessful (Bruhn et al. 2009; Lefevre 2008).

In 1993, the first harvest of Périgord black truffles in the southern hemisphere came from a truffière established in New Zealand in 1987. More than 100 truffières have been established in both the North and South islands of New Zealand since 1987. So far more than ten truffières have produced truffles. The latest one to become productive was established in 2002. All successful truffières are in the warmer parts of the country (Guerin-Laguette et al. 2009).

In Australia, the first black truffle truffière was established in Tasmania island in 1993 with the first harvest of truffles in 1999. Twenty-eight truffières have been established in the island, of which six truffières have produced truffles in a small amount since 2001. A total of 600 ha of truffle plantations have been established in Australia, including areas in New South Wales, Victoria and Western Australia

(Carol 2003; Cooper 2001; Malajczuk and Amaranthus 2007). According to Graham Duell (pers. comm.), the production of truffles of *T. melanosporum* in Australia is 4,900 kg. It is noted that a considerable proportion of truffles in truffières in both New Zealand and Australia have become rotten before they got matured. Unsuitable weather conditions could be the possible reasons for mushroom decay.

Culture techniques for *T. melanosporum* were introduced in Chile at around 2003 (Pérez et al. 2007; Ramírez et al. 2003; Santelices and Palfner 2010), and a few black truffle plantations were established on hazelnut (*Corylus avellana* L.) and produced a small quantity of truffles.

Israel has been reported to produce a small quantity of truffles in 2000 from one of the truffières established in 1993 (Kagan-Zur et al. 2002; Pinkas et al. 2000). There are no further reports on the productivity in Israel. Experimental black truffle plantations were established in Morocco in which truffles have been produced (Kahbar et al. 2008). In southern British Columbia, Canada, three experimental plantations of *T. melanosporum* and *T. aestivum* have been established with two sites limed to raise pH to 7.8 (Berch et al. 2009). In 2013, truffles were produced from the plantations (Berch S, pers. comm.)

In China, *Tuber indicum* and *T. aestivum* (*T. uncinatum*) have been collected and traded for centuries (Wang and Liu 2011), but it was not until the late 1980s that research on cultivation of truffles was initiated. The first successful cultivation of a *Tuber* species in China was *Tuber formosanum* which is closely related to *T. indicum* in a truffière established in 1989, producing truffles in 1996 (Huang et al. 2009). Since then, a few plantations have been established in Guizhou, Hunan, Sichuan and Yunnan provinces. They have no production yet except one report of harvest of *T. indicum* and *T. melanosporum* in Guizhou in 2008. Research on the cultivation of *T. melanosporum* and *T. aestivum* in China is in progress (Chen 2002; Wang and Liu 2011).

Experimental plots of *T. uncinatum* were established in Sweden and produced truffles in 2005 (Wedén and Danell 2008). In Finland, the first plantation of *T. aestivum* (=*T. uncinatum*) was established in 2002 with oak, hazel and *Tilia cordata*. The seedlings were protected during the cold winter and the mycorrhizas survived under −7 °C in 2007 (Shamekb and Leisola 2008). A few small plantations of *T. uncinatum* were also established in New Zealand and one of them produced truffles in 2007 (Guerin-Laguette et al. 2009). Research on cultivation of *T. uncinatum* is carried out in the USA, Germany, Austria, the UK, Slovenia, Slovakia, Serbia and Switzerland (Wehrlen et al. 2009).

Since the successful cultivation of *T. borchii* (bianchetto) in Italy in 1994, a few truffières have also been established with *T. borchii* mycorrhised trees of *Quercus robur*, *Corylus avellana*, *Pinus radiata*, *P. pinea and P. pinaster* in modified acid soils in New Zealand. The first bianchetto was produced from the demo plantation of the Plant and Food Research in New Zealand in 2006 and five *T. borchii* plantations are producing bianchetto truffles. The bianchetto truffles are well accepted by the New Zealand markets.

23.2.1.2 Desert Truffles

Desert truffles are species from the genus of *Terfezia*, *Tirmania* and *Leucangium* which are used as food in Africa and the Middle East (Trappe 1990). They can form ectomycorrhizas, endomycorrhizas and ectendomycorrhizas with *Helianthemum* and other members of Cistaceae under various conditions (Awameh 1981; Fortas and Chevalier 1992; Kagan-Zur 1998; Honrubia et al. 2002). Mycorrhizas of desert truffles have been produced in semi-axenic culture and in vitro (Awameh 1981; Fortas and Chevalier 1992; Kagan-Zur 1998; Honrubia et al. 2002; Morte and Honrubia 1995). Plantations were established in Spain in 2000, and desert truffles were harvested 2 years later. Yields of desert truffles from natural bushes typically range from 50 to 170 kg ha^{-1} annually in Spain. However, irrigated truffières (e.g. 90 L m^{-2}) produced truffles as high as 300 kg ha^{-1} indicating a potentially high profitable industry in semi-desert areas of warm countries (Honrubia, pers. comm.). Unfortunately, little progress in cultivation of desert truffles has been made since 2002.

23.2.1.3 Shoro

The shoro (*Rhizopogon roseolus* Corda) as delicacy has been harvested and eaten for many years in Japan and recorded in ancient fungal books (Wang et al. 2008). Shoro is also collected and traded in China. Shoro was the fourth most commonly consumable mushroom in Japan 200 years ago (Okumura 1989). However, production of shoro has declined since the nineteenth century and hence attempts for cultivation of shoro commenced in the 1980s. In the Shimane and Kyoto Prefecture, fruiting bodies were produced from infected seedlings in 1988 and 1991, respectively (Iwase, pers. comm.) A few plantations have been established in New Zealand using pine seedlings mycorrhised with spores from a shoro species that was accidentally introduced to this country with European settlers. Since 1999 all plantations have produced mushrooms (Wang and Hall 2002). Recently, a group of New Zealand scientists from Plant and Food Research used multiplex PCR to analysis phylogeographic variation among collections in the *Rhizopogon* subgenus *Roseoli* and showed that the shoro species which was reported as *Rhizopogon rubescens* and commonly found in New Zealand is different from the Japanese shoro species, *R. roseolus* (Visnovsky et al. 2010). New Zealand shoro species is more closely related to the American collections in the subgenus. However, data were insufficient to determine whether the genetic differences observed between the two types of shoro were of significance at the species or subspecies levels. The Japanese isolates of shoro have since been introduced into New Zealand for producing mycorrhizal seedlings. A small experimental trial established in 2007 has produced fruiting bodies since 2009 (Visnovsky et al. 2010).

23.2.2 Epigeous EMMs

Compared to truffles, cultivation of epigeous EMMs is more difficult and has been much less successful. So far, only saffron milk cap (*Lactarius deliciosus*) in France (Poitou et al. 1984) and then in New Zealand (Wang and Hall 2002), *Lactarius hatsutake* in China (Tan et al. 2008) and honshimeji (*Lyophyllum shimeji*) in Japan (Yamanaka 2008) have been successfully cultivated (Wang et al. 2012).

23.2.2.1 Saffron Milk Cap

Poitou et al. (1984) pioneered the cultivation of *Lactarius deliciosus*. They produced fruiting bodies in the field from outplanted mycorrhizal seedlings of *Pinus pinaster*. After a silent period for the development of commercial cultivation of saffron milk cap, a New Zealand *Pinus radiata* plantation produced the first saffron milk cap fruiting body in 2002, 18 months after outplanting (Wang and Hall 2002). Presently hundreds of hectares of saffron milk cap plantations have been established in New Zealand and nearly all of them are producing fruiting bodies every year. Recently, New Zealand mycologists have found out that the production of saffron milk cap is significantly related to initial mycorrhizal level and to plantation management including irrigation and mulching (Wang et al. 2011; Wang et al. 2012). Seedlings of *Pinus massoniana* mycorrhised with *L. hatsutake* in a nursery in Hunan, China, have produced fruiting bodies in plantations since 2001 (3–4 years after inoculation) with an average yearly production of 670 kg ha^{-1} (Tan et al. 2008). The fruiting bodies of several *Lactarius* species were also obtained in open pot containers under growth chamber conditions, i.e. *L. deliciosus* on *P. sylvestris* seedlings (Guerin-Laguette et al. 2000a) and *L. akahatsu* on *P. densiflora* seedlings (Yamada et al. 2001). *Lactarius sanguifluus* mycorrhised plants have been produced in vitro (González-Ochoa et al. 2003), but infections failed to develop after outplanting.

23.2.2.2 Honshimeji

Honshimeji (*Lyophyllum shimeji*) is a delicacy in Japan and China, which is equally famous as matsutake in some regions in Japan. In Nara and Kyoto Prefecture, Japan, fruiting bodies of shimeji were produced from artificial mycorrhised seedlings in 1998 and 1996, respectively (Iwase, pers. comm.). Fruiting bodies of *Lyophyllum shimeji* have been also produced from inoculated seedlings growing in open pots in a greenhouse and pure cultures (Kawai 1997; Ohta 1994, 1998; Yoshida and Fujimoto 1994). Research on *L. shimeji* in New Zealand has made good progress. Seedlings of *Pinus radiata*, *P. densiflora* and *Picea alba* formed mycorrhizas with shimeji isolates from Japan and China.

23.2.2.3 Chanterelle

The chanterelle (*Cantharellus cibarius*) and related species are one of the most popular EMMs. Their fruiting bodies are found to have bacteria living inside which makes pure culture of their mycelia very difficult. Danell (2002) successfully obtained pure cultures from fruiting bodies of *C. cibarius* and produced mycorrhizal seedlings. The growth and establishment of mycorrhizal formation by *C. cibarius* depended on the co-bacteria (Danell 1994). Fruiting bodies of *C. cibarius* have formed on inoculated seedlings growing in open pots in a greenhouse (Danell 2002; Danell and Camacho 1997). However, experimental plots of *Cantharellus cibarius* in Sweden failed to produce fruiting bodies even though the mycorrhizal formation has spread onto new roots (Danell 2002).

23.2.2.4 Matsutake

Matsutake, pine mushroom (*Tricholoma matsutake*) and related species are the most appreciated EMM in Japan. There has been a high demand for this mushroom due to production decline since WWII. Cultivation of matsutake has become a hot spot in research in Japan and Korea, but no successful attempt is reported so far although *T. matsutake* mycorrhizal plants have been produced in vitro and mycorrhizal formation in situ (Hu 1994; González-Ochoa et al. 2003; Guerin-Laguette et al. 2000a; Wang et al. 1997; Yamada et al. 1999, 2006; Wang et al. 2012). Recently, South Korean mycologists transplanted 150 matsutake mycorrhised seedlings of *Pinus densiflora* into a non-matsutake-produced *P. densiflora* forest at Hongcheon, Korea. The matsutake seedlings were produced by planting the pine seedlings in mesh pots near the front shiro. The matsutake mycelia started to grow into neighbouring soils 1 year after planting (Ka, pers. comm. 2008). The Mountain Environmental Research Institute, Gyeongsangbuk, South Korea, patented their methods of producing matsutake mycorrhised pine seedlings in 2007 (Park et al. 2007, patent No.: US7269923). Under sterile conditions 15-day-old seedlings of *Pinus densiflora* were inoculated with matsutake mycelia and incubated at a clean room. The 2-month-old mycorrhised seedlings were transplanted into a greenhouse for 4 years before outplanting to exiting *P. densiflora* forests. More than 40,000 seedlings have been outplanted but there is no report on its progress.

With respect to the growth of matsutake in liquid medium, Kawagishi et al. (2004) found that the addition of D-isoleucine to the culture medium of matsutake significantly enhanced mycelia growth. Kim et al. (2010) investigated the optimal medium composition of liquid culture with the goal of shortening the culture period and to maximise polysaccharide production and mycelial growth of matsutake. The experimental results showed that the optimal medium contained 40 g L^{-1} glucose, 30 g L^{-1} yeast extract, 1.5 g L^{-1} KH_2PO_4 and 1 g L^{-1} $MgSO_4 \cdot 7H_2O$.

23.2.2.5 Porcini

Boletus edulis Bull.: Fr. sensu stricto, *B. aereus* Bull.: Fr, *B. aestivalis* Fr., *B. pinophilus* Pilát et Dermek and *B. reticulatus* Boud. are a group of allied porcini species that are often grouped together as *B. edulis* sensu lato. *B. edulis* sensu lato is one of the highly prized edible mushrooms, sold freshly or dried worldwide with approximately 20,000–100,000 tons consumption annually (Hall et al. 1998b). The commercialisation of *B. edulis* depends on the collection of fruiting bodies from natural forests.

Modifications of the techniques described by Molina and Palmer (1982) for other ectomycorrhizal fungi have been successful in producing laboratory-scale numbers of plants mycorrhised with *B. edulis* and other Boletaceae (Olivier et al. 1997; Wang et al. 1998; Zuccherelli 1988). However, mycorrhizas often collapse once infected plants are transferred into unsterile media. Although the germination of *B. edulis* spores can be enhanced with abietic acid (Fries et al. 1987), mycorrhisation failed to form even with the application rate of 10^7 spores per seedling (Guerin-Laguette et al. 2011). The New Zealand Plant and Food Research mycologists successfully produced porcini mycorrhised pine seedlings with Talon's method, which has been used to produce *T. melanosporum* mycorrhised seedlings. Several small experimental plantations have been established with mycorrhizal seedlings since 2007 (Wang and Guerin-Laguette, unpublished data). Future research is required for efficient production of large numbers of mycorrhised seedlings. Spanish mycologists produced porcini mycorrhised seedlings with *Cistus* species in vitro (Agueda et al. 2008) and produced fruiting bodies in *Cistus* plantation as early as 3 years after outplanting (Oria de Rueda et al. 2008). This discovery and achievement might be the new hope for cultivation of porcini.

23.2.2.6 Caesar's Mushroom

Amanita caesarea (Scop.: Fr.) personally known as Caesar's mushroom is another delicious EMM (Wang and Hall 2004). Daza et al. (2006) studied the effect of carbon and nitrogen sources, pH and temperature on in vitro culture of several isolates of *Amanita caesarea* in association with *Quercus suber* and *Castanea sativa* in southwest of Spain. The growth condition was optimised to maximise production of mycelia (24–28 °C, pH 6–7, mannitol and glucose as carbon sources and ammonium as a source of nitrogen). The knowledge of in vitro growth requirements of *A. caesarea* is a first step towards inoculum production for nursery and field applications to increase the sporocarp production and ecological benefits to trees.

23.3 Advance in Cultivation Technology

23.3.1 Production of Mycorrhizal Seedlings

The major challenge to cultivate EMMs is producing stable mycorrhizal seedlings. The method used to produce truffle mycorrhizal seedlings pioneered by Joseph Talon and Francolini in the early 1800s (Bencivenga et al. 2009; Sourzat 2009) is still widely used presently. This method has been used to produce matsutake-infected pine seedlings in Japan (Iwase 1997), Korea (Lee 1981) and China (Wang et al. 1997) and porcini trees in New Zealand (Wang and Guerin-Laguette, unpublished data). However, spore inoculation remains the most popular method and has been used successfully to produce trees mycorrhised by many common truffle species with the exception of *T. magnatum*, *T gibbosum* and *T. oregonense*. This method has also been used to produce trees infected with *Rhizopogon* and desert truffle species (Honrubia et al. 2002; Visnovsky et al. 2010). Generally the starting point is the preparation of spore suspension, mycorrhizal-free seedlings and suitable substrate (Bencivenga et al. 2009; Hall et al. 2007; Sourzat 2009). The use of mycelium inoculum to produce mycorrhizal seedlings is a successful practice in forestry (Grove and Malajczuk 1994). The use of mycelium inoculum has been reported to be successful for mycorrhisation of pine seedlings with *Lactarius deliciosus* (Diáz et al. 2009; Guerin-Laguette et al. 2000a; Wang and Hall 2002), *L. hatsutake* (Tan et al. 2008) and *Suillus bovinus* (Chen et al. 2004). However, mycelium inoculum has been shown ineffective in producing mycorrhizal seedlings with other EMMs.

Rossi et al. (2007) pointed out that the fungal inocula preparation is the crucial point in the production of mycorrhizal seedlings. Diáz et al. (2009) studied on the production of saffron milk cap mycorrhised *Pinus halepensis* seedlings under nursery conditions. They concluded that (1) mycelial slurry at a dose of 10 mL plant^{-1} was efficient when compared with mycelia entrapped in alginated beads and solid inoculum, (2) mycorrhizal formation performed better in sphagnum peat substract than the mixture of sphagnum peat and vermiculate and (3) addition of moderate N (35 mg plant^{-1}) and P (27 mg plant^{-1}) produced better mycorrhisation. Knowledge on mating type genes opens up the possibility of using mycelial inoculation technology to produce truffle mycorrhizal seedlings with compatible mating strains (Rubini et al. 2010). In addition, this technology provides the possibility to select the best genetic strains for improving the productivity or environmental adaptability of EMMs. Mycorrhiza helper bacteria (MHB) could be essential for EMM mycorrhisation as shown in a case of Danell and Camacho (1997). Kataoka et al. (2009) also confirmed that MHB strains of *Ralstonia basilensis* and *Bacillus subtilis* are fungal selective when tested with four ECM fungi including EMM fungi *Rhizopogon* sp. and *Suillus granulatus*. Savoie and Largeteau (2011) addressed that the MHB may help *Tuber magnatum* and other uncultivated EMM species to produce mycorrhizal trees. Appropriate selection of suitable host plant species is essential for successful mycorrhisation.

For the cultivation of Périgord black truffle (*T. melanosporum*), *Quercus pubescens* and *Q. ilex* are considered better candidates than hazels (*Corylus* spp.) in European counties. However, hazels performed better in EMM production than oaks in Australia and New Zealand. Furthermore, among the species of hazels, *Corylus colurna* was better than *C. avellana*. *T. uncinatum* (*T. aestivum*) has a wider range of host plants than the Périgord black truffle, including broad-leaf tree species such as oak, hazel and conifers such as *Pinus nigra*, *P. armandii* and *Cedrus atlantica*. Wang and Guerin-Laguette (unpublished data) have observed that *T. borchii* associated with pine species showed a better production of mycorrhizal seedlings with less contaminants than with broadleaved trees (hazels, oaks) in New Zealand environment. More research on the relative advantages of different host species or superior strains is needed. Is host plant cloning improving EMM mycorrhizal quality and sustainability? This respect has been less studied and needed further exploration. A recent report of Ortega-Martínez et al. (2010) demonstrates that tree age influences the speed of sporocarp growth of *Boletus edulis* and *Lactarius deliciosus* in a *Pinus sylvestris* stand.

The Asian black truffle *T. indicum* is considered to be a broad host spectrum, an ecological trait that may be important to its invasion ecology. *T. indicum* was found fruiting in a forest in Oregon, USA, and was invaded into a nearby truffle orchard in which only *T. melanosporum* was introduced via molecular authentication (Bonito et al. 2010). Asian *T. indicum* was also observed on the roots of several North American endemic trees, such as loblolly pine (*Pinus taeda*) and pecan (*Carya illinoinensis*).

The quality control of EMM seedlings is an important issue but hard to do both technically and legally. Reyna et al. (2002) discussed methods for root sampling and the measurement levels of infection suggesting 250–500 infected root tips equivalent to 10–25 % of a root system were acceptable. Bencivenga et al. (1995) believed that the infection rate of about 33 % was acceptable and contaminations should never be higher than 25 %. However, performance on sampling seedlings and root subsamples is problematic. Morphological identification of mycorrhizal roots of different *Tuber* species is sometimes difficult or impossible (Mabru et al. 2001). Molecular methods may provide more accurate for quality control in a large scale. This practice and the associated cost require further study.

23.3.2 Establishing and Managing Plantations

The term "mycosilviculture" has been recently used to refer to the development of a science-based production of EMMs as a new industrial crop (see Sect. 21.3.5). Soil properties and climatic characters are the most important factors for truffières establishing. The climatic condition is more decisive than the soil because soil conditions are more manageable than climatic conditions (Chevalier 1998; Olivier 2000; Hall et al. 2007; Sourzat 2009). For instance, adding lime to acidic soils to raise the pH can make it suitable for the cultivation of the Périgord black truffle

(Chevalier 1998; Hall et al. 2007; Olivier 2000). Sourzat (2009) generated the details of soil requirements for growing truffles. The physical soil characteristics are perhaps even more important because the Périgord black truffle requires good drainage to avoid the soil conditions favouring competitive ectomycorrhizal fungi (Chevalier 1998; Hall et al. 2007; Olivier 2000). Spanish mycologists recently showed that the content of active Ca in soil played an important role for black truffle mycorrhisation and production (Garcia-Montero et al. 2008). In general, warm and moist spring and fall, hot summer with rainstorms and mild winter without heavy frost are good for growing black truffles (Sourzat 2009). Too much rain or snow in late fall and early winter and less sunny hours in summer are not suitable for cultivation of black truffles.

Methods of management of truffières are variable, from intensive procedures termed the Pallier technique to minimal management—the Tanguy method (Bencivenga et al. 2009; Chevalier 1998; Sourzat 2009). The Pallier method, adapted from orchard management, includes soil tillage, irrigation, weed control and tree pruning. It is expensive, but sometimes gives good production in return. Sourzat (2009) described a new method, "the Tanguy method," as an alternative cultivation method for black truffles. This method is based on natural truffle grounds and was created by Marcel Tanguy in Le Périgord, France. The method manages trees growing slower and produces truffles later with high production of 1 kg per trees on average.

Irrigation has been proved to be important for the Périgord black truffle (Hall et al. 2007), burgundy truffles (Wehrlen et al. 2009), the desert truffle (Honrubia et al. 2002) and saffron milk cap (Wang and Hall 2002; Wang et al. 2012). Irrigation is, in particular, necessary in the first 2 years after establishment and during truffle formation period. But heavy irrigation encourages *T. brumale* development that will lead to the replacement of *T. melanosporum* by *T. brumale* (Bencivenga et al. 2009; Sourzat 2009). The soil physiology and biology (microflora, micro- and mesofauna) are probably particularly important, but our knowledge about them is very limited. Recent research revealed that irrigation is not only important but also closely related to mushroom production of saffron milk cap (Wang et al. 2012).

23.3.3 Molecular Technology

Recent advances in genetics and molecular biological techniques in EMMs have provided better understanding of biology for an enhanced production of EMM. Most significantly, the genome of *Tuber melanosporum* has recently been sequenced which provided information to identify genes involved in the reproductive processes of this truffle. It is known that *T. melanosporum* is heterothallic and requires two master genes for mating. Mating type-specific primer pairs were developed to screen asci and gleba to provide definitive evidence of the presence of the mating genes (Rubini et al. 2010). The comparison of genomes of *Tuber melanosporum* and *Laccaria bicolor* showed that their genetic predispositions for

symbiosis evolved in different ways between Ascomycetes and Basidiomycetes (Martin et al. 2010). It might be a good idea to apply the technology of growing truffles to cultivate EMMs of Basidiomycetes (Savoie and Largeteau 2011).

Molecular methods, such as PCR with specific primers and multiplex PCR and sequencing, have potential in verifying inoculum and mycorrhizal trees. Molecular technologies also provide a new approach for understanding mycorrhizal associations and therefore have implications for cultivation and management of EMM plantations (Amicucci et al. 1998; Franken and Requena 2001; Martin 2001; Nehls et al. 2001; Visnovsky et al. 2010). Murata et al. (2008) found four genotypes and some sub-genotypes in Asian matsutake based on retroelement-based DNA markers. Using single-nucleotide polymorphic (SNP) DNA markers, Amend et al. (2009a) investigated isolation by distance patterns on eight populations of matsutake mushrooms within and between watersheds suggesting an important determinant of air-dispersed ectomycorrhizal species population structure in heterogeneous landscape. The nuclear-encoded large subunit ribosomal DNA of isolates in the genus *Amanita* including Caesar's mushroom was sequenced to explore phylogenetic relationships among collections (Drehmel et al. 1999). Visnovsky et al. (2010) investigated the phylogenetic relationships of *Rhizopogon roseolus* and other closely related fungi belonging to *Rhizopogon* subgenus *Roseoli* by sequencing the ITS1-5.8S-ITS2 region of rRNA. The designed multiplex PCR approach is used to track the establishment of ectomycorrhizal symbioses on *Pinus radiata* seedlings inoculated with commercially valuable *R. roseolus*. This diagnostic demonstrated the first fruiting of Japanese shoro cultivated on *P. radiata* in the southern hemisphere. The haplotype networking method was employed to assess intraspecific ITS rDNA diversity among Asian and North American *T. indicum* group B isolates (Bonito et al. 2010).

Great progress in molecular studies has been made in particular for the *Périgord* truffle in recent decades. Phylogenetic information such as single-based polymorphisms has been used to design species-specific primers for white (Mello et al. 1999) and black truffles (Mabru et al. 2001; Paolocci et al. 1999; Rubini et al. 1998). PCR-RFLP using a SNP on the mitochondrial LSU-rDNA is an easy method to differentiate *Tuber melanosporum* from other truffle species like *T. aestivum*, *T. brumale* or *T. indicum* (Mabru et al. 2004). The genetic differentiation among *T. melanosporum* populations, highlighted by Murat et al. (2004) and Riccioni et al. (2008), suggested that the characterisation of molecular markers to identify the regional origin of ascocarps is within reach (Murat and Martin 2008). In the fungal genome, several thousands of simple sequence repeat (SSR) motifs can be identified (Lim et al. 2004) given a large set of polymorphic markers. By analysing SSRs, Murat et al. (2011) claimed that *T. melanosporum* genomes is rich and highly polymorphic in SSRs. Out of the 139 isolates, 132 different multilocus genotypes were identified indicating high genotypic diversity (0.999). Furthermore, Tisserant et al. (2011) applied high-throughput Illumina RNA sequencing (RNA-Seq) to the transcriptome of *T. melanosporum* at different major developmental stages and identified a substantial number of novel transcripts, antisense transcripts, new exons, untranslated regions (UTRs), alternative

upstream initiation codons and upstream open reading frames. These researches provide new molecular markers to analyse the natural populations of this truffle and to authenticate mycorrhised seedlings.

23.4 Management and Conservation of EMM Resources

Since most EMMs, including *Tuber magnatum* and *Tricholoma matsutake*, are not able to be cultivated, their products are all gathered from the natural forests. The last decade's environmental deterioration has become a worldwide problem due to varied reasons, causing a general decline of EMM production. And thus protection and management of EMM nature resources has become an urgent matter particularly in developing countries.

Most EEMs are found growing in remote mountainous regions where EEMs are important sources of food and revenue to local people. Under the pressure of hunger and cold, deforestation and overharvesting are common. Environmental deterioration has been the cause for EEM species to become endangered or to disappear from some areas. For example, commercial harvest of matsutake began in the late 1970s in northeast China and the late 1980s in southwest China. Production of matsutake in both regions has been dramatically declined since the time commercial harvest started. Matsutake production in northeast China dropped from several hundreds to less than 100 tonnes per year. The southwest China matsutake production followed the similar pattern of depletion. For example, the production of matsutake in Chuxiong Prefecture of Yunnan Province, southwest China, reduced from more than 1,000 tonnes in the 1980s to around 250 tonnes in 2005. Matsutake production in Diqing Prefecture, another productive area of Yunnan, dropped from 865 tonnes in 1999 to 469 tonnes in 2005 (Yang et al. 2008, 2009). Nevertheless, production of the Chinese black truffle (the *Tuber indicum* complex) in southwest China is decreasing due to environmental deterioration caused by large-scale commercial harvesting. The environmental conditions have been damaged so much by unrestricted plundering of these natural resources during large-scale commercial harvesting since the 1990s that the black truffle species disappeared or became endangered in many counties of Sichuan and Yunnan Province (Chen 2004b; Wang and Liu 2009). Similar situations of deforestation and overharvesting EMMs occurred in most developing countries, where the range of EMM species present and their market value are poorly understood (Wang and Hall 2004). Compared with developing countries, Canada, South Korea and the USA show better management of their matsutake forests, and a relatively sustainable production of matsutake is maintained. The decline of matsutake production in South Korea by 7 % annually since the middle of the 1980s is regarded as forest ageing rather than commercial harvesting. Tree ageing caused the similar problem in Japan (Wang and Hall 2004). Therefore, improved forest management and conservation of existing EEM environments is an urgent matter particularly in developing countries.

Savoie and Largeteau (2011) termed the management of forest stands for mushroom production as "mycosilviculture" which is becoming increasingly important in Europe (Honrubia et al. 2011; Rondet 2011). Egli and Ayer (2009) found that thinning old-growth forest induced a significant increase of fungal diversity and their production, especially EMMs. Pilz et al. (2006) found that the number and weight of *Cantharellus formosus* were significantly decreased by thinning in the first year after logging, but the differences disappeared within the following 6 years. In Japan, management techniques for maximising *T. matsutake* production in existing forests are developed. Practical measurements include reducing the thickness of the litter layer; tree thinning removing competing ectomycorrhizal fungal fruiting bodies; protecting *T. matsutake* forests from diseases, insects, birds and other animals; and inoculating the soil by spraying *T. matsutake* spores and/or retaining some of the mature fruiting bodies on the forest floor. Similar management technologies were also employed in South Korea and China (Wang and Hall 2004). Commercial harvesting of EMMs has removed the opportunity for new trees or roots to become mycorrhised by spores and damaged growth environment for fungal hyphae in the topsoils (Chen 2004b). In order to correct this problem, the method for inoculating existing mature trees was developed (Guerin-Laguette et al. 2000b; Wang and Hall 2004). The results showed that black truffle mycorrhizas were successfully established on the new roots. Spores to reinoculate existing trees have also been used in attempts to replace *Tuber brumale* with *T. uncinatum* in France (Frochot et al. 1999). Similar methods have been used successfully to inoculate pine trees with saffron milk cap spores in Spain (Marcos, pers. comm.). Liu and co-workers used spores of *Lactarius volemus* to inoculate existing mature pine trees in Yunnan, China, and observed increased production of the mushroom from the inoculated trees (Liu et al. 2007).

Basic research on fungal succession as vegetation successions and growth in natural forests and plantations has provided better understanding of the dynamics of diversity and productivity of EMMs (Savoie and Largeteau 2011; Wang and He 2004; Wang and Hall 2004). Molecular analysis of EMM populations revealed that the matsutake population structure is related to heterogeneous landscape and its dispersal strategy (Amend et al. 2009a, b). Ecological studies on EMMs, including mycorrhizal communities and relationship of fructification to climatic and soil conditions, have provided the scientific basis for management of EMM plantations (Savoie and Largeteau 2011; Wang and Hall 2004).

23.5 Conclusion

EMMs are not only a gourmet food but also significant sources of livelihood. The most expensive and sought-after edible mushrooms belong to this group, for example, *Tuber melanosporum*, *T. magnatum*, *Tricholoma matsutake*, *Boletus edulis*, *Cantharellus cibarius*, *Amanita caesarea*, *Lyophyllum shimeji* and *Lactarius deliciosus*. Over the past 100 years, natural production of many mycorrhizal

mushrooms has declined dramatically. This has prompted interest and the need for developing appropriate methods for their cultivation. A few species of truffles, mostly in the genus *Tuber*, have been cultivated commercially. Techniques have been extended and developed for the cultivation of epigeous species, but few species, e.g. *Lactarius deliciosus* and *Lyophyllum shimeji*, are successful at a commercial scale. *Tuber magnatum* and *Tricholoma matsutake* and many valuable mycorrhizal mushrooms have defied cultivation. The last decade's environmental deterioration has become a worldwide problem due to varied reasons. Protection and management of EMM resources has become urgent matter, particularly in developing countries. Some new technologies have been developed for the management of EMM plantations, in order to maximise their production. Modem technologies involving the use of molecular approaches for truffle genome studies have provided better understanding of the biology and plant-fungus symbioses of EMMs. Cultivation and management of EMMs is in progress although cultivation on some mycorrhizal mushrooms remains challenging.

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