Fungal Biology

Marcela C. Pagano *Editor*

# Recent Advances on Mycorrhizal Fungi

Springer

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Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and nonliving is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and, therefore, may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of “one pot” microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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*Editor*
Marcela C. Pagano
Federal University of Minas Gerais
Belo Horizonte, Minas Gerais, Brazil

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# Contents

# Chapter 1
# Overview of the Recent Advances in Mycorrhizal Fungi

**Marcela C. Pagano and Vijai K. Gupta**

## 1.1 Introduction

Since 1999 researchers have noted the increasing decline of populations and loss of species in all ecosystems (Spellerberg and Sawyer 1999). Today, in spite of more financial resources available to conservation, there is a need of prioritization of good decisions by decision makers and how to make better choices. It is known the urgency in increasing knowledge on geography and ecology of present world biodiversity in order to do the best uses of limited resources for conservation (Spellerberg and Sawyer 1999) is known. This will help to reduce human impact on ecosystems and to improve sustainable managements.

Agropecuary activity, lumber extraction, and urban development are factors that act in forestry fragmentation, particularly in margin of rivers, and coast. Only at this time we are truly recognizing the importance of conservation of river forest cover and that volume of water stored in soil and organisms living in the biosphere is bigger than superficial and aquifer recharge waters (Pinto-Coelho and Havens 2015). Shifts in available water together with climatic change have become constraints not only in countries with arid climate but also in countries such as Brazil, which suffer limitations in water supply (Pinto-Coelho and Havens 2015). Thus, governmental decisions in this country focus, for example, on agroecosystems less dependent on rainfall. Moreover, there is increased interest to study the tropical riparian forests that have been extremely impacted in their important ecological functions (Ribeiro et al. 2009; Pagano and Cabello 2012a). The use of native species for restoration of riparian environments is increasingly accepted and initial management studies

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M.C. Pagano (✉)
Federal University of Minas Gerais, 3106200 Belo Horizonte, Minas Gerais, Brazil
e-mail: marpagano@gmail.com

V.K. Gupta
School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

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revealed that native flora present Arbuscular mycorrhizal fungi (AMF) in their roots as well as high spore number in the different associated soils (Stürmer et al. 2012; Pagano and Cabello 2012a). For example, recent studies by Mardhiah et al. (2014) in islands along a 40-year chronosequence of the Tagliamento River, Italy, showed increased water stable aggregates with site age confirming the essential role of soil biota and plant roots in aggregating soils subjected to physical disturbances.

Nowadays a whole management approach to soils is mandatory as undisturbed soils are necessary for food but also for clean air and water (drinking water depends on maintaining soil properties) and to attain better human health (Wall and Six 2015). As part of the soil biota and colonizing plant roots, the arbuscular mycorrhizas (AM) link the biotic and geochemical components of the ecosystems providing more important ecosystem services. Research on Mycorrhizas has gone through several stages (Stürmer 2012); however, the present period has revolutionized research on these fungi.

Thus, the importance of AMF and ectomycorrhizas (ECM) for soil organic matter and soil carbon conservation is nowadays more recognized (Bradford 2014) and the association of trees with different mycorrhizal fungi is highlighted to understand the biotic interactions in carbon dynamics (Averill et al. 2014). Therefore, their preservation is highly recommended (Lalaymia et al. 2014). Moreover, some analysis pointed mycorrhizal and dark septate endophyte fungal symbioses as critical to predicting ecosystem response to global change (Kivlin et al. 2013).

This chapter discusses advances on mycorrhizal fungi potential drawing on recent results of research worldwide and with special attention to developments in Latin America. Studies in mycorrhizas have developed largely; however, research on ecology and biogeography is still limited. Moreover, the applications of mycorrhizas in environmental issues are still incipient. These limitations are also discussed.

## 1.2 The Mycorrhizal Symbioses

Microorganisms are increasingly investigated for novel compounds from saprophytic terrestrial fungi to marine habitats and living plants with their endophytes (Schueffler and Anke 2014). A growing worldwide attention on fungi is noticed, as of 100,000 known fungal species more than one million are predictable to exist (Schueffler and Anke 2014).

Among soil microorganisms, AMF perform symbioses with more than 80 % of all terrestrial plant species (Smith and Read 2008; Pagano 2012). Additionally, some aquatic and wetland plants can associate with AMF (see Pagano 2012). The mycorrhizal symbioses can consist in the internalization of one of the partners into the plant host but also in changing the symbiotic partner when a variation in environmental conditions occurs (Buscot 2015). Mycorrhizas are related to most environmental issues facing us today: deforestation, climate change, pollution, and introduced or invasive species (Pagano and Cabello 2012b; Bellgard and Williams 2011; Pagano 2013; Yang et al. 2014).

Arbuscular mycorrhizas link the biotic and geochemical components of the ecosystem providing more than six ecosystem services: increasing plant/soil adherence and stability (the extra radical mycelial web improves rooting and plant establishment), promoting plant growth (reducing fertilizer requirement), increasing plant resistance to stresses (drought, salinity, heavy metals, and mineral nutrient exhaustion), increasing soil stability, structure, and water retention (through the secretion of glomalin and growth of the mycelial web), enhanced plant resistance against biotic stresses, bioregulation of vegetal development and better plant quality for human health (Modification of plant physiology), and increasing plant community diversity (van der Heijden et al. 1998; Gianinazzi et al. 2010).

It could be said that many regions remain unsampled for AMF and characterization of AMF life history and the community dynamics is yet little studied (Morton et al. 2004). For example, the consequences of AMF in the progress of plant invasions were poorly studied. Some works (Yang et al. 2014) tested the mycorrhization of invasive plants (*Solidago Canadensis*), showing changes in AMF species composition associated with native and exotic plant species and shifts in plant biomass and nutrient uptake. The authors discard allelopathic effects and highlight that altered AMF facilitated the invasion of this plant species. Lekberg et al. (2013) also noted that exotic mycotrophic plants can replace poorer native flora and suggested that AMF can respond more to plant functional group than plant provenance.

Some fungi are very attractive for use in mycoremediation due to their lignolytic enzymes that can degrade chemical pollutants (fossil fuels, wood, solid wastes) in the environment (Karaman et al. 2012). Various projects aiming at the restoration of degraded ecosystems or afforestation in semiarid areas and that have used mycorrhizal associations were located in Mexico (Allen et al. 1998; Carrillo-Garcia et al. 1999; Corkidi and Rincón 1997; Monroy-Ata et al. 2007) and in Spain (Lozano et al. 2014). For ecological restoration, the effect of climate change on species distribution must be taken into account (Gastón and García-Viñas 2013; Harris et al. 2006). If the selected species cannot tolerate the environmental modifications predicted under climate change, the restoration strategy will not work in the mid and long term. To date, only historical and current climatic conditions are taken into consideration in establishing the necessary requirements for restoration projects (Ravenscroft et al. 2010). Recently, Gelviz-Gelvez et al. (2015) showed eight potential plant species for use in ecological restoration projects in the semiarid region of Central Mexico. They based on suitable ecological attributes for restoration (cover, abundance, sociability, mycorrhization) and tolerance to projected future climate changes. Several species that would not tolerate environmental modifications caused by climate change will be inappropriate for use in long-term ecological restoration plans.

Increased interest on global change has led to a higher number of reports. With regard to climate change, it has been demonstrated that reduction in precipitation reduces crop production (Sanchez et al. 2001), increases weed invasibility (Kreyling

**Table 1.1** Number of reports dealing with AMF/ECM worldwide

| Topic | Number of reports |
|---|---|
| AMF | 7573 |
| ECM | 2815 |
| AMF and climate change | 104 |
| AMF and pesticides | 93 |
| AMF and invasive species | 58 |
| AMF and ECM | 7 |

Database survey conducted on April 2015 (SCOPUS); *AMF*, arbuscular mycorrhizal fungi; *ECM*, ectomycorrhizas

et al. 2008), and reduces the mycorrhization rates (Augé 2001; Smith and Read 2008). Moreover, different AMF communities can be found in degraded vs. native forest as well as in different tree plantations, crops, and vegetation types (see Pagano and Dhar 2014), as generally these fungi can occur in preferred associations with plant species (Helgason et al. 2002; Li et al. 2010; Torrecillas et al. 2012). Moreover, the impacts of herbicide-resistant weeds on soil biota of crop production fields are not well known (Kremer 2014).

Studies of the interactions between biological, physical, and chemical soil attributes and mycorrhizas remain scarce and are especially lacking in the context of long-term forest recovery and ecological restoration. Also studies on ecology and biogeography and the applications of mycorrhizas in agriculture and environmental issues are limited. Increasingly studies compare seminatural savanna, recovered savanna, and agroecosystem, in both dry and wet periods (Furrazola et al. 2015).

Of the over 7573 published references for AMF found in our Google Scholar search (Table 1.1), only 104, less than 2 %, investigated the effect of climate change on these fungi or the relations with pesticides, and less than 1 % regarded invasive plant species. Of over 2815 reports on ECM less than 3 % were related to climate change. Moreover, only seven reports included both AMF and ECM.

Additionally, with few exceptions, most studies assessed mycorrhizas in only one ecosystem type and generally at two seasons. Many plant species and different vegetational types in tropical regions including semiarid environments were found associated with AMF (Pagano et al. 2013; Silva et al. 2014) and this symbiosis requires to be understood under global change. Increasing interest on crops, specially corn, citrus, and coffee, and the soil biota enhancing agricultural sustainability is noted.

Among recent reviews, only three included ECM (Table 1.2). However, increased interest on ECM due to the use of nitrogen from soil organic matter that is otherwise unavailable to plants, whereas AM fungi, like roots, primarily take up inorganic nitrogen from the soil (Bradford 2014) is noted. This is due to the fact that studies have revealed that ecosystems dominated by trees that associate with

**Table 1.2** Some recent reviews and book chapters dealing with AMF and ECM

| Reports | References |
|---|---|
| Evolution and diversity in AMF and ECM | Buscot (2015) |
| Microbial inoculants in agriculture | Velivelli et al. (2015) |
| Ectomycorrhizal fungi as potential organic matter decomposers | Lindahl and Tunlid (2015) |
| Function in AMF symbioses | Bucher et al. (2014) |
| Mycorrhizal fungi and ecosystem responses to Global change | Mohan et al. (2014) |
| Implications of herbicide resistance and AMF | Kremer (2014) |
| Mycorrhizal and dark septate endophyte fungi and Global change | Kivlin et al. (2013) |
| Ecology of AMF | Willis et al. (2013) |
| Mycorrhiza as a biotechnological tool for improving orchid growth | Hossain et al. (2013) |
| Maintenance and preservation of ECM and AMF | Lalaymia et al. (2014) |
| Diversity of AMF in special habitats | Li et al. (2013) |
| AMF-induced tolerance to drought stress in citrus | Wu et al. (2013) |
| History of the taxonomy and systematics of AMF | Stürmer (2012) |
| Role of AMF in ecosystem services and Agroecology | Gianinazzi et al. (2010) |
| AMF technology in floriculture | Koltai (2010) |
| AMF association in coffee | Andrade et al. (2009) |
| Summary on AMF | Morton et al. (2004) |

ECM store more carbon per unit of nitrogen than plants associated with AMF (Averill et al. 2014).

The specific benefic role of AMF has received attention for cultivation of floricultural species. Some efforts concentrating towards the integration of AMF technology in floriculture practices to increase plant yield and the employment of commercial inocula in floriculture (with little success) were compiled by Koltai (2010).

## 1.3 Natural Environments

### 1.3.1 Spore Abundance and Diversity

AMF biodiversity in natural environments is typically higher than in agricultural systems, because of more plant diversity and a more complex habitat that can support a wider variety of organisms. However, it depends on the biome, vegetation type, and site (Douds and Millner 1999). Usually a universal pattern of AMF distribution (some abundant AMF species and rare species) is found, but dominant AMF can change with time (seasonal variations and succession) (Buscot 2015).

AMF spores (singly, in clusters or in sporocarps) are generally found in the soil and rhizosphere (some spores can be found within roots). Aggregate distribution is commonly found: thus some samples can present more spores than others. Therefore, sampling effort and strategy can affect our vision of AMF communities. For that reason Whitcomb and Stutz (2007) have suggested collecting 15 samples to detect ~80 % of AMF species present in one plot. They studied an experimental site associated with Central Arizona—Phoenix Long-Term Ecological Research at the Desert Botanical Garden in Phoenix, Arizona.

Nevertheless, sometimes it is very difficult to collect or analyze that amount of samples and the use of taxon accumulation curves is recommended to evaluate AMF diversity (Whitcomb and Stutz 2007). For example, if composite samples with little quantities of soils will be analyzed, we need to use less soil quantity (such as 10 or 20 g) to analyze the AMF abundances. Sometimes, few AMF species can be observed with one of them dominating, such as in one soil sample from Pará State, Brazil, where M. Pagano (unpublished) retrieved only one dominant species, *Acaulospora excavata*, at higher abundance (>4 spores $g^{-1}$ soil). Examples are shown by Pagano et al. (2013) who distinguished 32 AMF in semiarid vegetation types in Brazil, recovering ~72 % of the estimated AMF species richness for woody caatinga vegetation (unpublished results). Accumulation curves of AMF species found in the studies and the estimated richness based on the first-order Jackknife index (Jackknife 1) are compared in order to indicate the number of analyzed samples sufficient to detect the majority of AMF species present in the area. Furthermore, Silva et al. (2014) recovered a similar percent of the AMF richness in semiarid region of Brazil. Coutinho et al. (2015) reported a higher recovery of the total estimated richness through an altitude gradient in the rocky soils of the Serra do Cipó, Brazil.

Distributions of AMF spores vary both spatially and temporally within and between host plants and with soil types. In natural vegetation spore numbers can vary from 1 to 20 $g^{-1}$ dry soil (Lugo et al. 2008; Matias et al. 2009; Becerra et al. 2009; Velázquez and Cabello 2011; Velázquez et al. 2013; Carvalho et al. 2012; Soteras et al. 2012; Pagano et al. 2013; Freitas et al. 2014; Coutinho et al. 2015). However, some spores can be counted as fresh and be nonviable, especially those of *Glomus*.

Increasing works on pristine sites show higher AMF occurrence at intermediate compared to low altitudes while decreasing values at > 1100 m of altitude (Coutinho et al. 2015). Previous reports from Pampa de Achala (2250 m above sea level) in Sierras de Córdoba, Argentina, mentioned a total number of spores ranged between 0.08 and 40 spores $mL^{-1}$ soil (Lugo and Cabello 2002). In general, at elevated altitudes, higher values of spore abundances are found in the dry season which decreased in the wet period (Lugo and Cabello 2002). Oehl et al. (2006) found new AMF *Acaulospora alpina* in grasslands of the Swiss Alps at altitudes between 1800 and 2700 m above sea level.

It was reported that more than 20–25 taxa reported in field studies rarely occur (Willis et al. 2013). However, recent advances in spore identification and AMF classifications (which have increased the number of AMF species to 270, http://glomeromycota.wix.com/lbmicorrizas) have changed this picture.

Moreover, new AM species are described continuously, such as *Acaulospora entreriana*, retrieved from rhizosphere of native plant species from scrublands, grassland, and palm forest dominated by *Butia yatay* in Argentina (Velazquez et al. 2008, 2010) or *Septoglomus furcatum*, from rhizosphere of native tree species in Brazil (Błaszkowski et al. 2013), to name just a few. Therefore, new reports show increased number of AMF species. For example, Freitas et al. (2014) have identified 41 spore morphotypes in rhizosphere of three legume trees in Amazonian forest. Carvalho et al. (2012) reported 49 AMF species for six different natural habitats (highland fields, bogs, cerrado, etc.) and Silva et al. (2014) 50 AMF species for three semiarid sites (a dry forest, a transitional zone, and a moist forest).

Spores of AMF show seasonal patterns of abundance in natural environments: spores are greatest in the autumn (for Northern hemisphere) in areas where there are marked warm/cold seasons and lowest during the growing season. Thus, collecting spores in the autumn (or dry period), after they are produced, helps their identification (Douds and Millner 1999).

In Amazonian forest, Freitas et al. (2014) studied spores in the rhizosphere of three mycotrophic tree Leguminosae. They found that soil characteristics and plant host identity were responsible for affecting AMF communities. They observed less variation of species composition, showing that dominant AMF species can be recruited by plant communities. In Argentina, earlier studies coordinated by M. Cabello (Velazquez et al. 2008, 2010, 2013) in preserved areas such as El Palmar National Park, Entre Ríos province, reported 46 AMF species for five vegetation types.

In more stressed environments such as highlands and high altitude mountain ranges which have severe environmental conditions, we can find less AMF diversity. For example, Lugo and Cabello (2002) reported 17 AMF species from natural mountain grasslands at 2250 m above sea level, in Sierras de Córdoba, Argentina. In Natural saline soils of Central Argentina, Soteras et al. (2012) investigated the rhizosphere of the halophyte *Atriplex lampa* in two environments retrieving 18 AMF species. In Puna Grassland highland limited by the Cordillera Real to the east and the Andes to the west, varying in altitude from 2000 to 4400 m above sea level, Lugo et al. (2008) observed low species richness (10 AMF taxa). In rupestrian fields, Brazil, Pagano and Scotti (2009) and Pagano et al. (2010) also observed low AMF diversity (6 species) in some native plant species; however, few plant individuals were analyzed. However, 51 AMF species were reported (including 14 species possibly new to science and mostly from *Glomus* followed by *Acaulospora*) in four different vegetation types (Coutinho et al. 2015).

### *1.3.2 Root Colonization*

In tropical climates, higher root colonization is generally found during the rainy season. Usually, the colonization rate increased in the rainy period (Allen et al. 1998; Silva Junior and Cardoso 2006; Pagano et al. 2009), which may be related to the improvement of soluble nutrients and to a higher root activity in that season. However, in some vegetation types, such as the thorny dry woody savanna (caatinga) in Brazilian tropical semiarid zone, this pattern is not clearly followed (Pagano et al. 2013). This can be due to the short and sporadic rainy season when leaves, flowers, and the herbaceous vegetation grow (Rizzini 1997). Moreover, rates of root colonization from 2 to 79 % can be found depending on the plant host species, such as in rupestrian fields, Brazil, where Pagano and Scotti (2009) and Pagano et al. (2010) also observed high root colonization (>25 %) in some native plant species.

Additionally, in different stages of forest succession spores can increase in the rainy period compared to native forest area where sporulation cannot differ (Bonfim et al. 2013). Pagano et al. (2012) found a higher sporulation in managed forest in the rainy period. In the ECM symbiosis colonization rates usually increase in the rainy period (Pagano and Scotti 2008; Pagano et al. 2009). ECM fungi are particularly dominant in ecosystems where plants are limited by N (Read 1991), but elevated levels of N from fertilizers inhibit growth of external hyphae and fruit bodies (Olsson et al. 2003).

In the studies by Pagano (2012) on the AM communities in the Brazilian dry forest and in riparian forest restoration experiments, the choice of tree species for restoration purposes seems to have great implications on the conservation of AMF species; thus, highly dependent tree hosts could be selected over mycorrhizal-independent hosts. Researches by Urcelay, dedicated to study the Chaco Forest (South America's dry seasonal forest), in Argentina, showed that fire occurrence negatively affects AMF communities mainly as direct effects of fire on soil fungi (Longo et al. 2014).

In most studies, the *Arum*-type pattern of colonization (Dickson et al. 2007) was seen to be dominant for woody trees (Pagano 2012); however, sometimes both *Arum* and *Paris* types can be found indistinctly (Rodríguez-Rodríguez et al. 2013), but it depends on the host species, as Fernández et al. (2012) reported only Paris type for mycorrhizal species of pteridophytes. It is well known that the *Arum* type is formed in most plants that usually grow in sunlight, and that the spreading rate of colonization is faster in this type of colonization (Brundrett and Kendrick 1990). However, other environmental factors such as temperature and soil moisture content may model AM morphology in roots (Cavagnaro et al. 2001). In general, vesicles and hyphae are the most common AM structures observed in the plant roots. It has been shown that storage vesicles appear at later stages of colonization, being important contributors to carbon drain from the plant (Jakobsen et al. 2003), as well as propagules (Smith and Read 2008). Commonly, septate hyphae and microsclerotium of dark septate endophytic fungi can be found during root examinations (M. Pagano, personal observations; Fernández et al. 2012).

Commonly, higher AM colonization rate of plants occurs in mixed plantation, compared to monocultures, and increased intensity of root colonization of native host can be attained even if the native species are mixed with exotic *Eucalyptus species* (Pagano et al. 2009, 2012).

Today, a higher number of reports informed about AMF diversity and gradually this information is able to support decisions for ecosystem conservation. Some studies did examine mycorrhizal and ecosystem responses in multiple locations within an ecosystem type. However, nowadays there is need to further investigate the composition and distribution patterns of AMF species in different ecosystems/biomes. Such a study will facilitate the utilization and management of AMF to improve tree productivity, as well as to obtain other benefits. For example, in the semiarid region of Brazil, Pereira et al. (2014) studied the abundance of AMF in different economic plantations compared to native area of Atlantic forest in Brazil. They found that AMF community composition was more affected by different land uses than by soil characteristics.

Also important is to study the colonization of medicinal plant species such as those investigated by Songachan et al. (2015) in some *Clerodendrum* species, traditionally used for hypertension and for controlling rheumatism by the native people of India. The knowledge of their mycorrhizal status could help plant propagation and conservation.

The environmental benefits of AMF in soil aggregate stability are well documented (Rillig and Mummey 2006) and recently a meta-analysis confirmed that AMF commonly increase soil aggregation (Leifheit et al. 2014). However, this topic needs more studies in both natural and agricultural ecosystems. A better understanding of the mechanisms contributing to riparian soil development and stability will contribute to the management of natural rivers (Mardhiah et al. 2014). Despite research focused on AMF, recent works suggest that ECM also participate in a better soil structure (Zheng et al. 2014).

## 1.4 Conclusion

In this chapter, the need for more information to understand the importance of conservation forest cover and volume of water stored in soil and organisms living in the biosphere was mentioned. Moreover, the preservation of Arbuscular mycorrhizas and Ectomycorrhizas for soil carbon conservation was recommended. Advances on mycorrhizal fungi drawing on recent research worldwide were showed. Throughout the chapter, the applications of mycorrhizas in environmental issues were showed as still incipient, and the limitations of methodology were discussed.

Finally, this chapter argues that natural ecosystems present high AMF diversity; however, it decreases with higher altitude. Consequently, further research is necessary on this field, especially regarding the applications of mycorrhizas in environmental issues.

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# Chapter 2
# Advances in Arbuscular Mycorrhizal Taxonomy

**Marcela C. Pagano, F. Oehl, G.A. Silva, L.C. Maia, D.K. Silva, and M.N. Cabello**

## 2.1 Introduction

Taxonomy, the science of classifying organisms, describes names, identifies organisms, and generates tools for taxonomic identification of fungi. A review article by Shenoy et al. (2007) compiled the main activities of fungal systematics. The products of taxonomy are used by taxonomists and ecologists (Hodkinson and Parnell 2007). Correct species names are deliberately used for instance on land management systems to compare ecological interactions between the different components.

Current classification systems of Arbuscular mycorrhizal fungi (AMF, Glomeromycota) involve both morphological and molecular tools and their progress shows in some measure a natural organization and understanding of the relationships

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M.C. Pagano (✉)
Federal University of Minas Gerais, Belo Horizonte, Brazil
e-mail: marpagano@gmail.com

F. Oehl
Agroscope, Federal Research Institute for Sustainability Sciences, Plant–Soil-Interactions,
Reckenholzstrasse 191, Zürich 8046, Switzerland

G.A. Silva • L.C. Maia
Laboratório de Micorrizas, Departamento de Micologia, Universidade Federal de
Pernambuco, Avenida da Engenharia s/n, Cidade Universitária, 50740-600 Recife,
Pernambuco, Brazil

D.K. Silva
Laboratório de Microbiologia, Campus de Ciências Agrárias, Universidade Federal do
Vale do São Francisco, Rodovia BR 407, Km 12, Lote 543, Projeto de Irrigação Nilo
Coelho, s/n, "C1", CEP 56300-990 Petrolina, Pernambuco, Brazil

M.N. Cabello
Instituto Spegazzini (Fac. Cs Nat y Museo, UNLP),
aven 53 # 477, Comisión de Investigaciones Científicas de la Prov, Buenos Aires, Argentina

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among species. Several research groups have been discussing the taxonomy, systematics, and evolution of AMF. The researchers may propose their classification system; however, most biologists believe that these developing systems only reflect our knowledge at time (Goto 2014). This chapter presents an overview of data showing AMF classification, and recent advances are here compiled. Our goal was to provide nonspecialists such as researchers in other fields and land managers with information on historical and recent changes in AMF classification.

## 2.2 Advances in AMF Taxonomy

Taxonomy, the identifies and describes names, generating tools for taxonomic identification of fungi. The products of taxonomy (species lists, descriptions of formally delimited species, and identification keys, among others) are used by taxonomists and ecologists (Hodkinson and Parnell 2007). As previously stated, a review article by Shenoy et al. (2007) compiled the main activities of fungal systematics before and after the twentieth century.

With regard to the classification of AMF, it was based mostly in the spore morphologies (see Morton et al. 2004; Stürmer 2012). However, delimitation of taxa is very difficult and new information is continuously added. Stürmer (2012) reviewed the AMF systematics suggesting four periods, which the phylum Glomeromycota (Schüßler et al. 2001) has passed, that is, the discovery, alpha taxonomy, cladistics, and phylogenetic synthesis periods. The increasing AMF species reported within the course of time are shown in Table 2.1. One of the most widely accepted classification system for AMF proved to be that of Oehl et al. (2011a, b) (Table 2.2), who intended a further division of three AMF classes and the establishment of a fifth order, namely Gigasporales. They based their classification system on morphological (such as spore morphology and spore formation, and mycorrhizal and mycelial structures including staining processes) and genetic features (β-tubulin and rRNA sequences). A few important advances have been presented since then, e.g., in the Glomerales (e.g., Sieverding et al. 2014; Błaszkowski et al. 2015), in the Gigasporales (Silva et al. 2012; Pontes et al. 2013; Marinho et al. 2014), in the Diversisporales (Błaszkowski 2012; Medina et al. 2014), and in the Archaeosporales (Oehl et al. 2015). Therefore, we suggest the classification proposed by Oehl et al. (2011a) including the most recent progress for better understanding of the natural classification of AMF (see also Aguilera et al. 2015; www.agroscope.ch/saf).

**Table 2.1** Number of arbuscular mycorrhizal species reported by periods

| Period | Number of AMF species | References |
|---|---|---|
| 1845–1974 | >30 | Gerdemann and Trappe (1974) |
| 1975–1989 | ~97 | Schenck and Perez (1988) |
| 1990–2000 | 39 | Stürmer (2012) |
| 2001–2011 | 65 | Oehl et al. (2011a) |
| 2012 to present | ~40 | Goto (2014) |
| Total of species | ~270 | Goto (2014) |

**Table 2.2** Some recent reviews and papers dealing with arbuscular mycorrhizal fungi classification, identification, and phylogeny

| Focus | References |
|---|---|
| DNA-based detection and identification of Glomeromycota | Öpik et al. (2013) |
| Classification of arbuscular mycorrhizal fungi | Redecker et al. (2013)[a] |
| Systematic and phylotaxonomy of arbuscular mycorrhizal fungi | Krüger et al. (2012)[a] |
| A molecular guide to the taxonomy of arbuscular mycorrhizal fungi | Young (2012)[a] |
| A history of the taxonomy and systematics of arbuscular mycorrhizal fungi | Stürmer (2012) |
| Glomeromycota taxonomy | Oehl et al. (2011a) |
| Advances in Glomeromycota taxonomy | Oehl et al. (2011b)[a] |
| Glomerospores: a new denomination for the spores of Glomeromycota | Goto and Maia (2006) |
| Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi | Schüßler (2002) |

[a] Review

**Table 2.3** Journal articles dealing with arbuscular mycorrhizas and their taxonomy and ecology

| Key words | Number of journal articles |
|---|---|
| AMF | >4800 |
| AMF + ecology | >450 |
| AMF + taxonomy | >100 |
| New AMF species | 129 |

Database survey conducted on April 2015 (SCOPUS database since 1947) and other bases; *AMF*, arbuscular mycorrhizal fungi

Additionally, fungal taxonomists have recently integrated the modernization of its nomenclatural rules. The prerequisites of Latin descriptions, endorsement of electronic publication, and the use of the dual system of nomenclature (different names for the sexual and asexual phases of pleomorphic species) have ceased to be used (Hibbett and Taylor 2013).

## 2.3 Implications

In 2009, a total of 214 AMF species were described simply by morphological tools (few of them were successfully cultured) (Krüger et al. 2009). However, molecular characterization and morphological observations showed that AMF diversity is greater than the present estimations (Kottke et al. 2008; Öpik et al. 2008; Sudová et al. 2015).

A robust system of AMF classification will facilitate the research for specialists and nonspecialists (Table 2.3). There is a need to further explore the natural and nonnatural ecosystems for so far unknown AMF species and to describe them. This is for instance because almost all reports include a significant number of non-identified AMF species, often attributed to the genus *Glomus*, but also in other genera, such as *Acaulospora* and *Gigaspora*, we are still aware of several to many unidentified species (e.g., Velázquez and Cabello 2011; Zangaro et al. 2013; Silva et al. 2014; Coutinho et al. 2015). Such studies will facilitate the AMF identification in the different studied ecosystems as well as their utilization and management. AMF biodiversity in natural ecosystems such as tropical forests or permanent grasslands is typically higher than in conventional agricultural systems, because of higher number of plant species and more complex habitats that can support a wider diversity. In tropical regions such as Brazil the number of accepted new AMF species is increasing (Błaszkowski et al. 2013; Goto et al. 2013; Mello et al. 2013; Lima et al. 2014). Also in regions with few studies, such as aquatic sites with macrophytes, new AMF species such as *Rhizoglomus melanum* are found (Sudová et al. 2015).

Since 1990, when molecular analyses to AMF species identification have started (Table 2.1), more new AMF species (~143, Goto 2014) have been described than in the previous 150 years (~127) (Schenck and Perez 1988). Some authors estimate that the number of species can reach 300–1600 (Öpik et al. 2013; Kivlin et al. 2011; Sudová et al. 2015). The discovery, isolation, and morphological/molecular description of new species to science are required due to increase of environmental impacts in preserved areas, as well as the potential uses of these fungi as bio-inoculants (Sieverding et al. 2014). In this sense, studies need a better assessment of the AMF communities in natural, managed reforested and agronomic areas (Fig. 2.1).

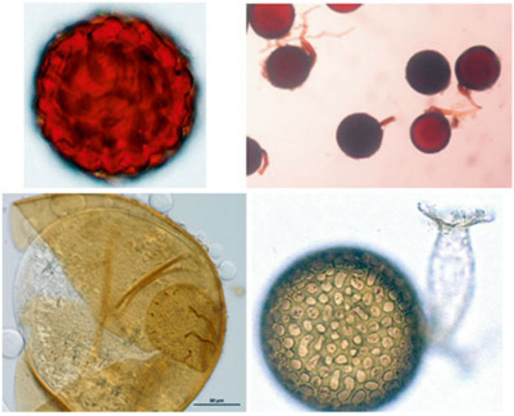

**Fig. 2.1** Spores of non-identified, difficult to identify, and recently identified AMF species found in field conditions: clockwise from upper left: unidentified spore, *Septoglomus furcatum* identified from semiarid region of Brazil, *Acaulospora* sp. found in Minas Gerais, and *Fuscutata aurea* from Amazonia. Photo credit: M. Pagano; Camilla M.R. Pereira

## 2.4 Conclusion

We briefly described advances in AMF classification based on both morphological and molecular characters and faster progress attained. We have mentioned that different research groups naturally adopt their preferred taxonomic system, which have been steadily improved by newly acquired knowledge. The different systems, however, have been approaching each other due to the concomitant progresses on morphological and molecular characterization of species belonging to the phylum. Both morphological and molecular characterization have still to be developed further. Moreover, a large portion, and probably the majority, of AMF species have still remained undiscovered and undescribed. Thus, AMF taxonomy and classification consequently have to develop further for at least another 50–100 years on both lower and higher taxon levels attempting to diminish the current knowledge gaps.

An advanced Glomeromycota system will facilitate ecological research and efforts to accrue benefits from mycorrhizas. Consequently, a better diagnosis of new AMF species will help to achieve maximum benefits from these microorganisms.

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# Chapter 3
# What Can the Arbuscular Mycorrhizal Fungi Community Tell Us About Plant Biodiversity Loss?

**Javier Álvarez-Sánchez, Irene Sánchez-Gallen, Laura Hernández-Cuevas, Lilian Hernández, and Carla Cruz**

## 3.1 Introduction

One of the main causes of biodiversity loss is the deforestation in tropical ecosystems (Achard et al. 2002); nowadays, these ecosystems occupy 7 % of total Earth surface but represent more than 50 % of total worldwide diversity (Pimm and Raven 2000); however, their mean cover loss rates fluctuated between 0.1 and 48 % (Skole and Tucker 1993). Mexico is not the exception; remnant cover of tropical rain and dry forests represents less than 20 % of their original cover.

In all cases, deforestation is due to several human activities that include forest exploitation and clearing for cropping and raising livestock (Lira-Noriega et al. 2007), as well as the creation of urban and communication infrastructure (Guevara et al. 2004a). The main result of these land use changes is the original habitat and species loss, which brings as consequences nutrient cycle modifications, such as atmospheric carbon accumulation, and loss of ecosystem services.

Currently, most of the tropical landscape is made up of fragments of mature rain forest, and secondary vegetation immersed into an "ocean" of cultivated and cattle ranching lands (Guevara et al. 2004b).

Fragmentation is a process that involves microenvironmental changes along the contact zone between remnant vegetation patches and other land uses (Laurance 2004), such as increased air and soil temperatures, higher evapotranspiration rates, and faster and more turbulent winds (Pohlman et al. 2007). These differences

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J. Álvarez-Sánchez (✉) • I. Sánchez-Gallen • L. Hernández • C. Cruz
Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, UNAM,
Circuito Exterior, Ciudad Universitaria, 04510 México, DF, Mexico
e-mail: javier.alvarez@ciencias.unam.mx

L. Hernández-Cuevas
Centro de Investigaciones en Ciencias Biológicas, Universidad Autónoma de Tlaxcala,
Tlaxcala, Mexico

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enhance light demanding species growth and recruitment in the edge zone (Benítez-Malvido and Martínez-Ramos 2003) and, therefore, plant structure and species composition around the edge change (Harper et al. 2005).

Another important outcome of fragmentation is related to the area–species richness relationship, where small fragments besides undergoing stronger microenvironmental changes usually harbor less species than large fragments, although there exist exceptions (Arroyo-Rodríguez et al. 2009; Sánchez-Gallen et al. 2010). Whether soil organisms follow these previous patterns is not clear, because some of the results of studies that have been carried out are contradictory.

### *3.1.1 Soil Biota and Land Use Change*

Mycorrhizal fungi and nitrogen-fixing bacteria are among the most important components of soil microbiota (Jeffries et al. 2003). They interact with plants forming a mutualistic relationship, where both parts receive benefits from each other, but also there are costs that can be very high; however, if it is a mutualism, costs will never surpass benefits; in other words, over time benefit–cost balance will remain positive.

In tropical ecosystems, the majority of plant species are associated with arbuscular mycorrhizal fungi (AMF) (Varela and Guadarrama 2003), forming the arbuscular mycorrhiza; this association allows plants to expand their nutrient absorption surface, increasing nutrient and water intake and therefore improving plant performance. Besides, plants gain other benefits from this interaction such as hormone production, pathogen and drought resistance, and heavy metal and interspecific-competition tolerance (Allen et al. 2003).

Recent ecological studies have highlighted the importance of AMF's presence to improve plant species establishment and, as a consequence, to modify plant species richness (van der Heijden 2002). This is the result of functional compatibility among fungi and plants.

The functional compatibility concept is based on some studies that have proven that the relationship among AMF and plants is mediated by the identity of both. AMF are capable of colonizing a great quantity of plants; however, there is only one plant species that will release more carbohydrates than the others; on the other hand, plants can receive nutrients from the interaction with any AM fungus; however, there is one fungus species that will contribute to more nutrients and more efficiently than the rest (van der Heijden 2002). Under this ecological framework, if it is valid to assume that land use changes in tropical rain forest (TRF) produce changes in belowground communities, how will these communities respond? And, if changes occur, which can be their direction and magnitude? We do not know.

### 3.1.2 Arbuscular Mycorrhizal Fungi and Land Use Changes in Tropical Rain Forest

Data from several land uses reported in the world show that the AMF species composition and richness can vary depending on vegetation type, soil physical properties, climate, and disturbance regimen, among others (Table 3.1). This implies that AMF communities under land use change pressure can take different directions.

For example, spore abundance is higher in cattle ranching lands than in the TRF (Picone 2000), or monoculture, maize crops, and vineyards (Oehl et al. 2003), but AMF diversity did not change when original TRF is transformed into cattle ranching land (Picone 2000) or it does decrease when TRF changed to crop lands (Oehl et al. 2003).

In Mexico, different studies on AMF and land use changes have been carried out. Violi et al. (2008) worked with TRF and adjacent disturbed zones in three localities of Chiapas and Veracruz; they assessed changes in AMF communities through time and space. Study sites include mature forests that were transformed into pine forest, "milpas" (local name for crops of different plants), pastures, and grasses and shrubs communities. They found *Acaulospora delicata*, *A. mellea*, *A. scrobiculata*, and *Rhizophagus clarus* associated with mature forest. Specifically, in Chiapas, they reported 35 species, 13 in burned areas, and 29 in mature forest; in another location in the surroundings of pine forest, they found 26 species, 22 in "milpas", 19 in mature forest, and two in pine forest; interestingly, *Gigaspora* species were not found in the mature forest. Besides, 22 in burned areas, 14 in disturbed areas, and 28 in mature forest were found in a third location. Sites with the same type of disturbance showed similar numbers of species for the genera *Acaulospora* and *Entrophospora*, while the genus *Glomus* showed the lower species richness in disturbed areas. These results suggest that the AMF communities in these environments are highly resistant and therefore these ecosystems can be restored quickly. However, this pattern is not necessarily the same for other areas with similar abiotic characteristics.

At the Los Tuxtlas region, Veracruz, eastern Mexico, results suggest a high AMF diversity as a whole; also, an increase in spore abundance in pastures and agroforestry sites, but a decrease in AMF diversity as forest cover diminishes, has been reported (Varela et al. 2009); Stürmer and Siqueira (2011) also found an AMF species richness decrease from mature forest to crop and agroforestry sites. In Los Tuxtlas, the main trend is as species richness increases, spore abundance decreases, and vice versa; therefore, TRF fragments and maize crops had the highest species richness but the lowest spore number; on the contrary, cattle ranching systems had the lowest species richness but the highest spore numbers. Probably, this happens because in the latter site environmental conditions are more stressful, with high light intensity and low water content that lead to a greater sporulation, whereas sites with more vegetation cover, such as TRF and agroforestry systems, have low light availability and plants do not allocate enough carbon to fungi; therefore, spore numbers are reduced (Zangaro et al. 2012).

**Table 3.1** Arbuscular mycorrhizal fungi presence in several tropical ecosystems

| Author (s) | Picone (2000) | Zhao et al. (2003) | Mangan et al. (2004) | Zhang et al. (2004) | Lovelock et al. (2003) | Peña-Venegas et al. (2007) | Guadarrama and Álvarez-Sánchez (1999), Guadarrama et al. (2007) | Stürmer and Siqueira (2011) |
|---|---|---|---|---|---|---|---|---|
| Country | Nicaragua and Costa Rica | China | Panama | China | Costa Rica | Colombia | Mexico | Brazil |
| Recorded genera | *Acaulospora*, *Glomus*, and *Gigaspora* | *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora* | *Glomus*, *Acaulospora*, *Sclerocystis*, and *Scutellospora* | *Glomus*, *Acaulospora*, *Entrophospora*, and *Archaeospora* | *Funneliformis*, *Acaulospora*, and *Scutellospora* | *Glomus*, *Acaulospora*, *Archaeospora*, *Scutellospora*, and *Gigaspora* | *Glomus*, *Sclerocystis*, *Acaulospora*, and *Gigaspora* | *Acaulospora*, *Glomus*, *Scutellospora*, and *Archaeospora* |
| Species richness | 28 | 27 | 27 | 44 | 16 | 18 | 16–44 | 61 |
| Vegetation type | TRF, and cattle ranching land | TRF | TRF | TRF, and deforested areas | Monocultures, and policultures | TRF, secondary forest, and pasture | TRF | TRF, old secondary TRF, agroforestry systems, cultures, and pasture |

*TRF*, Tropical rain forest

A comparative study between different land uses in forests of North America (cultures, grassland, scrubland) and Los Tuxtlas (agroforestry stands, maize crops, cattle ranching land, and tropical rain forest patches) (Álvarez-Sánchez et al. 2012) stressed that the factors which more accurately explained AMF species richness were total precipitation and pH; these results agree with some of the factors that Chaudhary et al. (2008) mentioned as important to understand AMF biogeographical distribution. Another relevant variable that might elucidate AMF presence is plant composition (Pringle et al. 2009).

Relationship among AMF and plant communities in Mexican tropical forest has been partially assessed, due to their inner biological complexity plus their diverse land transformations. It has been very difficult to conclude or elucidate the main factors that rule AMF and plant coexistence and dependence; however, important approaches have highlighted AMF dependence of secondary species (Guadarrama et al. 2004) and AMF's main role in TRF restoration (Álvarez-Sánchez et al. 2007).

We have studied AMF species richness and composition in Mexican tropical forests evaluating several different factors such as fragmentation, edge effect, succession stages, and land use changes. Our main goal was to understand the ecological relationships among AMF and plant species, and how they change under different land use conditions. Our main objective in this chapter is to present some of our recent findings and to discuss them in light of the ecosystem functioning and resource availability.

## 3.2 Study Sites

We chose five tropical forests and their associated land uses (such as secondary growth forest, agroforestry land, cattle ranching land, etc.). Four of them were located in Veracruz State, three were at the Los Tuxtlas Biosphere Reserve (LTBR), situated in southern and northern zones, the fourth was at CICOLMA station, and the fifth place was located in Chiapas State, inside the Lacandonian zone (Fig. 3.1).

At LTBR we worked with four different land uses: (1) tropical rain forest (TRF) (LTBR 1), (2) secondary growth forest (acahual) (LTBR 2), (3) cattle ranching lands (pasture) (LTBR 3), and (4) maize crops (milpa) (LTBR 4); besides, we evaluated the effect of the border zone between a fragment of TRF and a cattle ranching site (LTBR 5–7) and the influence of fragment size of seven remnant patches of TRF (LTBR 8–14). At the CICOLMA place, we have a tropical subperennial forest (TSF) instead of TRF (CICOLMA 1), together with "acahuales" derived from the TSF (CICOLMA 2).

At Marqués de Comillas, Chiapas, we established six land uses based on their regeneration stage; in this case, except for the TRF plots, all sites were used as cattle ranching parcels and now they are abandoned. Ordered from younger to older plots, we divided them into (1) herbs (HM), (2) herbs and shrubs (HSM), (3) herbs and leguminous (HLM), (4) herbs and trees (HTM), (5) Riparian vegetation (RVM), and (6) original TRF (TRFM).

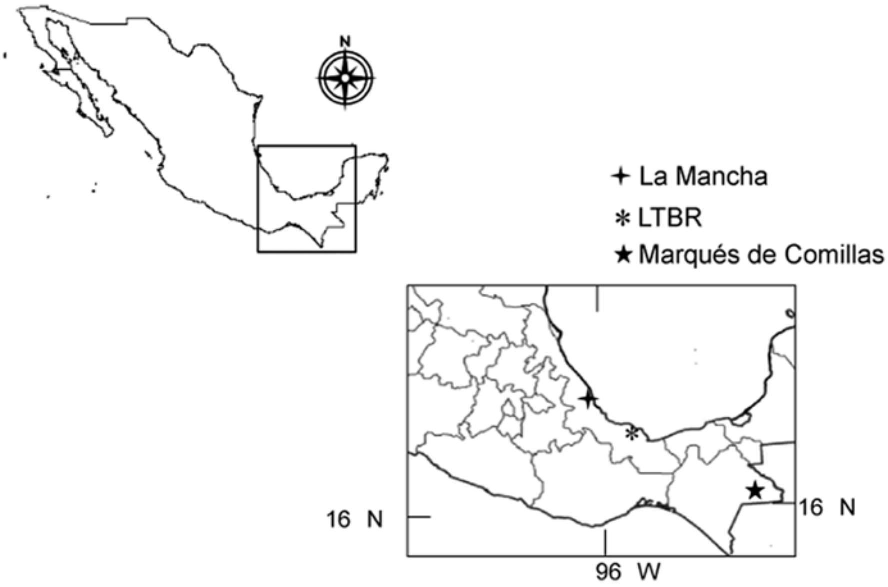

**Fig. 3.1** Site localization; CICOLMA (La Mancha) and Los Tuxtlas Biosphere Reserve (LTBR) are located in Veracruz State and Marqués de Comillas in Chiapas State

In total we had 22 sampling sites; here we collected soil from five randomly selected sample points in every plot. Later we transported to laboratory to separate spores from 100 g of dry soil; we treated all samples separately. We used a modified wet sieving and centrifuge method (Brundrett et al. 1996). Separated spores were placed on permanent slides. M.Sc. Laura Hernández-Cuevas identified them under light microscope, following the descriptions of Schenck and Pérez (1990), INVAM (invam.caf.wvu.edu/myc_info/taxonomy), Blaszkowski (http://www.agro.ar.szczecin.pljblaszkowski/index.html), and Schüβler and Walker (http://schuessler.userweb.mwn.de/amphylo/). We listed AMF species following the classification proposed by Schüβler and Walker (2010) (http://schuessler.userweb.mwn.de/amphylo).

We performed multivariate analyses using PCOrd 5 program (McCune and Mefford 2005) to group sites according to AMF species composition, we used Euclidean Distance index and the Ward method to join groups, and to order the samples in concordance with environmental variables, we applied the detrended component analysis (DCA).

## 3.3 Results

We found 132 species, 71 were identified to species and 63 were only identified to genus or family due to the conditions of the material from the field or that they were new species. The rest of the analyses were performed with the 71 species.

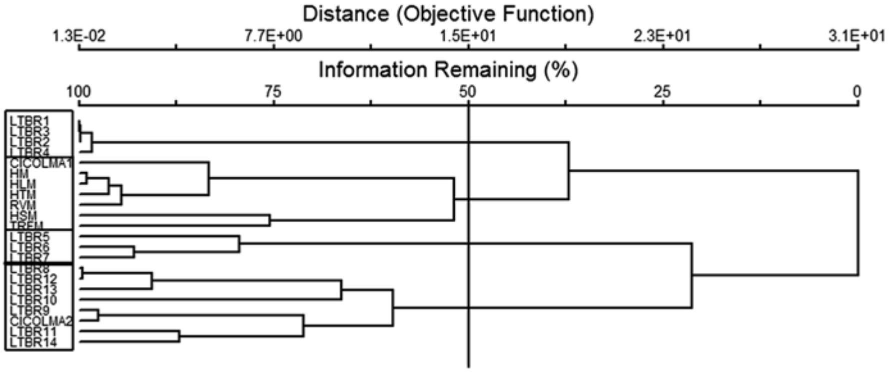

**Fig. 3.2** Classification tree analysis showed four groups at 50 % of similarity

*Glomus* and *Acaulospora* genera were the most abundant. We found *Funneliformis geosporus*, *Acaulospora scrobiculata, A. mellea, A. spinosa,* and *A. foveata* present in all sites, regardless of whether they came from original TRF or any other land used. On the other hand, *F. constrictus*, *A. morrowiae*, *A. laevis*, *Claroideoglomus claroideum*, and *A. delicata* were solely associated with TRF and acahual sites.

Species such as *Gigaspora rosea*, *Glomus microcarpum*, *Racocetra aff. verrucosa*, *Scutellospora calospora*, and *S. heterogama* were found once with one spore; they were rare species.

The classification analysis showed four discrete groups at 50 % of similarity: one belongs to the southern zone of the LTBR (LTBR 1–4), the second is formed by samples from CICOLMA acahual (CICOLMA1) and the Marqués de Comillas samples (HM, HLM, HTM, RVM, HSM, and TRFM), the third group includes patch-cattle ranching land gradient located at the northern zone of the LTBR (LTBR 5–7), and the fourth group comprises samples taken from fragments of different sizes at the northern zone of the LTBR (LTBR 8–14) and the CICOLMA site inside the TSF (CICOLMA2) (Fig. 3.2).

When we plotted our samples in the ordination space, we delimited almost the same groups as were defined by the classification analysis. Following sample distribution along the X- and Y-axis, we can propose that the X-axis is correlated with a disturbance gradient, while the Y-axis is correlated with a humid gradient (Fig. 3.3).

## 3.4 Site Evidence of Resource Stoichiometry Theory

Analyzing the behavior of other AMF structures that play an important functional role, such as the production of extraradical mycelium and root fungal colonization, can give us more information about the outcome of plants–AMF interaction, in the context of the demand for nutrients from the plants and the status of the natural ecosystem according to its physical and chemical properties.

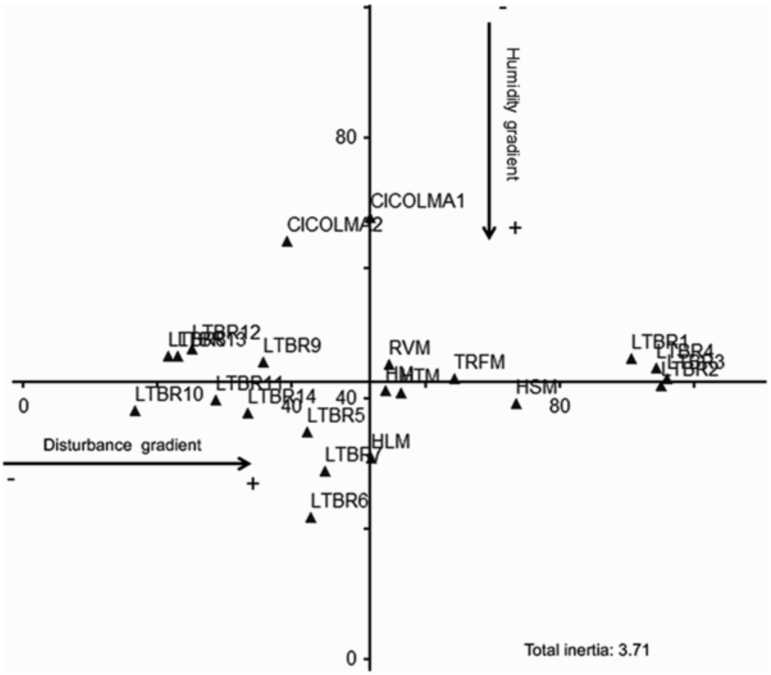

**Fig. 3.3** Ordination analysis and potential environmental variables (humidity and disturbance) that explain sample arrangement

We carried out this evaluation in CICOLMA sites, at eastern Mexico in Veracruz State. We analyzed vegetation types (tropical subperennial forest (TSF), secondary TSF, grassland, and shrubland), and climatic seasons (rainy and dry seasons) influence intraradical colonization and extraradical mycelium production. We found significant differences due to both factors and their interaction (Cruz et al. unpublished data).

The interaction grassland × rainy season had the highest mean value of total intraradical colonization (69.33 %), while the grassland × dry season interaction had the lowest (45.67 %). The colonization by vesicles was significantly higher in the forest in rainy season (23 %) and lower in the secondary vegetation in dry season (9.58 %). The arbuscular colonization showed the highest value in the secondary vegetation for dry season (6.58 %), while in the grassland in rainy season was 0 %.

Finally, mean mycelium length in shrubland during the dry season was the highest, whereas in the secondary vegetation for rainy season was the lowest; we had significant Spearman correlations between mycelium length and arbuscular colonization against pH, texture, different sources of nitrogen, and root biomass (Cruz et al. unpublished data). With a multivariate analysis, nutrient concentration and mycelium length grouped the sites as follows: forest with secondary vegetation and shrubland with grassland.

These results allowed us to understand the interaction among vegetation types and climatic seasons for the mutualistic interaction. Here we can hypothesize that during the rainy season the availability of ammonium was more critical, while in the dry season was the nitrate.

Recovery of degraded rain forest sites using AMF is a poorly explored strategy that is very attractive for all the advantages that these organisms bring about. Plant inoculation with AMF derived from original vegetation, prior to field transplant, can address the lack of AMF in deteriorated environments (Cuenca et al. 1998a, b).

Therefore, restoration of transformed and degraded ecosystems using AMF can improve plant establishment and growth (Álvarez-Sánchez et al. 2007; Siqueira et al. 1998), and this strategy will also accelerate successional process (Aziz et al. 1995).

## 3.5 Final Remarks

We are aware that our spatial scale is very large; however, we think that this approach can lead us to figure out global patterns that arbuscular mycorrhizal fungi (AMF) can show in tropical systems.

We have realized that the site is very important to define the AMF community, more than the individual abiotic factors, except for environmental humidity and the degree of disturbance, neither vegetation type nor land use explained AMF distribution or species composition, which contradicts our original framework. Probably, as other authors have sustained, AMF can survive these changes owing to their high degree of resilience (Stürmer and Siqueira 2011).

However, there are other aspects of AMF that we have started to address, such as multifunctionality, where AMF identity is important but much more the function that they have, and that it is related to nutrient array (Johnson 2010) and water soil absorption.

## 3.6 Conclusions

In conclusion, we can say that:

- AMF community is resistant and resilient.
- Site conditions determine the AMF community structure, but mainly the mutualistic function.

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# Chapter 4
# Mycorrhizal Symbiosis Organization of Dominant Tree *Prosopis laevigata* (Mesquite) in a Xeric Shrub of Central Mexico

**Arcadio Monroy-Ata, Juan Carlos Peña-Becerril, and Mariano García-Díaz**

## 4.1 Introduction

The majority of vascular plant species on Earth establish mutualistic symbioses with arbuscular mycorrhizal fungi (AMF) (Glomeromycota), particularly in arid and semiarid zones where soil water availability for plant development is a key issue for optimizing the stability and productivity of natural ecosystems (Barea et al. 2011; Giovannetti et al. 2004). AMF are obligate biotrophs that play a major role in soil fertility and plant nutrition, since their extraradical mycelia are able to uptake water and nutrients from soil and transfer them to the host plants (Smith and Read 1997); such mycelia also have an essential role in interplant nutrient transfer and for the exploitation and redistribution of soil resources within plant communities (Giovannetti 2008). These extraradical mycelia form a network that explores deep soil, beneath plant roots, for the acquisition of water and mineral nutrients (Barea et al. 2005); also, this network is a fundamental factor for the establishment, survival, maintenance, and succession of plant community diversity (Read 1997; Van der Heijden et al. 1998a).

In temperate forest, the soil flow of nutrients between plants via fungi is a significant feature of the "wood wide web" of ecosystem, where mature trees facilitate the growth of conspecific seedlings beneath them with the contribution of fungal network (Simard 2009; Whitfield 2007). Mycorrhizal symbiosis is important in stressful climates, because hyphal network facilitates plants to cope with stress and by helping seedlings survive, thus making plant guilds more stable in the face of environmental conditions (Simard 2009). With disturbance or drought stress, in a Douglas-fir forest, there is new plant seedling regeneration within the extensive mycorrhizal networks of old trees; in this way, old tree is the hub one that has a

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A. Monroy-Ata (✉) • J.C. Peña-Becerril • M. García-Díaz
Unidad de Investigación en Ecología Vegetal, Zaragoza Faculty, National Autonomous University of México, P.B. 9-020, México, DF CP 15000, Mexico
e-mail: arcadiom@unam.mx

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foundational role in structuring plant assemblages by means of hyphal networks (Simard et al. 2012; Simard 2009).

Notwithstanding, interactions between AMF and its hosts remain poorly known in dry ecosystems, where nutrient accessibility by plants is often low because water scarcity and below- (hyphal networks) and aboveground (i.e., fog condensation) strategies have been developed to face drought stress. In these ecosystems, shrubs and trees act as nurse plants (Niering et al. 1963), forming “fertility islands” (García-Moya and McKell 1970) or resource-rich patches under their coverage (Camargo-Ricalde and Dhillion 2003), which facilitate plant establishment of other species (Monroy-Ata et al. 2007). In such patches, AMF growth is enhanced and mycorrhizal potential increases in the rhizosphere of shrub and trees species (Azcón-Aguilar et al. 2003) by means of AMF inoculum (spores and roots). Also, AMF stimulate growth of shrubs and their responsiveness to drought (Goicoechea et al. 2005), generating a feedback where ligneous plants exert a selective pressure on AMF species (Alguacil et al. 2009) Thus, there are empirical works that show how AMF identity and diversity shape plant communities (Klironomos et al. 2000; van der Heijden et al. 1998a, b). Martínez-García et al. (2011) found, in a semiarid environment, differences in AMF communities in soils under shrubs and in gaps among them, whereas there were no differences among AMF communities colonizing roots.

In view of understanding interactions below- and aboveground, it is necessary to analyze dominant tree distribution patterns and their associated AMF diversity and abundance. In this work, the initial questions were as follows: (i) Which are the AMF species/genera under an old mesquite (*Prosopis laevigata*) tree and under canopy of their nearest mesquite neighbors? (ii) Is there a pattern in the richness and abundance of AMF species/genera around on old mesquite tree and under canopy of the same species neighbors?

## 4.2 Materials and Methods

### *4.2.1 Study Site*

The studied area was a xeric shrub ecosystem fragment with low perturbation, located at Tezontepec de Aldama Municipality, Hidalgo State, in Central México. The site coordinates are, in the analyzed core parcel: 20°12′6.12″ North latitude and 99°16′52.26″ West longitude, with an altitude of 2100 m above sea level; it is located within the Mezquital Valley, a semiarid zone (Fig. 4.1). The climate is dry and temperate, with 16.9 °C of average annual temperature and 462.7 mm of average annual precipitation, with rainy period ranging from May to October (Pavón and Meza-Sánchez 2009). The vegetation type is a thorny scrub with crassicaule plants (*Opuntia* and *Agave* spp.), dominated by mesquite trees (*P. laevigata*) and shrubs (*Acacia* spp., *Mimosa* spp.). Other component of plant mosaic is the herbaceous stratum, which is dominated by two *Bouteloua* species: *B. gracilis* and *B. curtipendula* (De la Rosa-Mera and Monroy-Ata 2006).

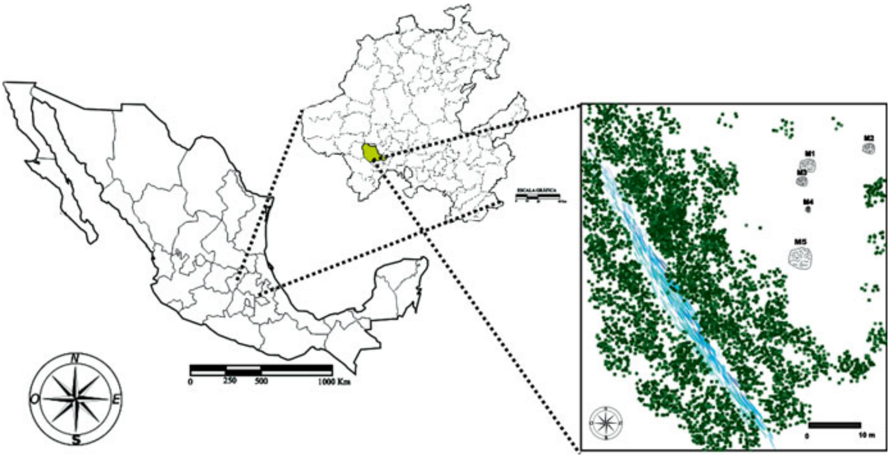

**Fig. 4.1** Study site in Tezontepec de Aldama locality, Hidalgo, Mexico

### 4.2.2 Sampling Technique

For this study a plot in a xeric shrub ecosystem, beside a seasonal creek, with low disturbance and old mesquite trees *Prosopis laevigata* (Humb. et Bonpl. ex Willd.) M.C. Johnst.), Fabaceae, was selected. The oldest mesquite tree was chosen and soils samples were taken, in the four cardinal points under its canopy, at the coverage edge and until 20 cm soil deep. The soil samples were taken in the proximity of bunch grass plants dominated by blue gramma (*Bouteloua gracilis* (Kunth) Lag. ex Griffiths.) Also, the four nearest mesquite trees were chosen in order to take four soil samples of each one, in the same way.

The sampling was made in the dry season (March 28, 2014), when there are arbuscular mycorrhizal fungi (AMF) in their spore form. The distance of each mesquite tree to the oldest one was registered; also, height and canopy and stem diameter were obtained for each tree. The coordinates of each tree were determined with a GPS device.

### 4.2.3 Spore Extraction and Taxonomic Determination

For each one of 4 soil samples obtained under the canopy of each mesquite tree, AMF spores were extracted in order to make their taxonomic determination. Arbuscular mycorrhizal fungi spores were obtained by wet sieving and decanting technique (Gerdemann and Nicolson 1963), modified by Brundrett et al. (1996). For this, 100 g of soil sample was suspended in water and sieved on a 44 μ mesh; the soil sample was resuspended in water, centrifuged at 2000 rpm by 5 min, resuspended in a 50 % sucrose water solution, and newly centrifuged at 1000 rpm by 3 min.

The supernatant was sieved on a 44 μ mesh, rinsed, and analyzed on a Petri dish with a dissecting microscope.

AMF spores were counted and taxonomically identified by each mesquite tree. For this, permanent slides were made with the spores; they were mounted in polyvinyl alcohol–lactic acid–glycerol (PVLG) (Koske and Tessier 1983) and PVLG mixed 1:1 (v/v) with Melzer's reagent (Brundrett et al. 1996). Identification to a species level was made based on characteristics of spore cell walls and comparison to voucher specimens (García-Sánchez et al. 2008). The spores were sized with a graduated scale slide. Finally, species richness and abundance were determined as a count of the different AMF species detected at each mesquite tree.

### *4.2.4 Statistical Analysis*

An analysis of variance, with the Kruskal–Wallis nonparametric test, was made for the total number of AMF spores, with $p < 0.05$ as significance level. Also, with AMF species abundance was obtained their relative abundance distribution (RAD) in accord with J.B. Wilson's (1999) technique for the characterization of plant-fungal community assemblages; this was made with the Table Curve 2D program (version 5.01) that generates the more likely curve-fitting. Too, a principal component analysis of AMF abundance spores and their associated mesquite trees was carried out, with the help of Infostat program (Di Rienzo et al. 2015).

## 4.3 Results

The oldest mesquite (M5) shows the major height, Diameter at Breast Height (DBH), and coverage in relation to their nearest neighbors; in contrast, M4 individual had minor values in the registered parameters and it was the nearest shrub to M5 too (Table 4.1). The longest distance measured from M5 was for M2 mesquite (15.9 m) in the study area.

Ten species of AMF were found in the mesquite trees sampled and three mycorrhizal species were common in the five individuals (*Glomus* sp.1, *Sclerocystis sinuosa*, and *Glomus* sp.2) (Table 4.2). According to the Kruskal–Wallis test,

**Table 4.1** Mesquite characterization in the experimental site

| Mesquite number | Height (m) | DBH[a] (cm) | Coverage ($m^2$) | Distance of M5 (m) |
|---|---|---|---|---|
| M1 | 3.2 | 10 | 24.6 | 13.5 |
| M2 | 2.1 | 6 | 15.1 | 15.9 |
| M3 | 2.6 | 7 | 11.9 | 10.7 |
| M4 | 1.7 | 3 | 3.5 | 7.4 |
| M5 | 4.8 | 20 | 59.2 | 0 |

[a] Diameter at breast height

**Table 4.2** Number of spores per mycorrhizal species in mesquite individuals[a]

| | Individuals | | | | | Total number of spores by species | | |
|---|---|---|---|---|---|---|---|---|
| Species | M1 | M2 | M3 | M4 | M5 | | | Frequency |
| *Glomus* sp.1 | 38 | 16 | 1 | 18 | 53 | 126 | a | 5 |
| *Sclerocystis sinuosa* | 21 | 6 | 14 | 10 | 44 | 95 | a | 5 |
| *Glomus* sp.2 | 41 | 9 | 1 | 5 | 37 | 93 | ab | 5 |
| *Entrophospora infrequens* | 12 | 70 | 0 | 2 | 6 | 90 | abc | 4 |
| *Funneliformis mosseae* | 0 | 5 | 0 | 38 | 54 | 97 | abc | 3 |
| *Glomus* sp.3 | 0 | 0 | 110 | 0 | 12 | 122 | abc | 2 |
| *Gigaspora* sp.1 | 6 | 0 | 1 | 0 | 0 | 7 | bc | 2 |
| *Gigaspora* sp.2 | 0 | 0 | 44 | 0 | 0 | 44 | bc | 1 |
| *Sclerocystis* sp. | 8 | 0 | 0 | 0 | 0 | 8 | c | 1 |
| *Acaulospora spinosa* | 0 | 0 | 0 | 2 | 0 | 2 | c | 1 |

[a] Number of spores in 100 g of soil. The number of spores of AMF species found in individuals mesquite is presented, sorted by frequency and second order by the number of spores

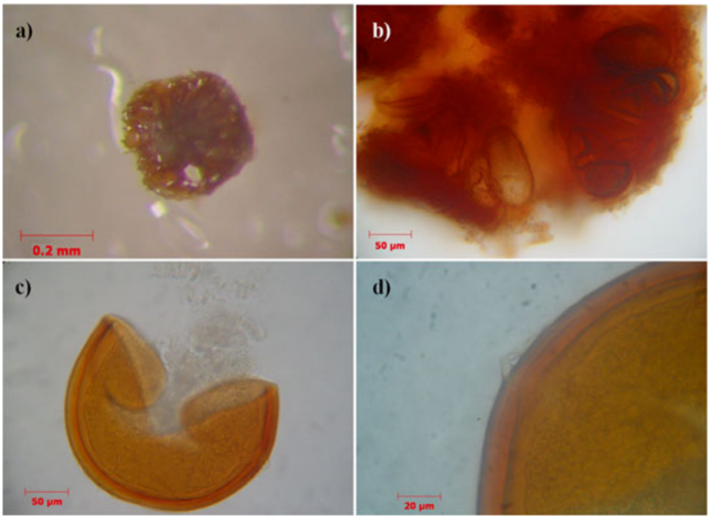

**Fig. 4.2** Spores of species of AMF in mesquite individuals: (**a**) and (**b**) *S. sinuosa*; (**c**) and (**d**) *Glomus* sp.1

significant differences in the total number of spores, by AMF species, were found ($H_{9,0.05} = 17.86$, $p = 0.023$), resulting in *Glomus* sp.1 and *S. sinuosa* having the highest number compared to other fungal species. Nevertheless, *Glomus* sp.3 had a high number of spores too; however, it only was present in two mesquite individuals (M3 and M5) (Table 4.2 and Fig. 4.2).

It was found that the oldest mesquite tree (M5) was the individual with the highest total number of AMF spores, with 206, followed by M3 tree, with 171 spores, due to a higher spore number in a single species: *Glomus* sp.3 (Fig. 4.3, Table 4.2).

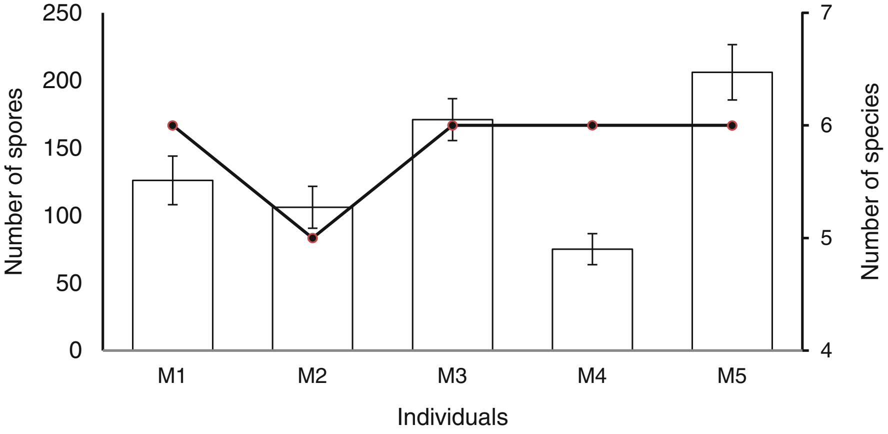

**Fig. 4.3** Total number of AMF spores (*bars*) and species (*line*) in the five mesquite individuals (bars representing standard error above and below the mean)

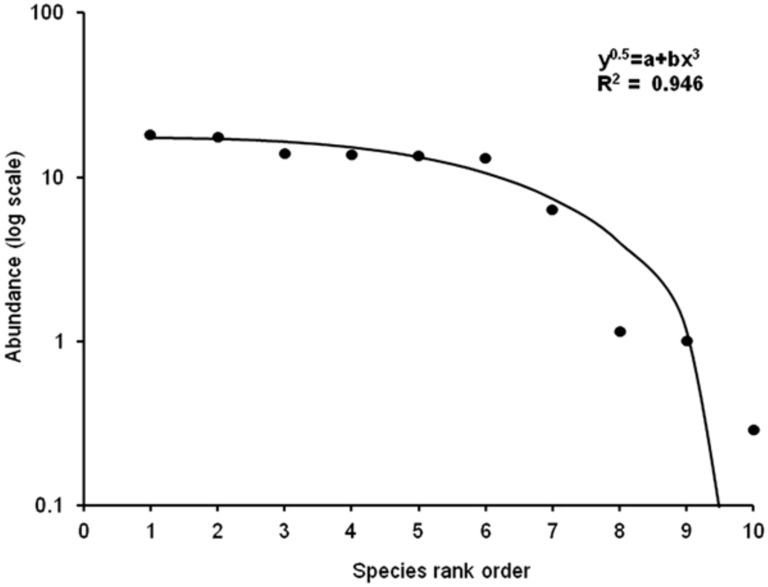

**Fig. 4.4** Relative abundance distribution (RAD) of AMF spores in the five mesquite trees

Four mesquite individuals had six AMF species and only in the M2 individual five mycorrhizal species were registered (Fig. 4.3). In relation to AMF species abundance by number of spores, six species had high counts, sorted from highest to lowest: *Glomus* sp.1, *Glomus* sp.3, *F. mosseae*, *S. sinuosa*, *Glomus* sp.2, and *E. infrequens* (Table 4.2, Fig. 4.4). Thus, the curve of relative abundance distribution (RAD) showed a concave-down-type shape (according to Wilson 1999), and the equation

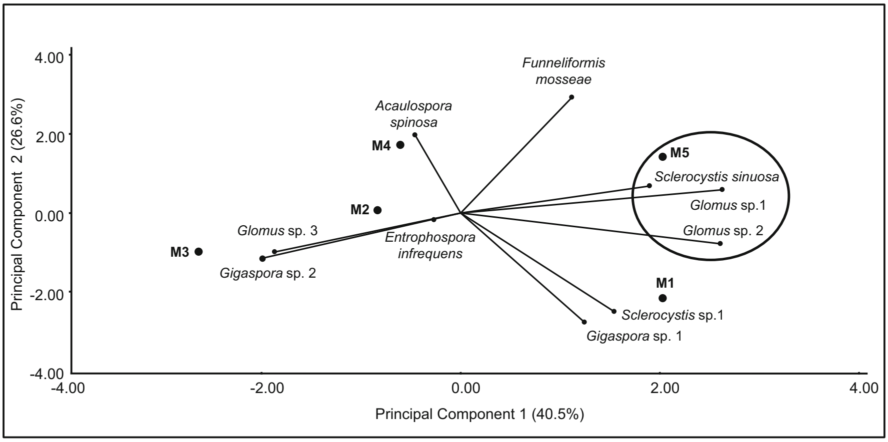

**Fig. 4.5** Principal component analysis of AMF species in the five mesquite individuals

**Table 4.3** Coordinates of principal component analysis graph for mesquite trees

| Axis: | CP1 | CP2 |
|---|---|---|
| Mesquite | | |
| M1 | 2.03 | –2.18 |
| M2 | –0.83 | 0.06 |
| M3 | –2.63 | –1.00 |
| M4 | –0.60 | 1.71 |
| M5 | 2.03 | 1.41 |

representing this particular behavior is as follows: $y^{0.5}=\mathrm{a}+\mathrm{bx}^3$, with $R^2=0.946$ ($F_{1,0.05}=140.58$, $p<0.0001$) (Fig. 4.4). This can be interpreted as a codominance of a six arbuscular mycorrhizal species consortium.

The principal component analysis for the ten AMF species clustered three of them (*Glomus* sp.1, *S. sinuosa* and *Glomus* sp.2) in M5 mesquite tree (Fig. 4.5); the same AMF species were present in the five mesquite individuals (Table 4.2).

## 4.4 Discussion

AMF–plant associations may not be always species specific (Öpik et al. 2009; Santos et al. 2006) due to factors such as climate and soil properties that can determine AMF consortia and their hosts (Lekberg et al. 2007; Landis et al. 2004). In arid and semiarid zones, the plant mosaics have geometric structures that have been characterized around the world as banded vegetation (*brousse tigrée*) in soils with slope or as plant patches (spotted) landscape in plains (Valentin et al. 1999; Cornet et al. 1992). This plant community structure reflects water dynamics due to precipitation pulses and space–time distribution of soil water potential; the plant patterns could be explained by the morphogenetic differentiation of soil water diffusion as a result of Turing instabilities (Fairén 2007); thus, in dry ecosystems plant mosaics are growing driven by evapotranspiration flows and this means that AMF networks are distributed according to soil water availability, because nutrient accessibility is limited by water scarcity (Aguiar and Sala 1999; Martínez-García et al. 2011).

In the semiarid shrubland of this work, the oldest mesquite tree sampled shows the highest AMF spore abundance (206) and the youngest (M4) the lowest number (75); in AMF species richness the furthest mesquite tree (M2) had only 5 genera/species and 6 all other trees. This could be correlated with the results of Teste et al. (2009), who found, in a temperate forest, that seedlings furthest from trees had a simpler ectomycorrhizal community composition and reduced EM richness and diversity, compared to seedlings in closer proximity. In this sense, the oldest tree is a "mother tree" for fungal propagules, as is shown by Thoen and Ba (1989) in a disturbed site of Southern Senegal, where ectomycorrhizal sporocarps have the ability to survive in harsh environmental conditions and to spread from the mother trees.

AMF communities are guilds with distinct functional traits during plant succession. For example, in Glomeraceae species, the majority of fungal biomass is found in hyphae growing inside the root, and, in contrast, the majority of Gigasporacea fungal biomass is found in the hyphae that are located outside the plant root (Maherali and Klironomos 2007). This could mean that *Glomus* spp. promote plant establishment and growth and *Gigaspora* spp. take part in hyphal network development (Monroy-Ata et al. 2007) and in nutrient mining from rocks and soil minerals (Landeweert et al. 2001). In this study, 6 AMF genera, with a dominance of 2 *Glomus* spp. and *S. sinuosa*, were found.

The relative abundance distribution of mycorrhizal spores shows that 4–5 AMF species coexist in the mesquite tree sampled, but the fact that the 3 dominant fungal species are present in all the trees points to a common origin in the oldest tree. This could be supported by the principal component analysis of the AMF spore abundance and the 5 mesquite trees, where a group with the oldest tree and the dominant mycorrhizal species was found (Fig. 4.5), and that this fungal consortium explains the 93 % of the variability of the data (Table 4.4).

**Table 4.4** Autovalues ($\lambda$) for mycorrhizal species ranged by frequency in mesquite trees

| $\lambda$ | Value | Proportion | Accumulated proportion |
|---|---|---|---|
| 1 | 4.49 | 0.45 | 0.45 |
| 2 | 2.95 | 0.30 | 0.74 |
| 3 | 1.81 | 0.18 | 0.93 |
| 4 | 0.75 | 0.07 | 1.00 |
| 5 | 0.00 | 0.00 | 1.00 |
| 6 | 0.00 | 0.00 | 1.00 |
| 7 | 0.00 | 0.00 | 1.00 |
| 8 | 0.00 | 0.00 | 1.00 |
| 9 | 0.00 | 0.00 | 1.00 |
| 10 | 0.00 | 0.00 | 1.00 |

## 4.5 Conclusions

This study was exploratory in search of organizational clues to understand mosaic plant assemblage, in a semiarid shrubland plot, linking a plant guild of dominant mesquite trees and their associated AMF. Plant communities can be linked by mycorrhizal network including distinct plant families (Kottke et al. 2008). In the resource islands of the 5 mesquite trees analyzed, 3 AMF genera/species had presence in the oldest and the youngest, the nearest and the furthest trees, showing that the first could be a "mother tree" for plant establishment of same species individuals. The 6 AMF genera found in the study area could be an indicator of fungal richness of mycorrhizal network and of its tendency towards an optimal exploitation of available resources, essentially water and nutrients, because soil hydric potential drives, in high ratio, mosaic plant assemblage in arid environments, to build a guild with functional complementarity among species, above- and belowground species.

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# Chapter 5
# Arbuscular Mycorrhizas in Different Successional Stages in Some Brazilian Ecosystems

**Waldemar Zangaro and Artur Berbel Lirio Rondina**

## 5.1 Introduction

During tropical succession, light-demanding pioneer and early-secondary tree species, which have very fast growth rates, replace the early-successional plant communities such as grasses, shrubs, and forbs. At later stages of succession, pioneer and early-secondary trees species, which are plants incapable of growing and reproducing under their own shadow (Saldarriaga et al. 1988), are replaced by late-secondary and climax tree species, which predominate in closed canopies and display intrinsic slow growth rates and tolerance to shading (Denslow and Guzman 2000; Guariguata and Ostertag 2001; Zangaro et al. 2003).

Many plant species belonging to different ecological groups of succession in tropical ecosystems rely on arbuscular mycorrhizal fungi (AMF) for water and nutrients uptake. The AMF external hyphae increase the volume of soil that can be explored beyond the depletion zone formed around the absorbing roots (Smith and Read 2008), mainly in relation to P, and receive carbohydrates from the host plant. The low diameter of AMF hyphae can uptake P from small sites that cannot be accessed by root hairs (Jakobsen 1995). AMF make symbiosis with many plant species, including herbaceous and woody (Janos 1983; Sanders et al. 1996; Zangaro et al. 2003). This symbiosis plays an important role in plant nutrient acquisition, growth, and survival (Smith and Read 2008), besides increasing the photosynthetic rate and roots longevity (Linderman 1988; Comas et al. 2002). The AMF association is important for rehabilitation of degraded lands and is highly promising for inoculation of native woody species, especially in low-fertility soils (Perry et al. 1987; Zangaro et al. 2000).

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W. Zangaro (✉) • A.B.L. Rondina
Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Ciências Biológicas, Departamento de Biologia Animal e Vegetal,
86051-990 Londrina, Paraná, Brazil
e-mail: wzangaro@uel.br; arturrondina@hotmail.com

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In this chapter, we will discuss differences in the relationships among distinct plant functional groups and AMF, as well as the implications of these relations for tropical ecological succession.

## 5.2 Differential Response of Tropical Herbaceous and Woody Species to AMF

AMF are an important biotic factor that influences differentially the establishment, survivor, growth, and reproduction of herbaceous and shrubby species of early phases of tropical succession. Rondina et al. (2014) assessed the effect of AMF on seedling development of 27 heliophilous herbaceous and shrubby tropical species grown in low- and high-fertility soils for 100 days. As shown in Fig. 5.1, most species grown in both soil types exhibited high AMF root colonization (80 %). In the low-fertility soil and non-AMF inoculation, the individuals of most plant species died between 50 and 70 days after the experiment installation. The individuals of the few plant species that survived in this condition grew little and displayed about 88 % less shoot dry mass than plants inoculated with AMF (Fig. 5.1). In the low-fertility soil, only six species flowered and flowering increased with AMF in one plant species and four species only flowered in the presence of AMF (Fig. 5.2). In the high-fertility soil, plant species non-inoculated with AMF exhibited seedlings about 13 % less shoot dry mass (Fig. 5.1), lower total leaf area, leaf area expansion, total root length, and nutrient concentrations in shoots than seedlings inoculated with AMF. Sixteen plant species flowered in the high-fertility soil, but 11 species displayed earlier flowering and 10 species exhibited more abundant flowering when grown with AMF (Fig. 5.2). Rondina et al. (2014) attributed the better flowering of mycorrhizal plants to the higher nutrient concentration in shoots (especially P and N) when compared with non-mycorrhizal plants. It possibly allowed more nutrients mobilized to the production of flowers. Furthermore, more nutrients in combination with the increase of total leaf area and leaf area expansion, also displayed by mycorrhizal plants, may enhance photosynthesis and C availability to flowering. The early and more abundant flowering exhibited by mycorrhizal plants may be very advantageous for these plants, which have short life span and are commonly highly prolific, in competition at the beginning of tropical succession.

Thus, AMF have different influences on the development of herbaceous and shrubby tropical species, depending on soil fertility: in low-fertility soil, AMF especially affect the survival, growth, and flowering. But in high-fertility soil, although mycorrhizal symbiosis did not provide large plant biomass accumulation, AMF still have a very important role in shoot nutrient concentrations and flowering. Some studies have found similar results for herbaceous species of other South America ecosystems. Urcelay et al. (2012) showed that in three typical Asteraceae species of Chaquean region (*Bidens pilosa*, *Tagetes minuta*, and *Zinnia peruviana*), mycorrhizal plants had lower shoot dry mass than non-mycorrhizal plants. Grilli et al. (2014) reported that the mycorrhizal colonization of *Euphorbia acerensis* and

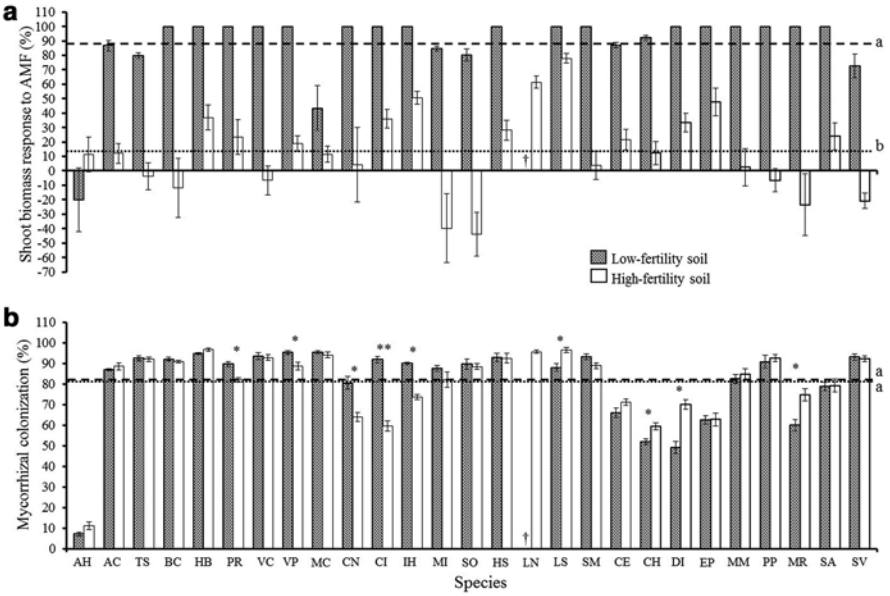

**Fig. 5.1** Shoot biomass response to AMF (**a**) and mycorrhizal colonization (**b**) of herbaceous and shrubby plant species of early stages of tropical succession grown in low- and high-fertility soils. *Vertical bars* indicate the standard error ($n=5$). *Dashed lines* represent the average of plants' response to AMF and mycorrhizal infection intensity considering all species in the low-fertility soil ($n=26$); *dotted lines* represent the same in the high-fertility soil ($n=27$). Means followed by the same letter do not differ from each other by Student's $t$ test at $P<0.05$. *Indicate significant differences in mycorrhizal infection intensity between soil fertilities within species by Student's $t$ test ($*P<0.05$; $**P<0.01$). *Dagger* represents death of the plants in the treatment. Species: AH=*Amaranthus hybridus*, AC=*Asclepias curassavica*, BC=*Baccharis* sp., HB=*Hypochaeris brasiliensis*, PR=*Porophyllum ruderale*, VC=*Vernonia cognata*, VP=*Vernonia polyanthes*, TS=*Tecoma stans*, MC=*Momordica charantia*, CN=*Chamaecrista nictitans*, CI=*Crotalaria incana*, IH=*Indigofera hirsuta*, MI=*Mimosa invisa*, SO=*Senna obtusifolia*, HS=*Hyptis spicigera*, LN=*Leonotis nepetifolia*, LS=*Leonurus sibiricus*, SM=*Sidastrum micranthum*, CE=*Cenchrus echinatus*, CH=*Chloris elata*, DI=*Digitaria insularis*, EP=*Eragrostis pilosa*, MM=*Melinis minutiflora*, MR=*Melinis repens*, PP=*Pennisetum purpureum*, SA=*Sorghum arundinaceum*, SV=*Solanum viarum*. Data from Rondina et al. (2014) with changes

*Euphorbia dentata* can be high (80 %), and plants associated with AMF exhibited lower or not different dry mass than plants without AMF. However, the high mycorrhizal colonization influenced negatively the reproduction traits of these Euphorbiaceae species, like inflorescence and fruits production.

The seedling growth response to AMF of approximately 150 native woody species belonging to different ecological successional groups from Brazilian tropical forests, studied by Carneiro et al. (1996), Siqueira et al. (1998), Zangaro et al. (2000, 2002, 2003, 2005, 2007), Pouyú-Rojas and Siqueira (2000), Siqueira and Saggin-Júnior (2001), Zangaro and Andrade (2002), Matsumoto et al. (2005), Patreze and Cordeiro (2005), Pasqualini et al. (2007), and Vandresen et al. (2007), are shown in Fig. 5.3. Plant woody species were classified into different ecological

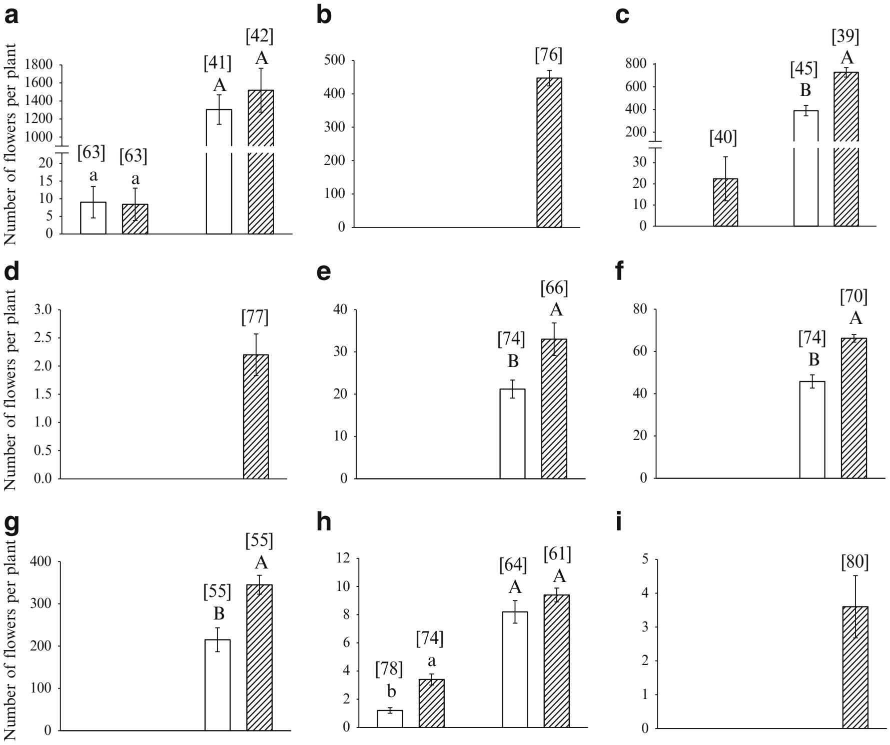

**Fig. 5.2** Number of flowers per plant and days elapsed for appearance of the first flower buds after plant emergence (number in brackets) of herbaceous and shrubby plant species of early stages of tropical succession grown in low- and high-fertility soils, with or without AMF. *Vertical bars* indicate the standard error ($n=5$). Means followed by the same lowercase (low-fertility soil) or uppercase (high-fertility soil) letters do not differ by Student's $t$-test at $P<0.05$. Species: *Amaranthus hybridus* (**a**), *Hypochaeris brasiliensis* (**b**), *Porophyllum ruderale* (**c**), *Momordica charantia* (**d**), *Chamaecrista nictitans* (**e**), *Crotalaria incana* (**f**), *Indigofera hirsuta* (**g**), *Senna obtusifolia* (**h**), *Hyptis spicigera* (**i**), *Leonotis nepetifolia* (**j**), *Leonurus sibiricus* (**k**), *Cenchrus echinatus* (**l**), *Digitaria insularis* (**m**), *Eragrostis pilosa* (**n**), *Melinis repens* (**o**), *Sorghum arundinaceum* (**p**). Data from Rondina et al. (2014)

successional groups such as pioneer, early-secondary, late-secondary, and climax. The response to AMF inoculation and the intensity of AMF root colonization of the native woody species decreased with the advance among successional ecological groups. Plant biomass response to AMF and AMF root colonization was very high among early-successional woody species, revealing the importance of arbuscular mycorrhizal association for the initial growth of this woody species, which are involved in the initial tropical forest structuring. In contrast, plant species belonging to late stages of succession that dominates in the mature forests showed low AMF root colonization and biomass response. These woody species display limited use of the AMF as tool for mineral acquisition during seedling stages.

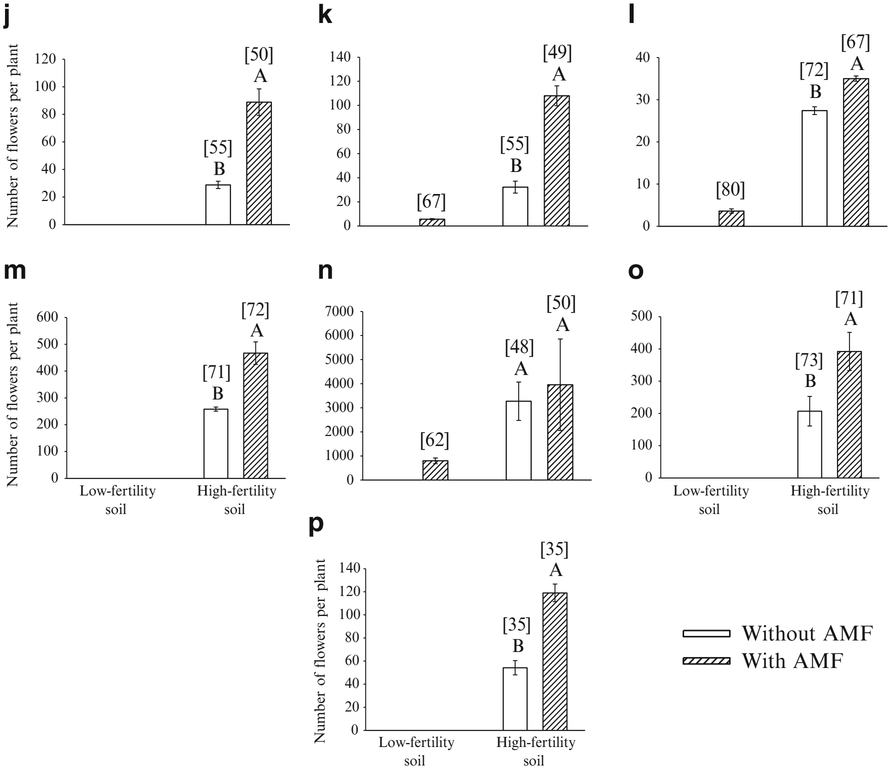

**Fig. 5.2** (continued)

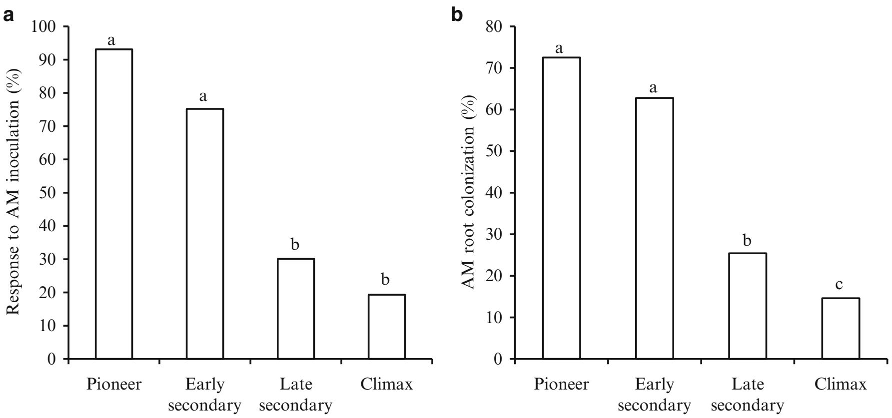

**Fig. 5.3** Plant response to AMF inoculation (**a**) and AMF root colonization (**b**) of native woody species belonging to different successional stages. Means followed by same letter are not different by Tukey–Kramer HSD test at 0.05 level. Data from 93 plant species for response to inoculation and 121 plant species for root colonization. Data from Zangaro (2012)

Pioneer and early-secondary woody species when grown in the absence of AMF presented lower nutrient concentrations in leaves. These concentrations increased strongly when the same plant species was grown with arbuscular mycorrhizas (Zangaro et al. 2003), indicating that the pioneer and early-secondary species are not able to acquire nutrients from a low-fertility soil when AMF are not present. Pioneer and early-secondary species show inherent high growth rate and their high nutrient accumulation when grown with AMF, suggesting that the high demand for nutrients can be reached only in the presence of AMF association. For late-secondary and climax species there was no alteration in nutrient concentration and accumulation on leaves in the presence or absence of AMF, which probably led to the absence of plant biomass response to AMF in both high- and low-fertility soils (Zangaro et al. 2007).

## 5.3 Fine Root Traits and AMF Colonization Intensity for Nutrient Acquisition

It has been widely accepted from research in temperate regions that the plant root architecture controlled mycorrhizal benefit. Plant species that explore large soil volume display long fine roots, highly branched, with low diameter, covered with numerous root hairs, and are expected to exhibit low levels of AMF root colonization (Manjunath and Habte 1991; Schweiger et al. 1995; Brundrett 2002). On the other hand, plant species with coarse root systems and few root hairs tend to have high AMF colonization (Baylis 1975; Graham and Syvertsen 1985; Hetrick et al. 1992; Manjunath and Habte 1991; Reinhardt and Miller 1990; Schweiger et al. 1995). The results of Rondina et al. (2014) and Zangaro et al. (2005, 2007), for seedlings grown in greenhouse, and Zangaro et al. (2008, 2012a, b, 2013, 2014), for field results from tropical herbaceous and native woody species, do not support this hypothesis. The AMF root colonization and spore production among tropical native woody species belonging to different phases of succession display relations with the morphological root characteristics and plant metabolic demand. Generally, plant species belonging to early-successional stages with fine roots and abundant and long root hairs displayed high AMF colonization and sporulation (Table 5.1). By contrast, late-successional woody species with coarse roots and few root hairs displayed low AMF colonization and spore production (Zangaro et al. 2005, 2007).

In tropical soils, which generally have low P available, the early-successional woody species with apparent root morphology for high uptake capacity are not able to ensure adequate nutrition for maintaining their inherent fast growth rate, becoming arbuscular mycorrhizas essential for their nutrient acquisition. Zangaro et al. (2005, 2007, 2008, 2014) suggested that, in addition to high carbon allocation to fine root construction, early-successional species maintain more AMF because they present morphological root traits (high root length, high specific root length, low diameter, long and dense root hairs) with high interface, which favor the contact with mycorrhizal propagules in the soil. Indeed, the high nutrient accumulation exhibited for early-successional species expresses its high external demand for nutrients. Thus, fast-growing woody species display inherent intense metabolism that demands

**Table 5.1** The Pearson's correlation coefficients among AMF root colonization (AMC) and AMF spore density in soil (AMS) with fine root dry mass (RDM), fine root length (RL), specific root length (SRL), fine root diameter (RD), root-hair incidence (RHI), and root-hair length (RHL) in the grassland, secondary, and mature forest from Pantanal, Atlantic, and Araucaria ecosystems in Brazil. *P*-values are in parentheses. Data for fine roots ($n=18$) are means from 0 to 10 cm and 10–20 cm depth

| Fine root traits | | | | | | | |
|---|---|---|---|---|---|---|---|
| | AMS | RDM | RL | SRL | RD | RHI | RHL |
| AMC | 0.83<br>(<0.0001) | –0.16<br>(0.54) | 0.62<br>(0.0056) | 0.86<br>(<0.0001) | –0.86<br>(<0.0001) | 0.91<br>(<0.0001) | 0.94<br>(<0.0001) |
| AMS | – | –0.08<br>(0.75) | 0.67<br>(0.0023) | 0.66<br>(0.0031) | –0.81<br>(<0.0001) | 0.77<br>(0.0002) | 0.67<br>(0.0023) |

Data from Zangaro et al. (2012b)

high amounts of nutrients to support the high growth rates and, therefore, maintain more AMF in root and soil to supply their nutritional needs. The relatively higher investment in leaves by the early-successional species increases the amount of photoassimilates that can be allocated to roots (Nielsen et al. 1998; Lynch and Ho 2005) and more carbohydrates may be provided to AMF in roots.

By contrast, plants from mature forest present less AMF colonization in fine roots in addition to less sporulation in soil. Several features such as shading, low growth rates, and metabolic demands may result in a reduced availability of carbohydrates to AMF in roots and consequently less mycorrhizal root colonization and sporulation in the mature forest (Zangaro et al. 2005, 2007, 2012a, 2014). Late-successional woody species display root morphology for low nutrient acquisition capacities (low root length, low specific root length, short and sparse root hairs) and are able to maintain their growth in the absence of AMF, even in deficient P soils (Zangaro et al. 2007). Therefore, the fine roots alone may be responsible for the nutrient acquisition among late-successional trees species. This indicates that slow-growing species may exhibit other strategies for nutrient acquisition instead of AMF association, as an additional enzymatic nutrient acquisition mechanisms (Chapin 1980), nutrient use efficiency (Manjunath and Habte 1991; Koide 1991), low requirement due to both low growth rate and metabolic demand (Zangaro et al. 2007), and high seed reserves for seedlings growth (Siqueira et al. 1998; Zangaro et al. 2000; Pasqualini et al. 2007).

## 5.4 Abundance of AMF in Different Stages of Succession

Mycorrhizal variables such as AMF root colonization and AMF spores density in soil were accessed over several years from sites covered with grasses, secondary forests, and mature forests in Atlantic, Araucaria, and Pantanal ecosystems in Brazil (Fig. 5.4). Mycorrhizal root colonization and mycorrhizal spore number over several months were also accessed from grassland, scrub, secondary forest, and mature forest of the Atlantic rainforest biome, located at Londrina municipality, Paraná state, Southern Brazil (Fig. 5.5). The AMF root colonization and the AMF spore density in

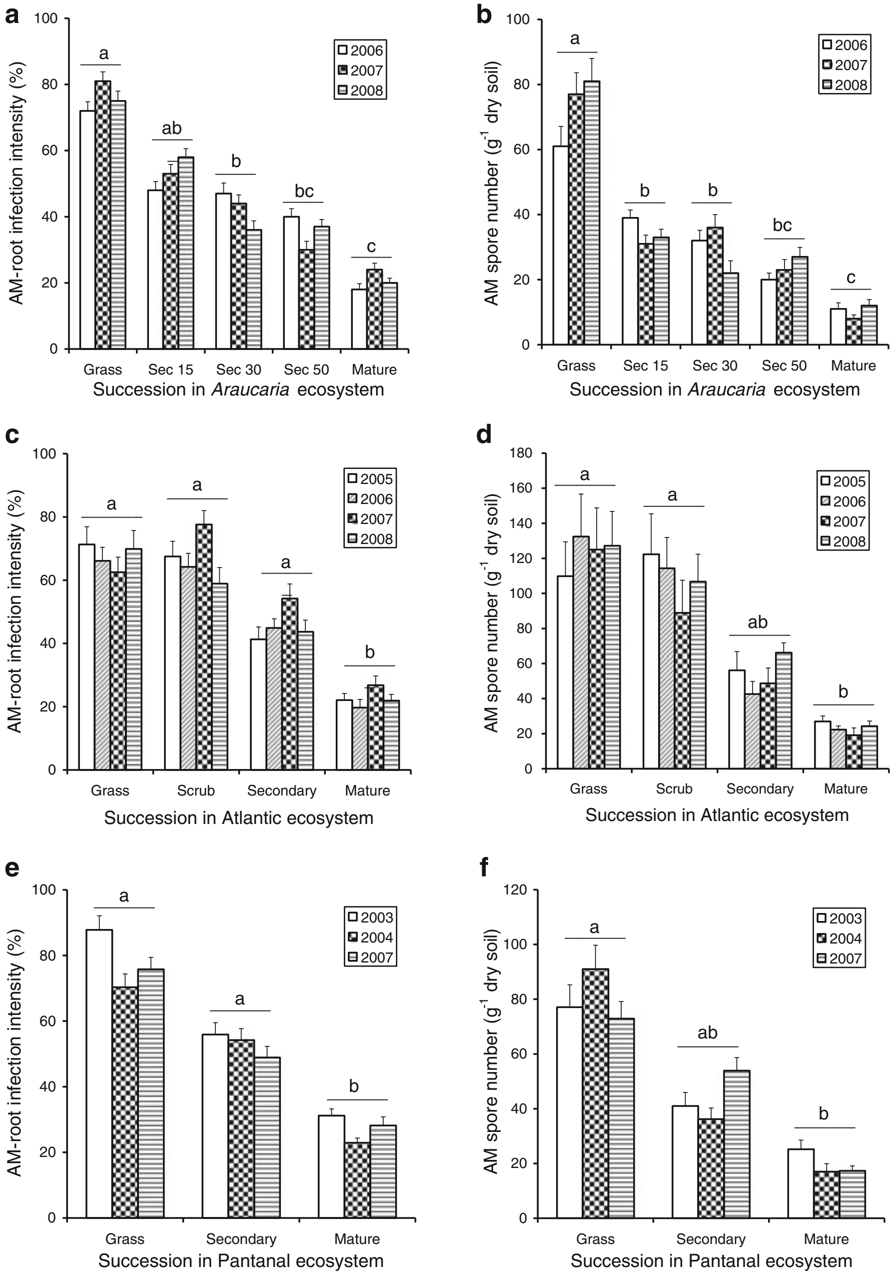

**Fig. 5.4** Mycorrhizal root infection intensity and mycorrhizal spore number over several years from *Araucaria* ecosystem (**a**, **b**), Atlantic ecosystem (**c**, **d**), and Pantanal ecosystem (**e**, **f**) in Brazil. In *Araucaria* ecosystem Grass (grassland site), Sec 15 (15-year-old secondary forest), Sec 30 (30-year-old secondary forest), Sec 50 (50-year-old secondary forest), and Mature (mature forest). In Atlantic ecosystem Grass (grassland site), Scrub (5-year-old scrub vegetation), Secondary (20-year-old secondary forest), and Mature (mature forest). In Pantanal ecosystem Grass (grassland site), Secondary (15-year-old secondary forest), and Mature (mature forest). *Error bars* are + SE of the means ($n = 15$). Means followed by the same letter among successional sites are not different by Tukey's test at 0.05 level. Data from Zangaro et al. (2012a)

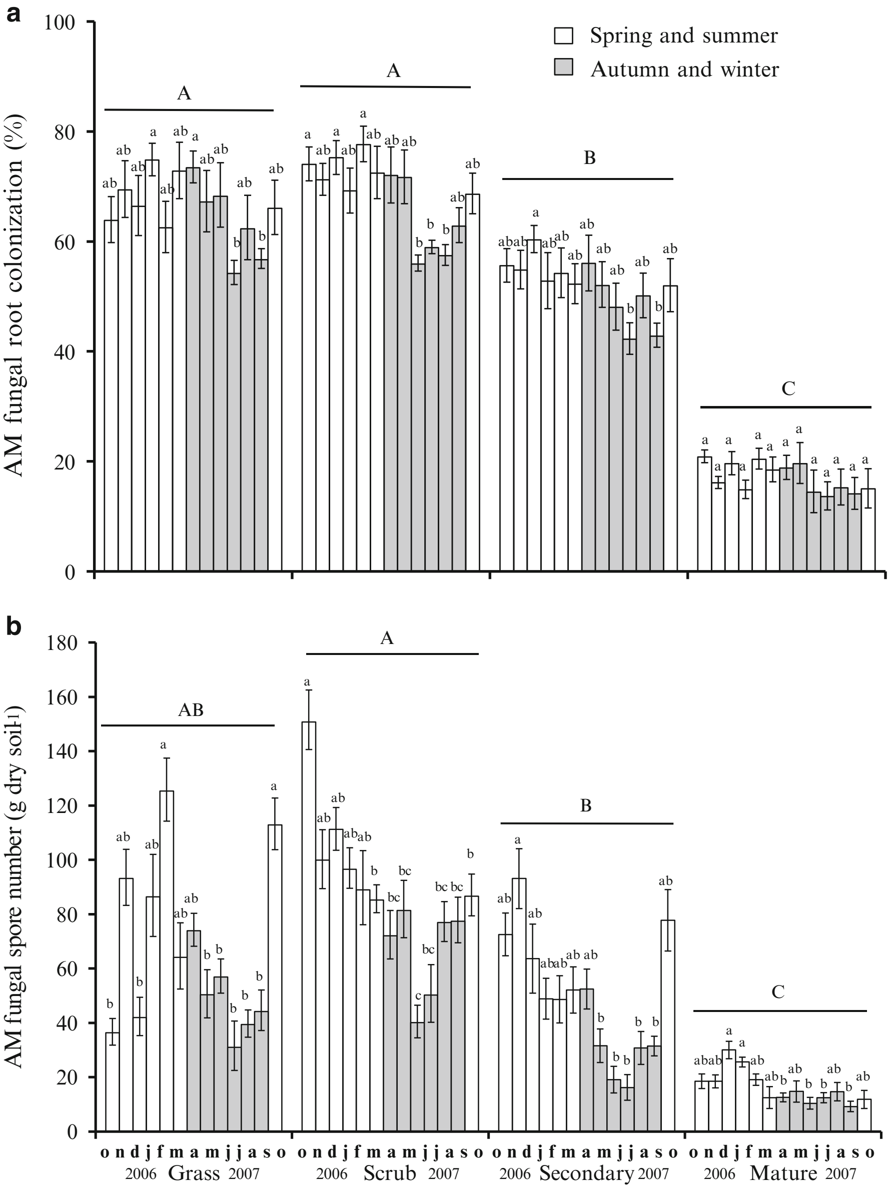

**Fig. 5.5** Mycorrhizal root colonization (**a**) and mycorrhizal spore number (**b**) over several months from grassland, scrub, secondary forest, and mature forest of the Atlantic rainforest biome, located at Londrina municipality, Paraná state, Southern Brazil. Soils and fine root samples were assessed at 0–5 cm depth from October 2006 until November 2007. *Error bars* are ± 1 SE. Means followed by the same letter are not different by Tukey's test at 0.05 level. Small letters compare means within a same successional site. Capital letters compare among successional sites ($n = 13$, $P < 0.001$ for AMF root colonization and $n = 13$, $P < 0.001$ for AMF spore number). Data from Zangaro et al. (2013)

soil decreased over succession, independently of studied biome. A positive correlation between spore numbers and mycorrhizal colonization was found, and these AMF variables can be considered as indicators of mycorrhizal incidence in soil (Picone 2000; Cardoso et al. 2003). The high and positive correlations found between AMF root colonization and AMF spore density may indicate the mycelial biomass of AMF in the soil, the AMF inoculum potential in the field, and the potential of plant species from different functional ecological groups to support AMF association. Mycorrhizal colonization and AMF spores in soils showed a high close correlation with parameters of fine root morphology (Table 5.1), but low correlation with root dry mass, suggesting that the morphological root characteristics are more important to the symbiosis than the fine root mass. These results toward plant investment in fine root morphology and AMF root colonization as an important way for plant nutrient acquisition.

The AMF root colonization and AMF spores density in soil exhibited strong reduction during succession progress in all areas studied. These results reflect the greater investment in arbuscular mycorrhizal symbiosis by host plants of the early-successional phases than native woody species of mature forest. The higher AMF fungi variables in early-successional stages than in mature forests comply with the higher AMF spores density found in pasture or natural grasses sites than in forests in Australia (Jasper et al. 1991), in addition to humid secondary forest (Fischer et al. 1994) and mature forest (Johnson and Wedin 1997) in Costa Rica, dry forest in Mexico (Allen et al. 1998), natural sites in Venezuela (Cuenca et al. 1998), lowland evergreen forests in Nicaragua and Costa Rica (Picone 2000), tropical forest in southern Brazil (Zangaro and Andrade 2002), and low-fertility soil at mature forest in Costa Rica (Lovelock et al. 2003). Besides, Zangaro et al. (2000) found low density of AMF spores and root colonization in plants from mature forest in southern Brazil and suggested that slow-growing species are less able to keep AMF due to growth in relative high soil fertility and shaded environments and exhibit low metabolic activity. Aidar et al. (2004) verified that AMF root colonization and AMF spores density decreased with increasing soil fertility in a chronosequence of an Atlantic forest in southeast Brazil. Powers et al. (2005) related that the amount of AMF hyphae in the soils of four tropical forests in Central and South America was unexpectedly quite low and suggested that plants of mature forests must rely on their fine roots instead of AMF for nutrient uptake. In an analysis in 15 published papers, Zangaro and Moreira (2010) verified that the amount of AMF spores in soils from mature forests and old secondary forests of the Brazilian Atlantic forest biome was lower than in recent secondary forests and open areas.

## 5.5 Inoculum of AMF Available in Soil

The soil inoculum potential of AMF decreased strongly over succession in the soils from the mature tropical forest, in a gap in the same mature forest, and in a recent secondary forest (Zangaro et al. 2000). The inoculum potential of AMF in the soil of the secondary forest was approximately five times greater than in the mature

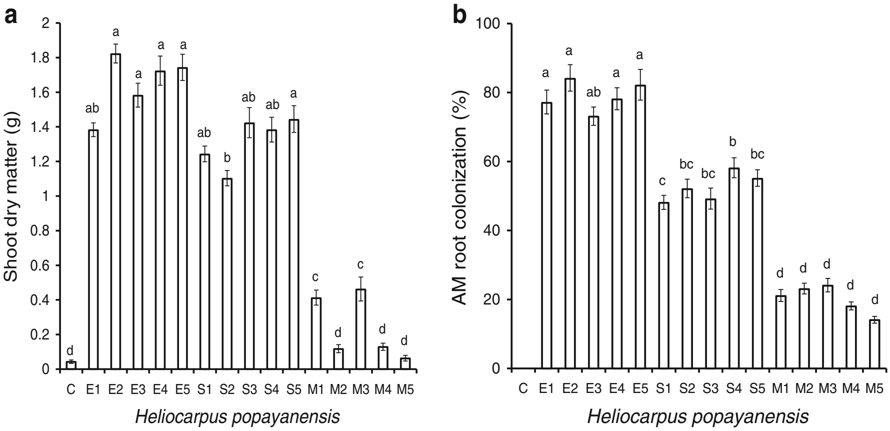

**Fig. 5.6** Shoot dry matter (**a**) and root colonization (**b**) of the tropical native woody *Heliocarpus popayanensis* grown in infertile soil. AM fungi inoculums are from five early-successional areas (E), five secondary forests (S), and five mature forests (M). *Error bars* represent ± 1 SE. Means followed by same letter are not different by Tukey test at 0.05 level

forest and in its gap. In another experiment (Zangaro et al. 2012a) the response to inoculation and the root colonization were accessed in seedlings of the woody mycotrophic species *Heliocarpus popayanensis* (Malvaceae) grown in 15 soils classes for 40 days and subsequently planted in infertile clay soil containing 1.66 mg P $dm^{-3}$, 1.24 mg N $dm^{-3}$, and 2.82 g C $dm^{-3}$ (Fig. 5.6). Seedlings grown in soils from early stages of succession and secondary forests displayed higher AMF root colonization and biomass response to AMF than seedlings grown in mature forests soils. These results emphasize the high AMF inoculum potential in the soils from early stages of tropical succession and the young secondary forests and the low potential in mature forest conditions. Thus, the herbaceous plant from open environments and pioneer and early-secondary woody species may be able to multiply the AMF in large amounts (Zangaro et al. 2013, 2014), allowing high inoculum potential for their offsprings (Rondina et al. 2014). On the other hand, in the mature forest, the inoculum potential was very low, indicating that the late-secondary and climax species that compose the most part of the vegetation in a mature forest have weak mycotrophy and, as a consequence, the potential of AMF inoculum is low in mature forest conditions.

## 5.6 Implication of the AMF Inoculum Potential for Secondary Forest Formation

The high mycorrhizal colonization and plant biomass response to AMF among early-successional woody species grown in greenhouse, in addition to high AMF root colonization and AMF spore abundance in soils from sites covered with grasses and young secondary forests, reflect the host plant potential for multiplying the AMF among heliophilous herbaceous plants and fast-growing woody species. The intense host investment to maintain high AMF soil inoculum potential toward large density of AMF propagules production, which may be favorable for the plant installation and recruitment of fast-growing species. The early-successional woody species are highly responsive to AMF colonization, regardless of soil fertility level (Siqueira et al. 1998; Zangaro et al. 2007), and exhibit great aggressiveness during establishment in open and disturbed areas (Zangaro et al. 2003). Thus, AMF may be the main biotic factor for the establishment and growth acceleration of the native woody species that lead for initial tropical forest structuring (Zangaro et al. 2000). In the later stages of succession, the light limitation increases and early-successional woody species with typical fast growth rate and shade intolerance has difficulty to grow, reproduce, and maintain AMF association, attributed to low irradiance and its effect on fine roots carbohydrate availability to these fungi. The reduction of AMF in later stages of succession under light limitation is connected with concomitant decrease of the shade-intolerant host plant species. These declines have important implications for tropical forest succession, because the incapacity of the early-successional woody species to maintain AMF associations limits the acquisition of water and nutrient reducing their regeneration potential, recruitment, and competitive ability in later stages of succession (Zangaro et al. 2012a).

During succession progress, the fast growth and turnover of these shade-intolerant woody species provides a continuous soil organic enrichment and improves the soil structure (Uhl et al. 1982; Zangaro et al. 2003, 2009). The increase of soil surface fertility along the succession progress is attributable to the biomass accumulation and decomposition as the vegetation develops along the time (Silver et al. 1996; Guariguata and Ostertag 2001; Lugo and Helmer 2004; Boeger et al. 2005). Decomposition of soil organic matter produced by fast-growing woody species is important because of its critical role in the cycling of essential plant nutrients (Degens et al. 2000). The transformations above- and belowground during initial forest structuring allow the posterior establishment and growth of slow-growing woody species, which are dominant in mature forests. Therefore, the adult native woody species that dominate the late succession phases of the tropical forests maintain low amount of AMF due to low AMF association requirements. Plants in mature forests, under low light intensity, can be more limited by carbon than nutrient availability in soils, restricting the carbohydrate allocation to AMF and decreasing the root colonization, which suggests that the low levels of AMF in mature forests could be due to high carbon cost for maintaining the arbuscular mycorrhizal symbiosis in soils containing sufficient nutrient amounts (Zangaro 2012; Zangaro et al. 2014). Therefore, the low plant metabolic demand and the light availability appear to be important factors that determine AMF colonization and sporulation in mature forests. As large amount of plant photosynthetic products can be drained by AMF (Nielsen et al. 1998; Lynch and Ho 2005), the cost for maintaining high level of AMF can be significant for plant species in mature forests. Thus, the symbiotic limitations are an important means for plant energy conservation in mature forests (Zangaro et al. 2012b). Besides, lipid-rich spores and fungal hyphae are subject to predation and parasitism, since they serve as a food source for a wide range of soil animals (Rabatin and Stinner 1988; Stürmer et al. 2006). The soil organisms increase during succession (Coleman et al. 2004), and the competition with soil organisms and the hyphae and spores predation may be other important aspects that contribute to the decrease of the AMF in soils of mature forests.

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# Chapter 6
# Stress and Mycorrhizal Plant

**Mohammad Miransari**

## 6.1 Introduction

The definition of stress is deviation from favorite conditions, which eventually decrease plant growth and yield (Grativol et al. 2011). Most of the fields in the world are subjected to some kind of stress including salinity, acidity, drought, heavy metal, compaction, suboptimal root heat, and flooding. Food production has to be doubled until the year 2050 to provide the world's ever increasing population with their essential food. Hence, it is important to find methods which may alleviate the adverse effects of stress on the growth and yield of plant (Qin et al. 2011).

The use of tolerant plants is among the efficient techniques, which can enhance plant survival under stress. Tolerant plants are equipped with some physiological and biochemical responses, which make the plant survive under stress. Activation of different signaling pathway and production of different organic and inorganic compounds under stress are among the responses of tolerant plant species (Boyko and Kovalchuk 2008).

Under stress plants can alter their morphology and physiology to resist the stress. Tolerant plants have developed some mechanisms with time which make them survive the stress (Boyer 1982). The related mechanisms include the activity of different cellular channels, regulators, signals, and methylation of DNA. Under stress, reactive oxygen species are produced in plant cells, which adversely affect plant cellular structure and growth. Although reactive oxygen species are essential for the activity of plant cells, at high rate they negatively affect cell growth and activity. As previously mentioned, plant response is by the activation of a set of signaling pathway and production of biochemical products (Zhang and Blumwald 2001; Wu et al. 2012).

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M. Miransari (✉)
Department of Book and Article, AbtinBerkeh Ltd. Company,
#8, Fourth floor, Sadaf Building, Hakim Nezami Ave., Isfahan 8175676486, Iran
e-mail: Miransari1@gmail.com; info@abtinberkeh.com

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Soil microbes, including arbuscular mycorrhizal (AM) fungi, plant growth promoting rhizobacteria (PGPR), and endophytic microbes, are also able to increase plant efficiency under stress, by developing symbiotic and nonsymbiotic association with their host plants. AM fungi are soil fungi establishing symbiotic association with their host plant and significantly enhancing plant growth and yield (Glick 2012). When realizing the presence of the host plant, fungal spores germinate in the soil and by establishing an extensive hyphal network make a symbiotic association with their host plant. Although the fungal spores are also able to germinate in the absence of their host plant, the spores are not able to proceed with the other stages of symbiosis (Marschener 1998).

AM fungi use different mechanisms to make the plant survive under stress by (1) enhancing plant water and nutrient uptake, (2) production of plant hormone, (3) interactions with the other soil microbes, (4) controlling pathogens, (5) activation of different signaling pathway in the host plant, and (6) affecting the rhizosphere environment and activities (Miransari 2010; Sajedi et al. 2010, 2011; Qin et al. 2011).

In the following some of the most important findings and advancement related to the use of mycorrhizal fungi on the growth of plant under stress are presented and analyzed. It is important to enhance the efficiency and productivity of the environment using appropriate microbial species including mycorrhizal fungi. The right combination of AM fungi and the host plant may result in the highest level of efficiency, especially under stress.

## 6.2 Plant and Stress

As sessile organisms, plants have evolved mechanisms with time, which make them tolerate the stress. According to the following details the related mechanisms include (1) morphological and physiological alterations, (2) development of signaling pathway such as plant hormonal signaling, (3) interactions with soil microbes such as AM fungi and PGPR, (4) exudation of different root products, and (5) affecting the properties of soil (Xiong and Zhu 2002; Qin et al. 2011; Miransari 2011a).

A number of cellular responses are resulted by plant under salinity stress, which are: (1) increasing the plant membrane thickness and the number of vesicle in plant cells, (2) enhancing the cell wall thickness and the number of vacuoles in cytoplasm and plasmodesmata, and (3) disrupting the production of thylakoid and thylakoid membrane (Mittler 2002; Apel and Hirt 2004; Fujita et al. 2006).

The findings that have been so far obtained under stress related to the response of plant at cellular, morphological, and physiological level can be used for the development of methods and practices, which can result in the production of tolerant plants. Accordingly, the expression and activation of stress genes can make the plant to handle the stress (Gupta et al. 2013). Among the important abilities of plant to survive under the stress is to minimize the concentration of sodium in their leaf by the exclusion of sodium (James et al. 2006).

One of the major mechanisms by which the plant is able to alleviate stresses such as salinity and heavy metal is cellular homeostasis. If plant cells are able to regulate the concentration of different heavy metals and salt elements in the cell including sodium, calcium, magnesium, and chloride the plant will be able to tolerate the stress. For example, the following sodium proteins, *SOS1*, *SOS2*, and *SOS3*, are able to regulate the concentration of sodium in plant cells resulting in cellular homeostasis. The compartmentalization of sodium into the plant cells is also regulated by the abovementioned gene (Zhang and Blumwald 2001; Zhu 2002; Chinnusamy and Zhu 2009; Cuin et al. 2012).

Plant is able to resist the salinity stress by increasing water efficiency and uptake. Accordingly, under salinity stress the changed osmotic potential of plant cytoplasm and cytosol to tolerate the stress results from the accumulation of nontoxic molecules including sucrose, glycine, proline, trehalose, betaine, alanine betaine, polyols, choline, *O*sulfate, pipecolate betaine, proline betaine, and hydroxyl proline betaine (Serraj and Sinclair 2002).

Although proline results in the osmotic adjustment of plant cells under stress, the following important mechanisms can also help the plant to survive the salinity stress: (1) unchanged redox potential of plant cells, (2) stabilizing the structure of plant cells, (3) scavenging the reactive oxygen species produced under stress, and (4) the balanced rate of NADP+/NADPH, which is essential for cellular metabolisms and activities (Gill and Tuteja 2010; Zhou et al. 2010; Wang et al. 2013).

Under stress the production of reactive oxygen species including super oxide radicals ($O^{2-}$), hydroxyl (OH-), and hydrogen proxide ($H_2O_2$) increases in plant, which is due to the alteration of metabolism activities in mitochondria and chloroplast. As a result the plant cellular structure is adversely affected. Plant response to such a stress is by production of antioxidant products such as ascorbate and glutamate and enzymes including super oxide dismutase, catalase, glutathione peroxidase, etc. (Sajedi et al. 2010, 2011).

With respect to the previously mentioned details, the related plant signaling pathways, which are activated under stress, include reactive oxygen species, hormonal signaling, mitogen-activated protein kinase, phospholipids, sugars, etc. Under stress the stress genes in plant are activated and result in a cross talk between plant hormone and hence alleviation of stress. The signaling of mitogen-activated protein kinase in plant under stress can also make the plant to be responsive to the stress as a result of the stimuli production and the related hormonal signaling (Smekalova et al. 2014; Miransari 2014a).

## 6.3 Plant Physiological and Signaling Pathway

Plants use different physiological responses under stress among which the activation of the related signaling pathway such as plant hormonal signaling is the most important one. Different plant hormones, including auxin, ABA, ethylene, cytokinins, gibberellins, jasmonate, and brassinosteroids, make the plant survive under stress by activating stress genes. The functions of hormones in plant include (1) regulation of seed germination and dormancy, (2) growth regulation, (3) stomatal activities, and (4) abscission of fruits (Qin et al. 2011; Ha et al. 2012; Ljung 2013; Miransari and Smith 2014). They alleviate effects of stress by affecting (1) plant growth and development, (2) nutrient uptake and behavior by plant, and (3) transitions of sink and source (Peleg and Blumwald 2011).

There is some kind of cross talk between plant hormones affecting their production and activity in plant (Li et al. 2006). Hormonal activities in plant are essential especially under stress, which make the plant to adopt itself under stress. They can rapidly influence gene expression in plant under stress. For example, under water stress ABA activity and signaling is able to alleviate the stress and hence make the plant to grow under stress. Such effects are by controlling stomata activity in plant and hence plant water efficiency (Wilkinson and Davies 2010). However, other hormones in plant can also affect the activity of stomata and hence water behavior. Investigating the behavior of plant hormones in plant including their production and activity is useful for the production of transgenic plants, which can tolerate stress conditions more efficiently (Peleg and Blumwald 2011).

## 6.4 Microbes and Stress

Among the most important benefits of soil microbes are their alleviating effects under stress. A wide range of soil microbes including AM fungi, PGPR, and endophytic bacteria are able to alleviate soil stresses by (1) enhancing the availability of soil nutrients, (2) production of plant hormone, (3) controlling pathogens, (4) adjusting and regulating the concentrations of toxic ions, and (5) production of different biochemicals (Kennedy and Smith 1995; Hamilton and Frank 2001; Miransari 2014b, c).

It has been indicated that PGPR are able to alleviate soil stresses by the production of an enzyme called 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Under stress the production of stress hormone, ethylene, increases, adversely affecting plant growth. However, PGPR are able to produce ACC deaminase, which is able to catabolize the prerequisite for the production of aminocyclopropane-1-carboxylate and hence alleviate the stress by decreased production of ethylene (Glick 2014).

## 6.5 Stress and Mycorrhizal Plant

As previously mentioned mycorrhizal fungi are able to make the plant tolerate the stress. The fungi can alter plant morphology and physiology in a way such that the plant can resist the stress (Miransari et al. 2008). Under stress the growth and activity of both the fungi and the plant are adversely affected. If the fungi are isolated from stress conditions, they will be more able to handle the stress (Hilderbrandt et al. 2001). Aliasgharzadeh et al. (2001) were able to isolate the tolerant species of mycorrhizal fungi from the saline soils of Tabriz, Iran.

### *6.5.1 Salinity*

Salinity is a major stress affecting both plant growth and microbial activities, worldwide. The related adverse effects are (1) unfavorable osmotic potential, (2) the toxic effects of ions such as sodium and chloride, and (3) negative effects on the morphology and physiology of plants and microbes (Tuteja 2007). Different methods have been tested and tried on the alleviation of salinity stress including (1) the leaching of soil, (2) using tolerant plants, and (3) using soil microbes (Läuchli and Grattan 2007).

Because of the tendency of ions such as sodium and chloride to bind the water molecule, the availability of water for the use of plants and microbes under salinity stress decreases and hence they both react to stress. The ions by themselves are also able to adversely affect plant and microbes as at higher concentrations they indicate some negative effects by replacing the ions, which are essential for plant and microbial growth. As a result the activity and production of enzymes and hence proteins decreases in plant and the related symptoms appear (Flowers and Yeo 1989; Abebe et al. 2003).

Although the leaching of soil decreases the soil salinity, such method may be laborious and expensive. The use of tolerant plants is among the most effective ones for the alleviation of salinity stress and hence increased yield under the stress. In tolerant plants, the presence of stress gene can help the plant to develop the related mechanisms including the activation of the related stress pathway and production of biochemicals such as proline, which can alleviate the stress by adjusting the plant water potential (Zhu 2001; Zhang and Blumwald 2001).

Interestingly, such ability of plants in alleviating the stress may be enhanced by using soil microbes such as AM fungi, which are able to develop a symbiotic association with their host plant. The fungi are able to utilize some mechanisms, which can help both the fungi and the host plant to tolerate the stress. If the fungal species are isolated from stress conditions, they will be able to alleviate the stress more efficiently (Daei et al. 2009). The other important point is that although the symbioses between the host plant and mycorrhizal fungi are not specific, using the right combination of the host plant and AM fungi may affect the alleviation of stress more efficiently (Miransari et al. 2008).

The related mechanisms of alleviation by soil microbes under stress include (1) the increased uptake of water and nutrients by the host plant, (2) development of an extensive hyphal network by mycorrhizal fungi, which is able to absorb water and nutrients from even the smallest soil pores, which are not accessible by root hairs, (3) adjusting the rate of stress elements in plant, (4) interactions with the other soil microbes, (5) a high number of vacuoles in the fungal hypha are able to absorb a high concentration of toxic ions, and (6) the mycorrhizal fungi are able to produce different biochemicals, such as plant hormones, which can alleviate the adverse effects of stress (Marschner 1995; Feng et al. 2002; Miransari 2014b, c).

The fungi are able to enhance the uptake of all essential nutrients for plant growth and yield production. However, phosphorous is among the most important nutrients, which is absorbed at a higher rate by the host plant. This is due to the activity of the fungi to enhance the availability of phosphorous in the soil by production of different enzymes such as phosphatases. The extensive hyphal network is also able to enhance nutrient uptake significantly. Under P nutrient conditions, plant roots are also able to produce phosphatases and organic products, which enhances the availability and hence the uptake of P by plant roots (Marschener 1998).

Nutrient uptake by mycorrhizal plants is affected by different parameters including: (1) plant and mycorrhizal species, (2) nutrient concentration in the soil, (3) climate, and (4) soil properties. At high nutrient concentration the uptake of nutrients especially P decreases by the host plant (Wang et al. 2011), which is due to the following: (1) the activity of transporter decreases in the plant, and (2) the host plant would not be willing to develop a symbiosis with the fungi (Miransari 2012; Schenk et al. 2012; Smith and Smith 2012).

The other important effect of P uptake by mycorrhizal plant is to enhance root growth. The host plant absorbs P by different mechanisms including the pathway of P uptake by plant roots and the pathway of P uptake by mycorrhizal fungi (Marschner and Dell 1994; Smith and Smith 2012). Mycorrhizal fungi are also able to maintain the integrity of vacuole membrane, which is essential for the compartmentalization of sodium and chloride under salinity stress (Maathuis 2009).

The fungi can also alter plant physiology so that the host plant can resist the stress. The fungal hyphae are able to enter plant roots and hence increase the uptake of water and nutrients by the host plant, as the vesicle and arbuscule are developed by the fungi. Vesicle is a fungal organelle with the great ability of absorbing high rate of different elements, which is especially a benefit under stress, and the arbuscule is the interface between the fungi and plant cells for the uptake of nutrients. Such kind of association requires the alteration of root structure including the microtubule (Gianinazzi-Pearson et al. 2004).

The fungi can make the plant to survive the stress by altering the host plant physiology. The following indicates how the fungi may alter plant physiology under stress. (1) Production of plant hormone, (2) enhanced production of chlorophyll and its fluorescence, (3) maintaining the permeability of cell, (4) improved water potential of plant, and (5) enhanced N fixing ability of legumes (Garg and Manchanda 2008; Jahromi et al. 2008; Sheng et al. 2008).

Salinity stress decreases the production of enzymes, which are essential for chlorophyll pigments. Due to the adverse effects of salinity elements on the growth of plant the uptake of magnesium as an important element for chlorophyll structure also decreases under salinity stress. Mycorrhizal fungi are able to increase the production of chlorophyll fluorescence content, as an indicator of plant photosynthesis efficiency, when the host plant is subjected to salinity stress (Sheng et al. 2008).

Mycorrhizal fungi are able to alleviate drought and salinity stress by absorbing higher rate of water and increased concentration of electrolyte in cytoplasm, which is due to the higher membrane integrity. Some molecular alterations also take place in plant under salinity stress. The aquaporin proteins in the transmembrane channels control different cellular activities. For example, the selective absorbance of water and other compounds and homeostasis of $H^+$ and other elements in the cells are controlled by such proteins (Hajiboland 2013).

The morphological and physiological properties of the fungi and hence the production of different products in the mycorrhizosphere (rhizodeposition) by the fungi is also affected. The fungi are able to produce a glycoprotein called glomalin, which is able to bind soil particle and hence improve the related properties (Singh et al. 2013). Hammer and Rillig (2011) hypothesized that due to the adverse correlation between the hyphal length and the production of glomalin, it may be a suitable indicator of fungal (*G. intraradices*) response under stress. In their experiments they tested the effect of different stresses including salinity (sodium chloride), drought (glycerol), and grazing (scissors) on the in vitro production of glomalin by fungal hyphae. Salinity significantly increased the production of glomalin, grazing fairly enhanced glomalin production, and drought did not affect its production.

Although most research work investigating the effects of salinity stress on the growth of mycorrhizal plants has been conducted under greenhouse conditions, a few research works have been conducted under field conditions. For example, Daei et al. (2009) tested the effects of different species of mycorrhizal fungi on the growth of different wheat genotypes including wheat yield and nutrient uptake under field conditions using saline soil and water. Mycorrhizal fungi significantly increased the uptake of different nutrients and resulted in the least uptake of salt by plant. The highest influence of mycorrhizal fungi on the growth enhancement of wheat under salinity stress was by *Glomus etunicatum*, *G. mosseae* and *G. intraradices*, respectively. Such interesting result under field conditions indicate that mycorrhizal fungi are able to efficiently increase wheat yield and nutrient uptake under salinity stress, however, some specific interactions may be more applicable under field conditions.

Salinity stress may increase or decrease the number of spores in the soil (Tressner and Hayes 1971; Aliasgharzadeh et al. 2001). Salt may delay the process of spore germination rather than inhibiting the process. However, if the host plant is present, the process of symbiotic association can alleviate the adverse effects of salinity on the process of spore germination and hyphal production. The process of hyphal growth is more sensitive to salinity related to the process of spore germination (Talaat and Shawky 2014).

Among the most important mechanisms by which AM fungi alleviate the effects of salinity stress on the growth and yield of the host plant is the homeostasis of salt elements in the cells. Such a process is regulated by the following: (1) the expression of the related stress genes, (2) the activity of the $H^+$ pumps, (3) the allocation of sodium and chloride to the cellular vacuole and apoplasm, and (4) the activation of the related signaling pathway (Daei et al. 2009; Miransari and Smith 2009; Bothe 2012).

### *6.5.2 Drought*

Some similarities exist between the adverse effects of drought and salinity on the growth and activity of plants and microbes, among which the most important one is the decreased efficiency of water by plant. Under drought stress there is not enough water for the use of plant and under salinity stress the presence of ions such as sodium and chloride decreases the availability of water to the plant. Accordingly, the related physiological mechanisms including the related signaling pathway may be similar under the stress of drought and salinity (Zhu 2002).

It is greatly indicated that AM fungi are able to enhance plant growth and yield under drought stress. Accordingly, most plants of arid and semiarid areas develop a symbiotic association with mycorrhizal fungi and hence such plants are able to tolerate drought stress more efficiently. Such details show the importance of soil microbes, specifically AM fungi for the proper functioning of ecosystem (Pagano 2014).

Water deficit is among the most stressful parameters in grassland and hence makes the grassland vulnerable under climate change conditions. AM fungi promote plant growth and yield under drought stress by enhancing the plant's ability to tolerate and avoid the stress. However, such responses must be indicated for different plant species. Using a factorial meta-analysis of previously published data, Worchel et al. (2013) determined how grass species may be responsive under stress in symbiotic or nonsymbiotic association with mycorrhizal fungi.

Worchel et al. (2013) accordingly indicated how the response of mycorrhizal plant to stress may be affected by biotic (the pathway of plant photosynthesis) and abiotic (drought level) factors. They also determined the phylogenetically produced signals produced by the fungi in symbiosis with their grass host plant under control and drought conditions. Mycorrhizal fungi improved grass growth under drought stress related to non-mycorrhizal grasses. Such a response was more evident at the most stressful conditions of drought stress. The fungi significantly increased the growth of C3 grasses under control and stress conditions; however, it was not the case for C4 grasses. Accordingly, Worchel et al. (2013) indicated that because AM fungi are able to alter plant response under drought stress, evaluating such responses can be useful for the prediction of grassland functioning under climate change conditions.

Under drought, AM fungi are able to increase the uptake of water and nutrients by their extensive network of hyphae. The fungi are also able to produce the plant hormone ABA and increase its production in the host plant and as a result regulate the activity of plant stomata and enhance water efficiency (Augé 2000; Ludwig-Müller 2000). Plant hormones are essential for plant growth and development. Although much more details have yet to be indicated, the research work has indicated that plant hormones are also essential for the development of symbiotic association between the microbes and the host plant. Most details related to the effects of plant hormones have been illustrated using mutants and transgenic plants (Ludwig-Müller 2010).

Abdelmoneim et al. (2014) conducted an experiment investigating the effect of mycorrhizal fungi on the growth of corn (*Zea mays* L.) under drought stress. They used *G. mosseae* at three different concentrations (300, 600, and 900 spore pot$^{-1}$). With the exception of plant root weight, the other plant growth factors were adversely affected under drought stress, related to the control treatment. Under both stressed and non-stressed conditions, *G. mosseae* at 900 spore pot$^{-1}$ significantly resulted in the highest values of plant growth related to the control treatment. Water deficit decreased plant-soluble proteins by 29 %; the three levels of *G. mosseae* increased plant-soluble proteins at 13, 22, and 29 %, respectively, under water control conditions and by 25, 36, and 45 % under water-deficient conditions, respectively, related to the control treatment.

While the level of prolin in plant leaf increased by 22 %, under drought stress the fungal treatments increased the prolin level by 29, 38, and 43 %, respectively; plant P uptake was decreased by 72 % under drought stress, related to the water control treatment. Under water control conditions, the fungal treatments increased plant P uptake at 43, 76, and 79 % and under water-stressed conditions the related values were 88, 94, and 95 %, respectively (Abdelmoneim et al. 2014).

Fernández et al. (2012) investigated the effects of drought stress on (1) the growth of two native plants (*Tetraclinis articulata* and *Crithmum maritimum*) and (2) composition and activity of microbes in the rhizosphere. The experimental treatments of sugar beet residue (SBR, organic) and mycorrhizal fungi, *G. mosseae* (isolated from heavy metal contaminated soils), were tested in a non-sterile soil contaminated with heavy metals. The fungi did not affect the growth of plant species. SBR significantly increased the morphological and physiological properties of *T. articulata* including (1) plant aerial growth, (2) uptake of foliar P, (3) lipid content in the plant and in the soil fungi and bacteria, and (4) the activity of enzymes.

Under drought stress the activity of phosphatase increased in *T. articulata* by SBR and AM fungal treatments. The colonization of AM fungi was synergistically affected by the interactions between SBR and AM fungi under stress. With respect to their results the authors accordingly suggested that the effectiveness of organic treatment is adversely affected by drought stress, especially for plants, which are not able to develop an efficient symbiosis with AM fungi (Fernández et al. 2012).

As a lignocellulosic material, SBR is the by-product of sugar industry. The fungus *Aspergillus niger* is able to fermentate SBR supplemented with rock phosphate resulting in the production of high source of polysaccharide and available P. While the fermented SBR by *A. niger* can increase the growth and P uptake of fungal hyphae, nonfermented SBR may adversely affect the fungal activities due to the production of some unfavorable products. SBR fermentation is also of environmental significance because the non-fermented SBR may result in the contamination of the environment (Azcón et al. 2009).

The activity of enzymes in the soil can be a great indicator of soil ecological properties including the rehabilitation process. Such kind of responses is affected by the presence of soil microbes including AM fungi and PGPR affecting plant behavior under stress. Accordingly the activities of soil microbes are a great indicator of soil response under stress, as the responses of soil microbes to stress are quick (Fernández et al. 2012).

### *6.5.3 Heavy Metals*

Due to the increased industrial activities of human, the concentrations of heavy metals have increased in the environment adversely affecting plant and microbial activities. Some heavy metals such as iron, zinc, manganese, and copper are essential for plant growth and microbial activities. However, at higher concentrations such heavy metals may adversely affect plant growth. The other heavy metals, including cobalt, cadmium, vanadium, chromium, etc., can also have negative effects on plant growth and microbial activities. The unfavorable effects of heavy metals on plant growth and yield include the decreased uptake of essential nutrients and disruption of enzymes activities (Forgy 2012; Zaidi et al. 2012).

Different methods including physical, chemical, and biological ones have been used for the alleviation of heavy metals on the growth of plant and on the environment. In the physical methods: (1) a new soil is used and mixed with the contaminated soil, (2) the contaminant is diluted, and (3) a lot of clean soil is mixed with the contaminated soil (Zhou et al. 2004; Yao et al. 2012). In the chemical method: (1) the soil is leached using water and chemicals, (2) the contaminants are fixed (insoluble, or with little solubility) using chemicals, and (3) the contaminant is volatilized or analyzed using heat at 1400 °C (Xia and Chen 1997; Zhou et al. 2004; Ehsan et al. 2007; Fu 2008).

The biological ones include the use of: (1) bioorganic products, which are able to absorb heavy metals in the environment, (2) tolerant plants and specifically hyperaccumulators, which are able to absorb high rate of heavy metals, while their growth remains unaffected, and (3) soil microbes (Shen and Chen 2000; Yao et al. 2012). Four types of plants have been recognized with respect to their response under heavy metal stress: (1) metal sensitive species, (2) metal resistant excluder species, (3) metal tolerant non-hyperaccumulator species, and (4) metal hypertolerant hyperaccumulator species.

Such plants utilize different molecular mechanisms and signaling pathways to tolerate/resist the stress and alleviate it to a tolerable level. By the process of metal homeostasis including the regulation of the pathway related to the production of reactive oxygen species, plants adjust their response to the stress. The generation of reactive oxygen species and the related signaling pathways are important parameters affecting metal detoxification and hence plant tolerance under stress (Lin and Aarts 2012).

Bioorganic products can remove heavy metals from the environment; however, such a method may be expensive and laborious. Use of hyperaccumulators is among the most suitable methods for treating the stress of heavy metals. Such plants are able to absorb high rate of heavy metals without affecting their growth. The important point about hyperaccumulators is that most of them are not able to develop a symbiotic association with their host plant, which is due to the production of some organic products by their roots. It can importantly be among the research subjects that how it is possible to establish and enhance the symbiotic ability of hyperaccumulators with mycorrhizal fungi, especially under the stress of heavy metals (Miransari 2011b).

Different mechanisms are used by plants to alleviate the heavy metal stress including the stabilization, volatilization, degradation, and absorption of heavy metals. Mycorrhizal fungi are able to alleviate the stress of heavy metals on the growth of the host plant. The fungi are able to absorb significant amounts of heavy metals in their hyphae and hence make the host plant grow more efficiently. The two most important mechanisms by which the AM fungi are able to alleviate the stress of heavy metals on the environment (bioremediation) are phytoextraction and phytostabilization, which can increase plant uptake of heavy metals and hence their allocation to different parts of the plant (Forgy 2012; Zaidi et al. 2012).

Using 19 different experiments, Audet and Charest (2008) determined the method of metal allocation to plant roots and aerial parts. They calculated the related parameters using different metals and for the plant species belonging to Fabaceae, Brassicaceae, Solanaceae, and Poaceae. They accordingly indicated that more plant biomass and higher concentration of heavy metals are allocated to the roots, which make the aerial parts of the plant survive under stress. Such a response by plant is dependent on plants species, especially their rate of growth, which indicates their alleviating strategies.

Feng et al. (2013) investigated the role of nanoparticles including silver and iron oxide in affecting plant growth by mycorrhizal fungi. Silver at the concentration of 3.2 mg/kg significantly decreased the growth of mycorrhizal plant by 34 %, which was due to the reduction of glomalin content and uptake of nutrients by the host plant roots. With increasing the concentration of silver the ability of the fungi to alleviate the stress of heavy metal enhanced by decreasing silver concentration and the activity of antioxidant enzymes in plants. The authors indicated that the behavior of nanoparticles in soil is complicated and more investigations are essential to show their effects on the growth of plant and the symbiotic microbes.

Lebeau et al. (2008) investigated the effects of soil microbes including AM fungi and PGPR on the bioremediation of soil polluted with heavy metals using the process of Bioaugmentation-assisted phytoextraction. They used hyperaccumulators and non-hyper-accumulators and indicated their effect on the stress of heavy metal alleviation by determining (1) heavy metal concentrations and the amounts absorbed by plant, (2) the method of heavy metal allocation and bio-concentration, and (3) plant biomass.

The results by Lebeau et al. (2008) indicated that the process of bioaugmentation by soil microbes resulted in a two- and fivefold increased metal concentration and amount, respectively, by the plant aerial part, without significant differences between the fungi and PGPR. They accordingly suggested that (1) such responses and effects must be tested using more field experiments and different combination of plant and microbial species with similar experimental methods, (2) the use of appropriate plant and microbial species are essential to enhance the process of bioaugmentation and microbial survival, and (3) efficient microbial consortium may be used for the bioremediation of soils polluted with multi heavy metals.

### *6.5.4 Compaction*

Due to the high use of agricultural machinery, especially at high soil moisture, the field soil becomes compacted. Under compaction stress the number of soil macropores decreases and the number of micropores increases. The circulation of air in the soil reduces and as a result plants face oxygen deficiency and hence plant leaf turn pale, and due to decreased plant growth plants become stunt and the plant yield reduces; the production of cluster roots is also the result of soil compaction (Chen et al. 2014). The emission of greenhouse gases including N from the soil reduces the available N in the soil and hence decreases plant growth. Soil compaction also decreases the growth and activity of soil microbes (Miransari et al. 2006, 2008, 2009a, b; Miransari 2013).

The stress of compaction must be alleviated so that plant can grow and produce yield. Although mechanical methods such as using subsoiler have been tested for the alleviation of compaction stress (Chen et al. 2014), such methods are expensive and laborious. Hence, the use of biological methods such as using mycorrhizal fungi have been tested and proved to be effective on the growth and yield of plants under field and greenhouse conditions.

Miransari et al. (2006, 2008, 2009a, b), Miransari (2013) hypothesized and proved that with respect to the properties of mycorrhizal fungi under stress, it is possible to alleviate the stress of compaction on the growth and yield of corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) under field and greenhouse experiments. Two field and two greenhouse experiments were conducted and compacted soil was treated with different species of mycorrhizal fungi.

In the field experiments tractor passes were used to compact the soil at different levels. After compacting the soil corn seeds were treated with the propagule of mycorrhizal fungi and during the corn growth soil properties such as bulk density and resistance were determined. Moreover, during the growing season, corn leaf was collected to be analyzed for the uptake of different nutrients. Plant height was also determined for different treatments. At harvest corn yield was determined. Interestingly, mycorrhizal fungi were able to alleviate the stress of compaction on the growth and yield of corn, although the alleviating effects of fungi decreased with increasing the level of compaction.

Under greenhouse conditions both corn and wheat plants were tested for their tolerance under compaction stress and the uptake of nutrients was also determined. The fungi were able to significantly increase plant growth and nutrient uptake under compaction and root growth and phosphorous uptake were the parameters, which were affected the most by the fungi and hence significantly enhanced plant growth under compaction stress.

It has been interestingly indicated that under compaction stress while the production of enzymes such as urease decreases the production of phosphatase increases (Pupin et al. 2009). The authors also mentioned that under compaction stress the population of fungi is stimulated, but the population and activities of bacteria including the nitrifying bacteria decrease.

Siczek and Frac (2009) investigated the effects of soil compaction and straw mulching on the activities of soil microbes under field conditions. Field soil was subjected to different levels of compaction using mulch and control treatments. The total number of bacteria and the activities of enzymes such as protease and phosphatase were determined. While under high level of compaction the activity of enzymes decreased, under medium level of compaction such activities increased related to the control treatment. Mulch treatment enhanced the number of bacteria and stimulated the activity of enzymes under compaction stress.

## 6.6 Conclusion and Future Perspectives

Arbuscular mycorrhizal fungi are among the most important soil microbes affecting plant growth and yield production under different conditions including stress. As a symbiotic and beneficial microbe to their host plant, they are able to alter plant physiology in such a way that the host plant can survive the stress. Different mechanisms are used by the fungi to alleviate the stress such as the increased uptake of water and nutrients by the host plant, production of different biochemicals by the fungi and the host plant roots, interaction with other soil microbes, etc. In this chapter some of the most important and most recent details related to the effects of mycorrhizal fungi on the growth of their host plant under stresses such as salinity, drought, heavy metals, and compaction have been presented. Such details include the responses of mycorrhizal and non-mycorrhizal plants under stress by using different mechanisms and signaling pathways. Although the fungi are nonspecific symbionts to their host plant, the use of appropriate fungal species with the right plant species may enhance their alleviating potentials under stress. Future research may further investigate the potential of mycorrhizal fungi under stress and how such potentials may be enhanced by modifying the related molecular and signaling pathway. The host plant may also be modified so that its symbioses with the fungi may result in a higher and more efficient survival under stress.

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# Chapter 7
# Taxonomic and Functional Response of Arbuscular Mycorrhizal Fungi to Land Use Change in Central Argentina

**Silvana Longo, Noelia Cofré, Florencia Soteras, Gabriel Grilli, Mónica Lugo, and Carlos Urcelay**

## 7.1 Introduction

It has been predicted that land use change will pose the main threat to biodiversity worldwide (Sala et al. 2000). A recent meta-analysis shows that, at local scale, conversion and degradation of habitats promote on average a global decline of 8.1 % of species richness and 10.1 % of abundance (Newbold et al. 2015). These human-mediated changes in biodiversity strongly affect ecosystem stability (Hautier et al. 2015). Given the importance of biodiversity on ecosystem functions and services, studies on the effects of land use on species assemblages are highly relevant in current global context.

Despite that soil is an important reservoir of biodiversity (van der Heijden et al. 2008) and that belowground communities are important drivers of aboveground communities and ecosystem processes (Wardle et al. 2004), soil biota is generally underrepresented in studies linking land use–biodiversity–ecosystem processes.

Arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) are one of the main components of the soil biota. They are present in most terrestrial ecosystems and establish obligate symbiosis with more than the 80 % of land plants (Smith and Read 2008). These fungi depend on plant photosynthetic carbon while providing

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S. Longo (✉) • N. Cofré • F. Soteras • G. Grilli • C. Urcelay (✉)
Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET,
Universidad Nacional de Córdoba, Córdoba, Argentina
e-mail: longosil@yahoo.com.ar; curcelay@imbiv.unc.edu.ar

M. Lugo
Diversidad e Interacciones Fúngicas, IMIBIO-CONICET, Universidad Nacional de San Luis,
San Luis, Argentina

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them with soil nutrients, among other benefits (Smith and Read 2008). The outcome of the plant–fungus interaction highly depends on the fungal and plant identity and the environmental context.

It has been widely documented that AMF affects plant community structure (e.g., van der Heijden et al. 1998) and ecosystem processes such as productivity (e.g., Klironomos et al. 2000), decomposition (e.g., Urcelay et al. 2011), and soil aggregation (e.g., Rillig and Mummey 2006). Then, local decline of AMF diversity under land use would have consequences on plant communities and ecosystem functioning.

It has been recognized that not only richness or diversity per se but also functional traits of species are important for understanding the response of biotic communities to land use change as well as their impacts on ecosystem processes and services (e.g., Díaz et al. 2007). This trait-based approach has also been recognized as a useful framework to understand fungal ecology (e.g., van der Heijden and Scheublin 2007; Koide et al. 2014; Aguilar-Trigueros et al. 2014).

The functional characteristics of AMF are considered phylogenetically fairly conserved (Hart and Reader 2002; Powell et al. 2009; Maherali and Klironomos 2012). Based on C–S–R triangle (competitor, stress tolerator, ruderal) framework (Grime 1979), Chagnon et al. (2013) recently assigned life history strategies to the three main lineages of AMF (Gigasporaceae, Glomeraceae, and Acaulosporaceae). Accordingly, Gigasporaceae are considered "competitors" characterized by high soil hyphal densities, late production of spores in the growing season, and higher nutritional benefits to hosts. In contrast, Glomeraceae are "ruderals" characterized by higher growth rates, higher intraradical colonization, early production of spores, and low soil hyphal densities. The higher intraradical colonization rates and lower soil hyphal densities imply less nutritional benefits to hosts (e.g., Maherali and Klironomos 2007). In turn, Acaulosporaceae is considered "stress tolerators" with low growth rates, long-lived mycelium, resistance to acidity and low temperature among other stressors, and probably more investment in constitutive defense (Chagnon et al. 2013). These life history strategies could also be useful to explain fungal assemblages in successional dynamics and their response to disturbances imposed by land use change (Chagnon et al. 2013).

In central Argentina (Fig. 7.1), land uses such as fire, grazing, and forest fragmentation are among the most important environmental changes (Zak et al. 2004). In the last 15 years, some studies were performed to assess the impact of those anthropogenic activities on AMF communities. Here we review those studies to analyze the response of AMF to land use. We particularly aimed to assess whether grazing, fire, and forest fragmentation (a) promote a decline of AMF taxonomic diversity and (b) negatively affect Gigasporaceae and Acaulosporaceae lineages' spore abundance and Glomeraceae remains unaffected as predicted by the C–S–R framework.

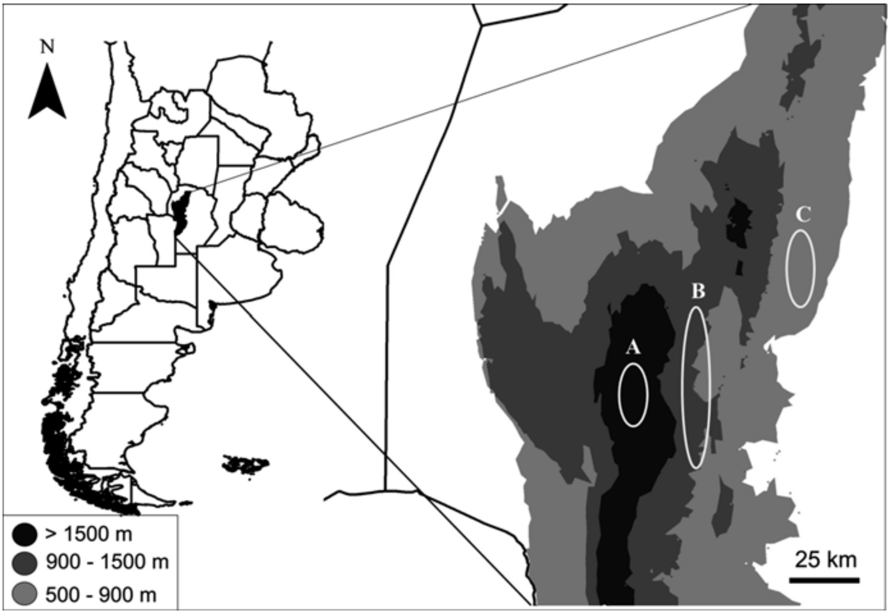

**Fig. 7.1** Location of studied areas with each land use in Córdoba, central Argentina. (**a**) Mountain Grasslands (grazing). (**b**) Chaco Serrano Forests (fire). (**c**) Chaco Forests (forest fragmentation)

## 7.2 Effects of Land Use on Diversity and Abundance of AMF Spores

To evaluate the effect of grazing on AMF spore communities, Lugo and Cabello (2002) conducted a study located in altitudinal grasslands in central Argentina (Fig. 7.2a). Six sampling sites were selected: three grazed and three ungrazed for a minimum of 20 years. Samplings were carried out over four seasons. The main findings of this study revealed no differences in spore richness, diversity, and abundance.

Instead, Longo et al. (2014) found that fire severely affects AMF diversity. In this landscape-level study, five locations were selected in Chaco Serrano Forests (Fig. 7.2b). In each location, five nearby burned and unburned sites were studied in two seasons (autumn and spring). Results showed that diversity, richness, and evenness of AMF spores consistently decreased in the five burned sites and in some cases nearly half. However, spore abundance was not significantly affected by the fire.

It has been widely documented that habitat loss promoted by human-mediated forest fragmentation strongly affects biological community's dynamics (e.g., Saunders et al. 1991). In this context, area size is an important factor affecting biodiversity (Haddad et al. 2015). Grilli et al. (2012) aimed to evaluate the relationship between area size of forest remnants and AMF spore communities at the

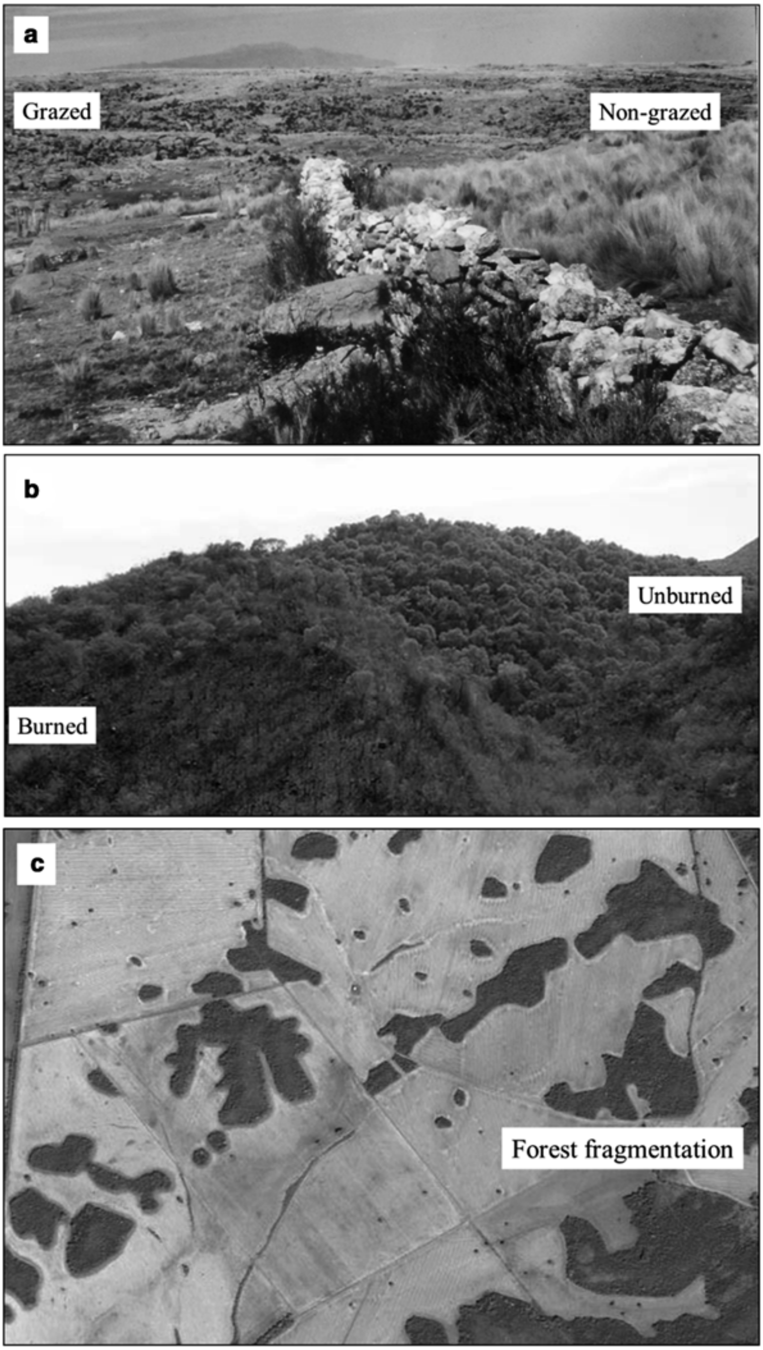

**Fig. 7.2** Photographs of the three studied land uses. (**a**) Grazing in mountain grasslands; (**b**) fire in Chaco Serrano forests; (**c**) forest fragmentation in Chaco

landscape level. To this end, eight forest fragments with different sizes (0.86–1000 ha) immersed in an agricultural matrix in the Chaco region were selected (Fig. 7.2c). In this study, diversity of spores decreased with decreasing area size, while abundance marginally did it.

In Table 7.1 we summarized the direction of the response of each species (positive, neutral, or negative) to each land use *per* sampling season in those studies. Two species occurred in more than one study: *Entrophospora infrequens* and *Scutellospora biornata*. They were negatively affected in one case (by fire in spring) but not affected in the other five. The remaining species were analyzed within each particular study.

Grazing significantly decreased the abundance of *Scutellospora* sp. in one out of four seasons (winter). In contrast, the following species increased in response to grazing in one season: *Acaulospora laevis* (winter), *A. mellea* (autumn), and *Glomus* sp. (autumn). The remaining species showed neutral response to grazing.

Fire significantly decreased the abundance of several species. *Acaulospora rehmii*, *A. scrobiculata*, *Gigaspora gigantea, Gigaspora* sp. 1, and *Gigaspora* sp. 3 consistently decreased their abundance in burned sites in both seasons. In turn, *Ambispora jimgerdemannii*, *Entrophospora infrequens, Dentiscutata heterogama, Scutellospora biornata,* and *Scutellospora* sp. declined with fire in spring, while *Racocetra gregaria* and *Acaulospora* sp. 1 did it in autumn. In contrast, *Septoglomus constrictum* consistently increased in abundance in burned sites over both seasons, while *Glomus* sp. 4 and *Glomus* sp. 5 only did it in spring.

In fragmented forest, three unidentified species of *Glomus* significantly declined their abundance with decreasing area size. The remaining species were not affected.

These results show that the response of AMF depends on the land use type. While fire and reduction of area size seem to strongly decrease AMF diversity, no important differences between grazed and non-grazed sites were observed.

In comparison to fire and forest size reduction, grazing might not strongly affect soil properties and plant diversity. Indeed, despite that livestock grazing removes plant biomass they increase plant diversity in the studied mountain grasslands (e.g., Pucheta et al. 1998). In contrast, fire imposes great impacts to soils such as reduction or elimination of the aboveground and belowground biomass, loss of soil organic horizon, and increase in soil temperature and ash deposition (Neary et al. 1999; Certini 2005), at least in the short term (Neary et al. 1999). Regarding forest fragmentation, it has been shown in the studied area that reduction in area size is accompanied by a decrease in plant diversity (Cagnolo et al. 2006). Then, different impacts on soil properties and plant communities might explain the differential response of AMF communities to the studied land uses.

When looking at particular AMF, several species were not affected by land use while others consistently decreased or increased. Examples of these last cases are some *Gigaspora* species that decreased their abundance in burned sites in both seasons, while *Septoglomus constrictum* behaved in the opposite way (Table 7.1). These species could be considered as indicators of land use in the studied region (Oehl et al. 2003).

**Table 7.1** Response of AMF spore species to land use (+: increased, −: decreased, and =: did not change)

| *Land use type* | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Fire spring | Fire autum | Grazing summer | Grazing autum | Grazing winter | Grazing spring | Area size |
| **Acaulosporaceae** | | | | | | | |
| *Acaulospora cavernata* | = | = | | | | | |
| *A. laevis* | | | = | = | + | = | |
| *A. mellea* | | | = | + | = | = | |
| *A. rehmii* | - | - | | | | | |
| *A. scrobiculata* | - | - | | | | | |
| **Ambisporaceae** | | | | | | | |
| *A. jimgerdemannii* | - | | | | | | |
| **Claroideoglomeraceae** | | | | | | | |
| *Claroideoglomus claroideum* | = | = | | | | | |
| **Entrophosporaceae** | | | | | | | |
| *Entrophospora infrequens* | - | = | = | = | = | = | |
| **Gigasporaceae** | | | | | | | |
| *Dentiscutata heterogama* | - | = | | | | | |
| *Gigaspora gigantea* | - | - | | | | | |
| *Racocetra gregaria* | = | - | | | | | |
| *Scutellospora biornata* | - | = | = | = | = | = | |
| **Glomeraceae** | | | | | | | |
| *Glomus aggregatum* | = | = | | | | | |
| *G. dimorphicum* | | | = | = | = | = | |
| *G. fuegianum* | | | = | = | = | = | |
| *Sclerocystis coremioides* | = | = | | | | | |
| *Sclerocystis rubiformis* | | | = | = | = | = | |
| *Septoglomus constrictum* | + | + | | | | | |
| **Unidentified (Longo et al. 2014)** | | | | | | | |
| *Glomus* sp. 1 | = | = | | | | | |
| *Glomus* sp. 2 | = | = | | | | | |
| *Glomus* sp. 3 | - | = | | | | | |
| *Glomus* sp. 4 | + | = | | | | | |
| *Glomus* sp. 5 | + | = | | | | | |
| *Glomus* sp. 6 | = | = | | | | | |
| *Glomus* sp. 7 | = | = | | | | | |
| *Glomus* sp. 8 | = | = | | | | | |
| *Glomus* sp. 9 | = | = | | | | | |
| *Glomus* sp.10 | = | = | | | | | |
| *Glomus* sp. 11 | = | = | | | | | |
| *Claroideoglomus* sp. | - | = | | | | | |

(continued)

**Table 7.1** (continued)

| *Land use type* | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Fire spring | Fire autum | Grazing summer | Grazing autum | Grazing winter | Grazing spring | Area size |
| *Acaulospora* sp. 1 | = | - | | | | | |
| *Acaulospora* sp. 2 | = | = | | | | | |
| *Acaulospora* sp. 3 | = | = | | | | | |
| *Gigaspora* sp. 1 | - | - | | | | | |
| *Gigaspora* sp. 2 | = | = | | | | | |
| *Gigaspora* sp. 3 | - | - | | | | | |
| *Scutellospora* sp. | - | = | | | | | |
| **Unidentified (Grilli et al. 2012)** | | | | | | | |
| *Glomus* sp. 1 | | | | | | | = |
| *Glomus* sp. 2 | | | | | | | - |
| *Glomus* sp. 3 | | | | | | | = |
| *Glomus* sp. 4 | | | | | | | = |
| *Glomus* sp. 5 | | | | | | | = |
| *Glomus* sp. 7 | | | | | | | - |
| *Glomus* sp. 8 | | | | | | | = |
| *Glomus* sp. 9 | | | | | | | = |
| *Glomus* sp. 10 | | | | | | | = |
| *Sclerocystis* sp. | | | | | | | - |
| *Gigaspora* sp. 1 | | | | | | | = |
| *Gigaspora* sp. 2 | | | | | | | = |
| **Unidentified (Lugo and Cabello 2002)** | | | | | | | |
| *Acaulospora2* | | | = | = | = | = | |
| *Acaulospora3* | | | = | = | = | = | |
| *Glomus* spp. | | | = | + | = | = | |
| *Glomus* sp. 7 | | | = | = | = | = | |
| *Glomus* sp. 3 | | | = | = | = | = | |
| *Scutellospora* sp. | | | = | = | - | = | |

## 7.3 Effects of Land Use on AMF Spores: A Functional Group Approach

Arbuscular mycorrhizal fungi were grouped into three functional groups according to their traits (*sensu* Chagnon et al. 2013): Gigasporaceae, Acaulosporaceae (Acaulosporaceae + Ambisporaceae + Entrophosporaceae), and Glomeraceae (Claroideoglomeraceae + Glomeraceae + Pacisporaceae). Then, the number of cases in each functional group that evidenced negative, neutral, or positive response to land use was computed and a Chi-square analysis on absolute frequency was applied (Table 7.2).

**Table 7.2** Number of positive, neutral, and negative response cases regarding the spore abundance of species belonging to three AMF functional groups

| Functional group | Land use effect | | |
|---|---|---|---|
| | Positive | Neutral | Negative |
| Glomeraceae | 5 | 53 | 5 |
| Acaulosporaceae | 2 | 26 | 7 |
| Gigasporaceae | 0 | 15 | 11 |
| Total | 7 | 94 | 23 |

Glomeraceae includes Claroideoglomeraceae, Glomeraceae, and Pacisporaceae. Acaulosporaceae includes Acaulosporaceae, Ambisporaceae, and Entrophosporaceae; see Table 7.1 for more details

The AMF functional groups were differentially affected by land use ($X_2 = 37.69$; $p = 0.0001$). Specifically, Glomeraceae was mainly unaffected (84 % of analyzed cases) and the few positive and negative responses were equally distributed in the remnant cases. In turn, 74 % of Acaulosporaceae revealed neutral responses, while 20 % and 6 % were negative and positive, respectively. In contrast, Gigasporaceae showed 58 % of neutral and 42 % of negative response cases.

It is worth to highlight that Glomeromycota as a whole seem to be fairly resistant to land use since spore abundance was mostly unaffected. Nevertheless, there were clear differences between functional groups.

In line with the C–S–R framework for strategies in AMF (see Fig. 1.1 in Chagnon et al. 2013), Gigasporaceace and Glomeraceae represented opposite trends in their response to land use. This is consistent with the competitor–ruderal life history axis, closely related to environmental conditions caused by disturbance intensity. In turn, Acaulosporaceae showed to be in mid position of the axis. This is not fully consistent with the scheme adapted by Chagnon et al. (2013).

The majority of Glomeraceae seems to be resistant to disturbance posed by land use such as fire, grazing, and forest fragmentation. This is consistent with their "ruderal" life strategy which is characterized by rapid growth, capacity to fuse hypha and restore integrity of the mycelium in soil, and earlier investment in spore production (Chagnon et al. 2013). On the other hand, an important proportion of cases in Gigasporaceae reveal that this group is particularly sensitive to disturbance and would behave as "competitors." They need to allocate large quantities of carbon to extraradical mycelia for soil exploration and sporulate later in the growing season (Chagnon et al. 2013). These traits make this functional group fairly incompatible with the disturbances generally associated with land use. The results suggest that Acaulosporaceae have traits situated in between "ruderal–competitor" axis, but this remains to be explicitly measured.

Overall, these results reveal that trait-based approaches, in particular the C–R–S framework, provide useful insight for ecological understanding of AMF ecology in face of global change.

## 7.4 Conclusions and Future Directions

Altogether, the results analyzed here reveal that land uses in central Argentina (grazing, fire, and forest fragmentation) tend to negatively affect AMF diversity, mainly those human activities that involve severe soil damaging and decrease plant diversity such as fire and forest fragmentation. When considering AMF functional groups, Glomeraceae seems to be resistant to these land uses, while Gigasporaceae seems to be fairly sensitive. It could be predicted that these changes in AMF taxonomic diversity and functional traits would have consequences on successional dynamics and ecosystem processes. For example, it has been shown that Glomeraceae invests more in intraradical colonization than extraradical mycelium and this may imply less nutritional benefits to plant hosts. In contrast, Gigasporaceae largely invests in extraradical mycelium allowing for better exploitation of soil resources, particularly P, thus enhancing benefits to plant hosts (Maherali and Klironomos 2007). In addition, the large amounts of soil mycelium might promote better soil aggregation through glomalin secretion by these fungi (Rillig and Mummey 2006).

Further studies using trait-based approaches would be useful to test hypothesis regarding the AMF-mediated effects of land use on successional dynamics. This seems to be a fruitful way forward to gain knowledge for making predictions about the consequences of global changes on plant community dynamics and ecosystem functioning.

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# Chapter 8
# Mycorrhizas in Agroecosystems

**Marcela C. Pagano, Belchior L. Dantas, Olmar B. Weber, Eduardo A. Correa, Fabio D. Tancredi, Neimar F. Duarte, Alberto Bago, and Marta N. Cabello**

## 8.1 Introduction

The agricultural expansion has led to an increase in the irrigated cropland area and the use of fertilizers enormously, thus generating water degradation, increased energy use, and common pollution (Foley et al. 2011). Moreover, dependence on soils for food and fuel production increases impacts of anthropogenic activities on soils such as warmer temperatures and altered plant allocation belowground due to elevated atmospheric $CO_2$ concentrations (Luo et al. 2006; Torn et al. 2015).

Of particular concern is the increased interest to study the water consumed in agricultural activities and to reduce the environmental impacts in the agricultural and livestock. High quantities of water are dedicated to irrigation in addition to the fact that rain-fed agriculture is the world's largest user of water (Foley et al. 2011). In Brazil, for example, c. 40 % of water is wasted after use by any agricultural activity.

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M.C. Pagano (✉)
Federal University of Minas Gerais, Belo Horizonte, Brazil
e-mail: marpagano@gmail.com

B.L. Dantas • O.B. Weber
EMBRAPA Agroindústria Tropical, Fortaleza, Ceará, Brazil

E.A. Correa • F.D. Tancredi
EPAMIG—URECO Fazenda Experimental de Pitangui, Pitangui, Minas Gerais, Brazil

N.F. Duarte
Instituto Federal de Minas Gerais, Belo Horizonte, Brazil

A. Bago
Estacion Experimental del Zaidin (CSIC), Granada, Spain

M.N. Cabello
Instituto Spegazzini (Fac. Cs Nat y Museo, UNLP), aven 53 # 477,
Comisión de Investigaciones Científicas de la Prov., Buenos Aires, Argentina

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Only at this time we are truly recognizing the importance of organic farmland cover and of volume of water stored in soils and vegetation. Nowadays, a whole management approach to understand surface and deep soil responses to global change is needed (Torn et al. 2015). A network of integrated manipulation experiments on soils was recently proposed in order to synthesize information and benefits especially to increase the resilience of soils (including the soil profile) and the soil organic matter stocks (Torn et al. 2015). It is known that crop rotation is an alternative to manage and treat soil-borne pathogens (Reeleder 2003), but increase in crop diversity is restricted and this usually results in crop yield decline (Bennett et al. 2012). However, creating biotic soil environments that promote plant health may contribute to improving the sustainability of food production (Chaparro et al. 2012). For example, susceptible crop plants do not develop diseases in certain soils (Mazzola and Gu 2000) being promising to use them to engineer the cultivable soil microbiome (Ellouze et al. 2013).

In the beginning of the twenty-first century, new alternatives for the management arose, such as inter-cropping, tillage, and organic amendments, which affect soils' physical and chemical properties, modifying the abundance, diversity, and activity of the soil microbiota including symbiotic fungal populations (Cardoso and Kuyper 2006; Kahiluoto et al. 2009; Nyfeler et al. 2011; Pagano et al. 2011).

As part of the soil biota and colonizing plant roots, the Arbuscular mycorrhizal fungi (AMF) link the biotic and geochemical components of the ecosystems providing capital ecosystem services. Research on Mycorrhizas has gone through several +stages (Stürmer 2012); however, the present period has revolutionized research on these fungi. Thus, the importance of AMF for soil health is nowadays recognized (Bradford 2014) and the association of trees with different mycorrhizal fungi is highlighted to understand the biotic interactions in global carbon dynamics (Averill et al. 2014). Therefore, it was urgently recommended to study their function under global change (Kivlin et al. 2013). This chapter discusses advances in mycorrhizal fungi potential in agroecosystems drawing on recent results of research worldwide and with special attention to new perceptions.

## 8.2 The Mycorrhizal Symbioses in Agroecosystems

Microorganisms are intensively investigated for novel compounds from saprophytic terrestrial fungi to marine habitats and living plants with their endophytes (Schueffler and Anke 2014). A growing worldwide attention on fungi is noted, as of 100,000 known fungal species more than one million are predicted to exist (Schueffler and Anke 2014). Among soil microorganisms, AMF are of special interest for agriculture and increasing investigation worldwide is continuously reported (Stürmer and Siqueira 2006; Pagano and Covacevich 2011; Wetzel et al. 2014).

As regards the AMF occurrence in agroecosystems, more information on indigenous mycorrhizal fungi is needed as well as enough understanding of inoculum persistence. An example by Astiz et al. (2014) showed differences in the indigenous mycorrhizal fungi tested in maize (See below, Sect. 8.3). Additionally, it is known that cover crops favor the indigenous AMF by means of active roots in the period when the soil has no crop (see Douds et al. 2005).

The roles of AMF and their interactions with other microorganisms in maintaining soil fertility and biocontrol of plant pathogens in sustainable agriculture are still poorly understood (Johansson et al. 2004). It is known that AMF may improve or decay yield in monoculture crops. However, Bender and van der Heijden (2015) confirmed that maize and wheat inoculated with soil organisms $\leq 2$ mm, including AMF, increase crop nutrient uptake and plant yield. Compared to grasslands, conventionally managed fields present low AMF diversity; however, organically managed fields are more similar to natural grasslands (Verbruggen et al. 2010). AMF spore abundance is also reduced (Oehl et al. 2003, 2004) as shown in the agroecosystems of Central Europe. One reason can be reduction of AMF diversity along with reduced diversity of host plants (Bennett et al. 2012). The impact of different agricultural practices on AMF in arable fields is also still poorly understood (Verbruggen et al. 2010; Oehl et al. 2010).

Spore populations of AMF communities in arable fields can vary from just one to 50 spores $g^{-1}$ soil (Sjöberg et al. 2004; Oehl et al. 2010), but it also depends on the soil type. Rarely more than 26 AMF species were reported in field studies (Schalamuk and Cabello 2010). Generally, Acaulosporaceae, Gigasporaceae, and Glomeraceae can be found in agricultural fields; however, *Glomus* predominate (Oehl et al. 2003, 2005; Schalamuk and Cabello 2010). This can lead to think on different types of AM inocula based on the proportions of their AM families (Acaulosporaceae, Gigasporaceae, Glomeraceae) between field and trap cultures. For instance, Czerniak and Stürmer (2015) tested two AMF species of different families, such as Gigasporaceae and Glomeraceae (*Dentiscutata heterogama* and *Claroideoglomus etunicatum*, respectively) in on-farm production of inoculum.

In the trap cultures from agroecosystems, more than 90 % of AM species belong to Glomeraceae (Oehl et al. 2005; Schalamuk and Cabello 2010). It is known that *Glomus* spp. (Glomeraceae) present more extensive root colonization than other families and lower soil colonization by extraradical hyphae besides rapid colonization of new plants also from colonized root fragments (Hart and Reader 2002). Thus, in the trap cultures from crop systems generally *Glomus* or *Acaulospora* species are recovered (Oehl et al. 2003).

AMF density and distribution vary both spatially and temporally within and between species, with soil types and with host plant species diversity. Investigating field samples in 16 sites around the plain of the upper Rhine valley, Oehl et al. (2010) found higher AMF species richness (58 taxa). Interestingly, Oehl et al. (2005) began detailed investigation of AMF through soil depth. They showed that AMF communities in deep soil layers are diverse and different from the topsoil. The species richness decreased in grasslands and in vineyards continuously with increasing soil depth. By contrast, in the maize fields the highest richness was found below plowing depth. Therefore, they stressed that deep soil layers should be included in studies to get a complete picture of AMF diversity. At present, research significantly expounding upon the results of the studies on crops, especially corn, has increased and new reports are continuously seen (Miransari 2013; Gomes et al. 2015).

In Chile, 24 AMF species were associated with different cultivars of *Triticum aestivum* and, differently, *Acaulospora* and *Scutellospora* predominate. In that study, AM fungal community structure differed among wheat cultivars: “Porfiado” and “Invento,” with 19 species in relation to “Otto” cultivar (15 species) (Aguilera et al. 2014).

In Argentina, earlier studies have found less management of AMF in order to increase plant productivity (Covacevich and Echeverría 2009). It is known that soils of the Pampas region present high native AMF that colonize crop plants under different management systems (Covacevich et al. 2006, 2007; Schalamuk et al. 2006; Covacevich and Echeverría 2008); however, they are not yet manipulated. More recent research on the impact of agricultural practices on AMF symbioses pointed to a selective decrease of viable spore number with glyphosate applications (Druille et al. 2015). Those authors evaluated the number and viability of AMF spores and changes in AMF species composition in native grasslands in the northeast of the Flooding Pampa, to face decreases in the grassland productivity, which leads to decline in livestock production. The determination was achieved primarily in the entire community and subsequently in the four dominant species, resulting in altered AMF community structure. However, the use of sublethal doses of the herbicide was more useful contributing to project sustainable land management agroecosystems (Druille et al. 2015).

In Southern Brazil, increasing studies of AMF in experimental farms and fruit plant orchards have extended the panorama of investigation with this type of soil fungi. Reports on AMF diversity in fruit orchards of Blueberry cultivars showed the prevalence of species of *Glomus* and *Acaulospora* and the potential benefit from inoculated AMF such as *Gigaspora margarita* and *Glomus etunicatum* (Farias 2012). In the semiarid region, Dantas et al. (2015) investigated the AMF occurrence in the establishment of fruit plants (pineapple, Sapota trees) under organic management (Fig. 8.1), detecting *Glomus* spores in all the areas and corroborated the fact that soil management in organic cropping systems reduced the AMF species richness and abundance in relation to natural vegetation areas.

The AMF occurrence was investigated in an experimental farm in Minas Gerais State (Fig. 8.2), where plots with corn planted in the spacing of 0.8 m between rows were established in an Oxisol (USDA Soil Taxonomy 2006). Spores of *Glomus* and *Acaulospora* were predominantly retrieved from crop field; however, the preserved adjacent Atlantic forest and sites at initial stage of regeneration presented more diverse AMF spores (Azevedo, unpublished data). High diversity and abundance were related to adjacent native forest.

Lastly, the work of Wetzel et al. (2014) merits discussion as they observed changes in AM fungal community structure and diversity in a long-term field experiment (wheat continuous crop rotation with sugar beet, and *Sinapis alba* as a cover crop). They compared results obtained by sequence analysis as well as by morphological spore identification, resulting in better detection of changes in community composition and diversity through the morphological methods.

**Fig. 8.1** Cultivated areas in Ceará, Brazil. Clockwise, from *upper left*: pineapple and sapota tree cv. BRS 228, colonized roots of *Cocos nucifera* and AMF spores of *Scutellospora* and *Glomus* isolated from soils (Photo-credit: B. Dantas and M. Pagano)

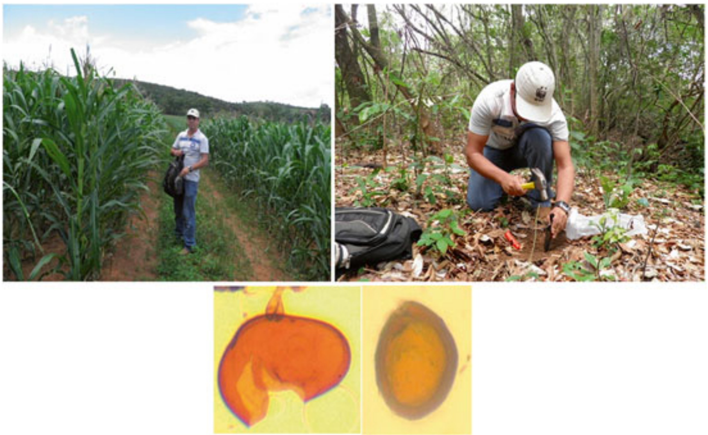

**Fig. 8.2** Cultivated areas with corn, in Minas Gerais, Brazil. Clockwise, from *upper left*: cultivated plot, preserved native forest, AMF spores of *Glomus* and *Racocetra* found in corn plantation and adjacent native Atlantic forest (Photo-credit: E. Azevedo and M. Pagano)

## 8.3 AM Inoculation Technology

The management of AMF in the rhizosphere provides an alternative to high inputs of fertilizers and pesticides in sustainable plant production systems [Reviewed by Gianinazzi and Schüepp (1994) and Azcón-Aguilar and Barea (1997)]. Moreover, crop yield increases showed the potential to be used by farmers (Douds et al. 2005). However, AM inoculation technology is limited by the lack of production of commercial inocula, because AMF cannot be multiplied on artificial growth media without a host (Sieverding 1991).

Since 1997, reviews such as by Azcón-Aguilar and Barea stressed the lack of better technology for commercial horticultural mycorrhizal inoculum. They suggested a cautious choice of compatible host/mycorrhiza/substrate combination for crop success. As AMF cannot be produced in large-scale containers due to recalcitrance to pure culture, many methods are used to handle these fungi, inoculating them on host plants, and replicating large amounts of inoculum. In this way, in vivo cultures of species from different regions are maintained in ex situ collections (Giovannetti and Avio 2002). For that purpose, the spores are inoculated nearby the roots of a host plant cultivated in soil, sand, expanded clay, peat, or other substrates (after sterilization by steam, fumigation, or irradiation). In general, new spores are produced in the pot cultures 3 months after inoculation (see Giovannetti and Avio 2002). Observations under microscope of stained fungal structures in the roots are indicated to appraise the percentage of mycorrhizal colonization and to check for fungal pathogens or nematodes. This is necessary before any further utilization of the pot culture, called "crude inoculum." If no contamination is present, ~20 g of that inoculum can be used for another cycle. More quantity of inoculum may be produced in around 6 months. Other techniques have been developed to produce large quantities of soil-free inoculum, based on hydroponic and aeroponic cultivation systems (Jarstfer and Sylvia 1995). The roots transformed by *Agrobacterium rhizogenes* are also effective as inocula which generally utilized carrot, but they are generally used as experimental model systems for research purposes (See Giovannetti and Avio 2002). But these inoculation procedures are highly expensive and only utilized in agriculture of high value products.

It has become customary to use AM spores as inoculum (Read 2003), and using three representative genera of AMF (mixed inocula) is a common inoculation strategy. Douds et al. (2005) indicate that a multispecies inoculum by using materials easily available to farmers will reduce costs compared to commercially inocula. Several works showed the feasibility and importance of AM inoculation in a large number of horticulture, fruit, and ornamental micropropagated plants (Kumari et al. 2005). The selection of appropriate fungal endophytes plays a fundamental role in preventing growth after transplant (Requena et al. 2001). Plant micropropagation can presently benefit from AM biotechnology, an appropriate and necessary tool.

The applications of mycorrhizas in agriculture and environmental issues are still incipient. AMF inoculant for farm application requires large-scale multiplication fungi, which is generally carried out in substrate-based or in vitro systems (Ijdo et al. 2011). Commercial inocula exist, but often these inoculants do not work or contribute satisfactorily, especially under field conditions (Weber 2014). As large-scale production of AMF has disadvantages in terms of inoculum certification and quality, in vitro production may confer more advantages especially for early inoculation on either seed or seedlings (Bago and Cano 2006). In this sense in some countries such as Spain ultrapure inoculants named GLOMYGEL® are on sale for different cultures. Those inoculants contain indigenous AMF species adapted to similar soil conditions (http://www.mycovitro.com/). In Germany, only *Rhizophagus* *irregularis* is offered by SYMPLANTA®, but other AMF may be provided on demand. The inoculum is based largely on fungal spores (some root fragments can be present) also produced in vitro (http://www.symplanta.com/).

The expensive technology of inoculum production comprises formation of single cultures of AMF. A cheaper method is the on-farm system (farmers on their own property can produce inocula) (Douds et al. 2008, 2010). Both indigenous and introduced AMF can be included; however, native AMF can be more efficient due to local adaptation to the environment (Sreenivassa 1992). Infective propagules of AMF (spores, hypha, and colonized roots) can be used as inocula (Sieverding 1991). Some experts tested the production of AMF inoculum using lignocellulosic agrowastes (biofertilizers) using the on-farm method. Pulp sludge residues exhibited great potential for use together with *Dentiscutata heterogama* obtaining higher spore abundance, root colonization, and infective propagules (Czerniak and Stürmer 2015). Moreover, those authors confirmed the probable use of Gigasporaceae as inoculants.

Maize crop in Argentina is, after soybeans, the second most important crop with a planted area of about 2.4 million hectares (Calviño and Monzon 2009) followed by wheat, citrus, sugarcane, and sunflower (Boix and Zinck 2008). However, non-tillage and contemporary hybrids with high yield that accumulate crop residues affect the balance of biological and chemical cycles disturbing the P and Zn levels (Ratto and Miguez 2006). In this sense, Astiz et al. (2014) suggested that soil characteristics could be used to select potentially beneficial inocula to compensate Zn deficiency in maize. The inoculum of indigenous AMF from sites presenting different levels of P and Zn resulted in changes in root colonization by AMF and response to inoculation in both Zn uptake and dry matter production. The inoculum indigenous from a site with low P and high Zn content was the less efficient than that from agricultural soils with higher P but lower Zn content. Thus, comparison of agricultural fields with high and low soil biota abundance and diversity to assess soil biota potential when soil communities are well developed is urgently needed (Bender and van der Heijden 2015).

## 8.4 Conclusion

In this chapter, the need for more information to understand agroecosystems and soils under different management has been highlighted. The examination and use of arbuscular mycorrhizas in different crop systems has been mentioned. Throughout the chapter, the applications of mycorrhizas in agriculture were shown as still incipient. Morphological identification procedure of AMF continues to be important, although this requires a specific training and experience. Better technology for commercial mycorrhizal inoculum was developed in a few countries. Finally, this chapter argues that agroecosystems present low AMF diversity; however, organically managed fields are more similar to natural ecosystems. Consequently, further research is necessary on this field, especially regarding the applications of mycorrhizas.

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# Chapter 9
# Arbuscular Mycorrhizae in Coastal Areas

**K. Jobim, I.R. Silva, D.M.A. Assis, L.C. Vieira, D.K.A. Silva, F. Oehl, L.C. Maia, and B.T. Goto**

## 9.1 Introduction

Coastal areas represent multifunctional ecosystems (Martínez and Psuty 2004), transitional between ocean and terrestrial environments (Williams 2007). These ecosystems are globally distributed, occurring along ocean coasts, estuary, and lakes from Arctic and Antarctic to Tropical zones (Nordstrom et al. 1990). Coastal sand dunes are characterized by diverse biotic and abiotic components under influence of anthropic activities, which results in habitat loss (Clark 1996). Consequently, the flora of the coastal areas has been drawing global attention for its protection, conservation, and rehabilitation (Rodrigues et al. 2011).

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K. Jobim (✉)
Universidade Federal do Rio Grande do Norte (UFRN),
Programa de pós-graduação em Sistemática e Evolução,
Av. Salgado Filho s/n CEP 59071-970, Lagoa Nova, Natal, RN, Brazil
e-mail: khadija_jobim@hotmail.com

I.R. Silva • D.M.A. Assis • L.C. Vieira • L.C. Maia
Laboratório de Micorrizas, Departamento de Micologia,
Universidade Federal de Pernambuco, Avenida da Engenharia s/n,
Cidade Universitária, 50740-600, Recife, Pernambuco, Brazil

D.K.A. Silva
Laboratório de Microbiologia, Universidade Federal do Vale do São Francisco,
Campus de Ciências Agrárias, Rodovia BR 407, Km 12, Lote 543,
Projeto de Irrigação Nilo Coelho, s/n, "C1",
CEP 56300-990, Petrolina, Pernambuco, Brazil

F. Oehl
Agroscope, Federal Research Institute for Sustainability Sciences, Plant–Soil-Systems,
Reckenholzstrasse 191, Zürich 8046, Switzerland

B.T. Goto
Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte
(UFRN), Av. Salgado Filho s/n CEP 59071-970, Lagoa Nova, Natal, RN, Brazil

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Plant species from these environments are subject to several stressful factors, such as low water and nutrient availability (Ievinsh 2006), substrate instability by sand movements (Barbour 1992), and salinity (Wilson and Sykes 1999). In such conditions, ecological strategies that favor the maintenance of plant communities are of great importance to maintain these areas. An important ecological strategy of plant species is to associate with arbuscular mycorrhizal fungi (AMF), which play an important role in the establishment and survival of coastal dune plant communities (Rodríguez-Echeverría et al. 2008).

Arbuscular mycorrhizal fungi form a ubiquitous mutualistic association with plant roots, improving nutrient absorption, especially P (Smith and Read 2008), and favoring the aggregation of substrate particles, enhancing edaphic stability (Beena et al. 2000). The importance of AMF for the establishment and growth of plants in coastal dunes was first reported by Nicolson (1959). Since then, studies on these fungi in dunes have been conducted in temperate (e.g., Gerdemann and Trappe 1974; Błaszkowski 1993, 1994a), tropical (e.g., Beena et al. 2000, 2001; de Souza et al. 2013; da Silva et al. 2012, 2015a, b; Stürmer and Bellei 1994), subtropical (e.g., Stürmer et al. 2013), desertic (e.g., McGee and Trappe 2002; Błaszkowski and Czerniawska 2008), and continental regions (e.g., Gerdemann and Trappe 1974; Berch and Trappe 1985).

The last four decades indicate a gradual increase in the number of publications on occurrence of AMF in dune areas (especially maritime dunes). Studies in these environments have enabled to document the mycorrhizal association and the AMF community associated with the plants, in addition to the description of new taxa (Błaszkowski et al. 2012; Goto et al. 2011). Considering the importance of the AMF for the maintenance of vegetation in maritime dunes, this chapter presents a brief history and current situation of research on diversity and taxonomy of AMF in these ecosystems.

## 9.2 AMF Occurrence in Coastal Areas Worldwide

Research on AMF occurring in coastal areas is not new, and apparently one of the first publications, as mentioned by Greipsson (2002), appeared in 1900. However, only in the 1970s more attention was given to this subject. In North America, studies have been concentrated in the USA, which holds 86 % of the publications analyzed for the continent with the majority developed by Koske and collaborators during the 1970–1990 decades. The remaining works were carried out in dunes of Canada (7 %) and Bahamas (5 %). These studies covered taxonomical review and species description (Gerdemann and Trappe 1974; Ames and Linderman 1976), checklists and mycorrhizal colonization (Koske and Halvorson 1981, 1989; Sylvia 1986; Koske 1987, 1988; Koske and Gemma 1996), sporulation patterns and species interaction (Koske 1981), ecological studies focusing on the role of AMF communities in plant succession (Koske 1987; Koske and Halvorson 1989), and species distribution patterns (Friese and Koske 1991).

As contributions to taxonomy, we highlight the review of Endogonaceae by Gerdemann and Trappe (1974), who registered the occurrence of 11 AMF species in dune areas. The focus of most studies was to evaluate the occurrence and diversity of AMF in dominant plants of US coastal dunes, especially *Ammophila breviligulata* Fern. and *Ammophila arenaria* (L.) Link (Koske and Halvorson 1981; Koske 1981; Bergen and Koske 1984; Koske 1987; Friese and Koske 1991; Koske and Gemma 1997). Dune areas with predominance of *A. arenaria* presented higher density and diversity of AMF than dunes where *A. breviligulata* predominates, and among the detected species, *Acaulospora scrobiculata* Trappe stood out with a significant spore production (Koske and Halvorson 1981).

In South America, the studies are concentrated in Brazil. Most reports were performed in the states of São Paulo (e.g., Trufem et al. 1989, 1990, 1995; Trufem et al. 1994), Santa Catarina (e.g., Stürmer and Bellei 1994; Córdoba et al. 2001), Paraíba (da Silva et al. 2012; Goto et al. 2009, 2010, 2012b; de Souza et al. 2013), Bahia (Santos et al. 1995; Goto et al. 2012b), and Rio Grande do Norte (Goto et al. 2012a, b; Błaszkowski et al. 2014, 2015). In São Paulo State, Trufem (1990) registered 46 AMF species, distributed in five genera: *Acaulospora*, *Gigaspora*, *Glomus lato*, *Sclerocystis*, and *Scutellospora (lato sensu).* The predominance of *Glomus* (*lato sensu*) species in low pH contrasted with the data available in the literature at this time, which mentioned that low pH favored species of *Acaulospora*, *Gigaspora*, and *Scutellospora* (Hayman and Tavares 1985; Wang et al. 1985), and Trufem (1990) suggested the presence of physio-ecological adaptations of AMF to the different environments (Trufem 1990). Trufem et al. (1994) observed a higher number of glomerospores with increasing temperature, precipitation, and insolation. High number of glomerospores was observed in the rhizosphere of *Diodia radula* Cham. & Schlecht., *Cordia verbenacea* DC., and *Symphopappus cuneatus* Sch.Bip., with greater AMF species diversity in the rhizosphere of *D. radula* and *Smilax elastica* Griseb (Trufem 1995).

In the coast of Santa Catarina state the first report of Stürmer and Bellei (1994) revealed the species composition and seasonal variation of AMF populations associated with *Spartina ciliata* (Aiton). Muhl. Twelve species were identified, with *Gigaspora albida N.C. Schenck & G.S. Sm., Racocetra weresubiae (Koske & C. Walker) Oehl, F.A. de Souza & Sieverd., A. scrobiculata*, and *Scutellospora* sp. being the most common. In this study, there was no significant seasonal variation. Subsequently, 12 AMF species were recorded, and Gigasporaceae (current Order Gigasporales) dominated in the fixed dunes whereas Acaulosporaceae dominated in the frontal dunes (Córdoba et al. 2001). The total number of glomerospores and species richness increased with the increase of dune stabilization, suggesting that glomerospores are important in the process of ecological succession in dune ecosystems. In contrast, Cordazzo and Stürmer (2007) observed that Gigasporaceae (current Order Gigasporales) dominated the fledgling dunes, while Glomeraceae dominated the fixed dunes with *Panicum racemosum* (P. Beauv.) Spreng, in Rio Grande do Sul. Afterward, Stürmer et al. (2013) observed predominance of *R. weresubiae, Dentiscutata cerradensis (Spain & J. Miranda) Sieverd., F.A. de Souza & Oehl, and Racocetra gregaria (N.C. Schenk & T.H. Nicolson) Oehl, F.A. de Souza & Sieverd* in three areas of coastal dunes with similar AMF community structure, despite their significant geographical distance (150 km). In Bahia state coastal dunes, most plants presented mycorrhizal association, and *Glomus microcarpum Tul. & C. Tul* showed higher density and frequency (Santos et al. 1995). Studies in Paraíba state performed by de Souza et al. (2013) found predominance of glomoid and gigasporoid spores in natural and revegetated dunes, which was confirmed by da Silva et al. (2012). Besides, new species were described from these areas—*Dentiscutata colliculosa* B.T. Goto & Oehl (Goto et al. 2010), *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto, Oehl & G.A. Silva (= *Racocetra intraornata*; Goto et al. 2009), *Racocetra tropicana* Oehl, B.T. Goto & G.A. Silva (Goto et al. 2011) and *Paradentiscutata maritima* B.T. Goto, D.K. Silva, Oehl & G.A. Silva (Goto et al. 2012a). In Rio Grande do Norte state, studies that started in 2012 allowed the discovery of three new species — *Glomus trufemii* B.T. Goto, G.A. Silva & Oehl (Goto et al. 2012b), *Rhizoglomus natalensis* (Błaszk., Chwat & B.T. Goto) Sieverd., G.A. Silva & Oehl (Błaszkowski et al. 2014), and *Acaulospora ignota* Błaszk., Góralska & B.T. Goto (Błaszkowski et al. 2015).

Few studies on the ecology and taxonomy of AMF were conducted in Europe, and Poland accounted for most of them (51 %), in general under the leadership of Janus Błaszkowski. In coastal dunes, he found more than 40 species (*Scutellospora dipurpurescens* J.B. Morton & Koske, *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl, and *Glomus microcarpum* being frequently retrieved) (Błaszkowski 1993, 1994a; Tadych and Błaszkowski 2000). Other reports showed predominantly the AMF association with *A. arenaria*. Blaszkowski and Czerniawska (2008) observed *Glomus lato sensu* and *Scutellospora lato sensu* in dunes from Holland and Rodríguez-Echeverría and Freitas (2006) found *S. constrictum, Corymbiglomus globiferum* (Koske & C. Walker) Błaszk. & Chwat, and *Racocetra persica* (Koske & C. Walker) Oehl, F.A. de Souza & Sieverd. in Portugal. In recent studies, *Glomus* and *Gigaspora* were found especially in preserved dunes (Camprubí et al. 2010) and *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker, *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl and *S. dipurpurescens* in roots of *A. arenaria* (Błaszkowski and Czerniawska 2011).

More evidence came from coastal dunes in Africa, including species description (Błaszkowski and Czerniawska 2008; Błaszkowski et al. 2010, 2012). In Asia, there are increasing reports from India (Kulkarni et al. 1997; Beena et al. 2000; D'Cunha and Sridhar 2009; Kamble et al. 2012a, b, c).

Few works on occurrence and diversity of AMF in coastal areas were performed in tropical areas, in comparison with those in temperate and subtropical regions (Sridhar and Beena 2001). South America (and especially Brazil) presented higher number of registered species (83), in spite of the limited number of reports compared to Europe (72) and North America (50). Asia also presents high species richness (74 species), with most reports limited to India. This suggests that tropical coastal areas can be considered *hotspots* of AMF diversity and need to be investigated considering the importance of these fungi for ecosystem conservation. In general, *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* are the most common genera in coastal areas (Maun 2009). In South America, *Glomus* is more represented, followed by *Acaulospora* and *Racocetra*. The most reported species are *A. scrobiculata*, *Sclerocystis sinuosa Gerd. & B.K. Bakshi,* and *Acaulospora foveata Trappe & Janos* (Trufem et al. 1989, 1994; Trufem 1990, 1995; Córdoba et al. 2001; Cordazzo and Stürmer 2007; da Silva et al. 2012; de Souza et al. 2013; Stürmer et al. 2013). However, *Glomus* predominates in Brazilian ecosystems, with the exception of maritime dunes areas, where species that produce larger spores, such as those of the order Gigasporales, notably *Gigaspora* and *Scutellospora*, are commonly found (da Silva et al. 2012; Stürmer and Siqueira 2008; Stürmer et al. 2013).

## 9.3 Mycorrhizal Colonisation and Number of Glomerospores Associated with Plants in Coastal Dunes Areas

Species of Asteraceae, Convolvulaceae, Fabaceae, and Poaceae are crucial for dune stabilization (Kamble et al. 2012a; Kulkarni et al. 1997; Sridhar and Beena 2001). In such habitats specimens of these families associate with AMF and with nitrogen-fixing bacteria (Martínez and Moreno-Casasola 1996; Moreno-Casasola and Espejel 1986; Beena et al. 2001), while roots of some plant species such as *Cyperus pedunculatus (R.Br.) J.Kerns (Cyperaceae)* and *Cakile maritima Scop. (Brassicaceae)* are not colonized. Moreover, glomerospores were not found in the rhizospheres of *Sesuvium portulacastrum L. (Acanthaceae), Wedelia biflora (L.) DC. (Asteraceae), and Cyperus maritimus Poir (Cyperaceae)*, among others (Santos et al., 1995; Beena et al., 2001).

In dune areas, root colonization can vary from <10 to 100 % (Giovannetti and Nicolson 1983; Koske 1988; Kamble et al. 2012b) being influenced by seasonal variations, plant host, edaphic conditions (Giovannetti and Nicolson 1983; Greipsson 2002) and increases with dune stabilization (Koske and Polson 1984). The number of glomerospores is also variable in sand dunes, with the rhizosphere of legumes frequently showing high levels (470 spores 100 $g^{-1}$ soil in *Prosopis juliflora*) (Selvaraj and Kim 2004), but higher spore density has also been detected, for example, in the rhizosphere of the Poaceae *Festuca arundinaceae* (547 100 $g^{-1}$ soil) (Błaszkowski 1994a). Conversely, few glomerospores were found in the rhizosphere of other species such as *Juncus conglomeratus L.*

## 9.4 Conclusions

Benefits of the association between coast dune plants and AMF were described worldwide (Greipsson 2002). Some studies showed the importance of AMF for degraded land restoration (Koske and Gemma 1997; de Souza et al. 2013) and for ecological succession (Rose 1988; Córdoba et al. 2001), being indicators of environmental quality compared to impacted sites (da Silva et al. 2015a). The AMF constitute important microorganisms in dunes, as most plant species occurring in these sites form mycorrhizal association. These fungi contribute to plant establishment and are essential to maintain ecosystem services. This highlights the importance of appropriated management in coastal areas in order to preserve their biota. The high diversity of AMF in tropical regions contributes to plant diversity, and in coastal areas which are continuously under pressure, more studies are needed to improve our knowledge on mycorrhizal association, a key component of ecosystem functioning and maintenance.

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# Chapter 10
# Arbuscular Mycorrhizal Diversity from Mangroves: A Review

**James D'Souza**

## 10.1 Introduction

Mangroves are a type of coastal woody vegetation that fringes muddy saline shores and estuaries in tropical and subtropical regions (Gopal and Chauhan 2006). They grow at the interface between land and sea in tropical and subtropical altitudes where extreme conditions such as high salinity, strong winds, high temperatures, and anaerobic soils are dominant. There may be no other group of plants with such highly developed morphological and physiological adaptations to extreme conditions (Kathiresan and Bingham 2001). Based on their habitats, they are classified into two groups, viz., true mangroves (TM) and mangrove associates (MA). True mangroves are species that specifically grow in intertidal zones, while mangrove associates occur in either littoral or terrestrial habitats. They enrich coastal biodiversity, yield commercial forest products, protect coastlines, and support coastal fisheries (Kathiresan and Bingham 2001). The biodiversity of mangroves has also been of increasingly greater interest, firstly, because of the Convention on biological diversity, and, secondly, because the mangrove ecosystems are among the most threatened by the global climate changes, particularly the sea level rise (Macintosh and Ashton 2004).

More than 41 % of the world's mangroves occur in South and Southeast Asia of which Indonesia alone accounts for 23 %. A further 20 % of the total mangrove area lies in Brazil, Australia, and Nigeria (Spalding et al. 1997). While practically all mangroves occur in small patches that develop in deltaic habitats, the mangroves in the Ganga-Brahmaputra-Meghna Delta, shared between India and Bangladesh, are the largest coastal wetland system in the world. Popularly known as Sundarban,

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J. D'Souza (✉)
Department of Botany, St. Xavier'S College (Goa University), Mapusa, Goa, India

235, Bamman Bhatti, Arambol, Pernem, Goa 403524, India
e-mail: james2442@gmail.com

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they currently cover about one million ha. of area (Gopal and Chauhan 2006). Mangroves have become the center of many conservation and environmental issues because of loss of beneficial effects on the coastal environment (D'Souza and Rodrigues 2013a). Anthropological pressures such as growing industrial units along the coastlines and discharge of domestic and industrial sewage are polluting these areas. Mangroves are destroyed in many countries, due to the growing demand for timber, fuel wood, fodder, and other non-wood forest products (NWFPs) (Saenger 1982). To ensure the conservation of mangroves for environmental benefits, management of mangrove ecosystems is the need of the hour. Many studies have highlighted these problems and the conservation efforts are being considered.

Arbuscular Mycorrhiza plays a vital role in controlling and directing plant community structure (Aziz et al. 1995). Lovelock et al. (2004) observed that the additions of N and P influenced tree growth in mangrove plants. Distribution of nutrients and periodic changes in the climate affect the growth of mangroves. These microorganisms have been known to have a positive effect on the composition of mineral nutrients, especially Phosphorus (P) with low mobility (Smith and Read 2008). These fungi play a significant role in soil N and C cycles and also help in the reduction in plant uptake of phytotoxic heavy metals (Willis et al. 2013) and aid in increasing the plant diversity, alleivating osmotic stress caused by exposure to salt via accumulation of soluble sugars in root cells (Feng et al. 2002).

Ecological functions attributable to arbuscular mycorrhizal (AM) fungi include increasing plant tolerance, influencing response to severe climatic conditions and increasing plant productivity in natural plant communities (Brundrett and Kendrick 1996). In such dynamic and in fluctuating soil physicochemical parameters, it will be interesting to study the role of AM fungi. In this chapter, I will be discussing the numerous AM fungal studies in the wetlands including mangrove ecosystems mostly focusing on their diversity pattern in various water levels, sporulation, csolonization pattern, potential role of beneficial symbiosis, and how they influence ecology of other organisms in these extreme environments. The objectives of this chapter are to summarize arbuscular mycorrhizal diversity across the wetlands including mangroves ecosystems and identify dominant factors affecting this symbiotic organisms.

## 10.2 Materials and Methods

Sixteen publications were surveyed that describe AM fungal communities in the wetlands. Different factors such as study sites, plant diversity, tide levels, edaphic factor, colonization intensity, and interaction with other organism which affect AM fungal diversity are considered, and how they affect AM diversity is discussed in this review. All the abovementioned factors which were taken into account for studying AM diversity in different wetlands habitat are listed in Table 10.1.

**Table 10.1** List of major arbuscular mycorrhizal studies in mangroves habitats

| Type of ecosystem | AM morphology observed | Number of AM species recorded | Factors studied | References |
|---|---|---|---|---|
| Mangroves (China) | – | 14 | Plant diversity, study sites, and colonization intensity | Wang et al (2014) |
| Mangroves (West coast of India) | *Paris* type and *Arum* type | 21 | Plant diversity and study sites | D'Souza and Rodrigues (2013a) |
| Mangroves (West coast of India) | *Paris* type and *Arum* type | 14 | Seasons<br>Study sites | D'Souza and Rodrigues (2013b) |
| Mangroves (Pearl river estuary, Southern China) | – | 23 | Tide levels | Wang et al 2011 |
| Mangroves (South China) | *Paris* type and *Arum* type | 6 | Plant diversity, study sites | Wang et al (2010) |
| Mangroves (Sundarbans) | *Paris* type, intermediate type, and *Arum* type | 42 | Tide levels<br>Edaphic factors | Kumar and Ghose (2008) |
| Mangroves (Great Nicobar Island, India) | *Paris* type and intermediate | 5 | Edaphic factors and Interaction of other microorganisms | Kothamasi et al. (2006) |
| Saline-alkaline soils of Yellow River Delta (China) | – | 7 | Study sites | Wang et al (2004) |
| Mangroves (Sundarbans) | *Paris* type and *Arum* type | 6 | Plant diversity | Sengupta and Chaudhuri (2002) |

### 10.2.1 Flooding

Wetland plant species are found in varying water depth and are associated with arbuscular mycorrhizal (AM) fungi. Miller and Bever (1999) assessed the distribution of AM fungi in two wetlands, dominated by *Panicum hemitomon* (grass species), and observed nine AM fungal species. Out of Nine AM fungal species, eight were common to the both sites. Within each wetlands there were significant variations in AM species density, suggesting that water depth is an important factor determining the distribution of the AM fungi, even if wetlands and the host plant species are similar along the dry-to-wet gradient. Wang et al (2011) in their study suggested that the effect of flooding on the mycorrhizal association in wetlands can be more informative when conducted in less fertile area with controlled water levels and can reveal more information on AM colonization in aquatic plant species. Besides, there are flooding effects known to decrease AM colonization and spore numbers, but sufficient inoculum survives to colonize (19–33 %) subsequent non-flooded crops (Wangiyana et al. 2006).

Rickerl et al. (1994) reported that no inhibitory effects of moderate tide level on diversity and colonization were observed. This might be due to high tolerance ability of roots of host plant species to salinity in MTL. Moderate tide level zone also promotes growth of mangroves by increasing their photosynthetic rates improving the efficiency of aerenchyma (Wolfe et al 2006).

## 10.3 AM Colonization

Host plant species get colonized by various AM species, belonging to different genera simultaneously (Bever et al. 2001) resulting in competition among them (Smith and Read 2008); most often it is observed that they complement each other (Jansa et al. 2008). Arbuscular mycorrhizal communities within a host can change significantly over various seasons. Dominant AM species during young stages of growth (seedlings stage), which help them in establishment, decreases in numbers as developments of host take place and rare and undetected species shows dominant association (Husband et al. 2002). This might be due to the importance of nutrients in early stages of plant development, particularly in nutrient limitation and stressful environments. Johnson et al. (2010) recently suggested that the primary driver of local adaptation of AM fungal species is edaphic factors in particular ecosystem. Most typical AMF structure, which gives the name to this group of fungi, is the arbuscule This structure, whose shape reminds that of a small shrub, forms inside the root cortical cells by branching in several very thin hyphae. In this way, the surface area, where the nutritional exchanges between the plant and fungus take place, is maximized. Aseptate AM hyphae that grow between root cortical cells produce structures, such as intercellular hyphae and vesicles, representing the intraradical phase of the fungus. Extraradical hyphae grow in rhizosphere forming the network over long distances and explore the soil beyond the nutrient depletion zone.

### *10.3.1 Nutritional Status*

Mycorrhizal fungi improve the nutritional status of plants from many habitats, primarily by increasing phosphorus (P) uptake through the extraradical hyphae. If mycorrhizal fungi contribute to the P nutrition of wetland plants, as has been demonstrated in terrestrial plants, their relative importance should increase in systems where P demand exceeds supply (Koide et al. 2000). The major nutrients phosphorus (P) and nitrogen (N) are deficient in mangrove ecosystems (Carr and Chambers 1998) and likely to limit the growth of mangrove plant species. Arbuscular mycorrhizal fungi-enhanced availability of nutrients is a primary factor affecting the abundance and composition of plant species communities (Klironomos 2003).

Microorganisms such as phosphate-solubilizing, N-fixing, and AM fungi are known to interact in rhizosphere soils and can solubilize the bound P into available form. AM fungal hyphae aid in the transport of nutrients by extending beyond the depletion zone (Cui and Caldwell 1996). Many wetlands are in low-lying areas that receive substantial nutrient inputs from surface runoff and groundwater and are therefore very fertile. Some wetlands, however, are on highly weathered, sandy, or peaty soils and/or receive water inputs mainly through precipitation (e.g., bogs, pocosins, Carolina bays, the Everglades). These nutrient-poor wetlands tend to have infertile soils and poor productivity relative to their nutrient-rich counterparts. Plants have two uptake pathways: one direct uptake pathway and other Arbuscular mycorrhizal (AM) pathway (Smith and Read 2008). The main source of phosphorous to plants is organic phosphate(Pi), which is the crucial limiting factor for plant development and growth (Smith and Read 1997). In the rhizosphere soil, available Pi occurs in low concentrations. One strategy of plants to counter low concentration of Pi is to form symbiosis with arbuscular mycorrhizal (AM) fungi belonging to phylum *Glomeromycota*. They efficiently take up phosphate and other micronutrinets like Zinc (Zn) and Copper(Cu) and depend on plants for photosynthates.

### *10.3.2 Stress*

Mycorrhizal fungi are also thought to ameliorate a variety of other stresses to the plants, from drought to infection by root pathogenic fungi (Newsham et al. 1995). It is unlikely, however, that the fungi are able to alleviate stress due to hypoxia. Some researchers have even suggested that these fungi act as benign parasites when the soil is saturated for extended periods and that mutualistic associations may redevelop when sites become seasonally dry (Anderson et al. 1984); however, evidence for this is very limited. The question remains, are mycorrhizal fungi performing similar functions in wetlands as they are in drier habitats?

### *10.3.3 Freshwater Ecosystem*

Freshwater wetland plant communities are one such type of ecosystem where the community-level effects of AM fungi are poorly understood. In the past, AMF have been considered to be of limited importance in wetland soils because they are thought to be obligately aerobic while many wetland soils are frequently anoxic. Recent researchers have demonstrated that AM fungi are present in the roots of various types of wetland plants (Rickerl et al. 1994). Wirsel (2004) studied AM fungal diversity in wetlands in various zones and observed that AM fungal species richness is more diverse as in upland systems. Although there have been some studies monitoring the effect of AM species on the growth of wetland plants (Miller 2000) very few are specific to mangroves.

**Ecological Implications** The wetlands plant community contains dominant nonmycorrhizal species and mycorrhizal subdominant plant species. There is evidence that in other systems with dominant nonmycorrhizal plants and subdominant mycorrhizal plants mycorrhizas can maintain plant species richness by allowing the persistence of some species of plants that would otherwise be outcompeted. Though more work needs to be done, compilation of all research papers in this review suggests that there is definite pattern across various plant species. The interaction between water and nutrient availability could be important in determining the extent of the mycorrhizal symbiosis among some species of coastal plant communities. Besides, variation in mycorrhizal colonization among different species of wetland plants and the role of AM fungi in competition for P could be very important in forming various wetlands plant communities and aid in the maintenance of the high species diversity. This review of AM fungal communities across selected worldwide locations and ecosystems suggests that soil microorganisms such as AM fungi may exhibit different distribution and colonization patterns, including taxa that are common, specific to a region/ecosystem, and possibly rare. Combination of data from a wider range of ecosystems with increase in durations of study periods in same locations in different seasons and plant host species may assist in establishing the link between assemblage of AM fungal species and ecosystem function.

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# Chapter 11
# Interaction of Rhizobacteria with Arbuscular Mycorrhizal Fungi (AMF) and Their Role in Stress Abetment in Agriculture

**B. Karthikeyan, B. Abitha, Allen John Henry, Tongmin Sa, and M. Melvin Joe**

## 11.1 Introduction

The term mycorrhiza (mykós, "fungus," and riza, "roots") refers to a symbiotic relationship between the fungus belonging to the order Glomales and the roots of vascular plants (Kirk et al. 2001). This association increases the nutrient absorption by increasing the root surface area, through soil exploration by the external mycelium, which in turn increases the access of nutrients and water to the host plant (Karthikeyan et al. 2007). Additionally, this association plays a major factor in influencing the community structure and the biomass of soil microorganisms (Kim et al. 2010). AM fungi interact synergistically with other microorganisms such as nitrogen-fixing bacteria (Brown and Carr 1984; Barea and Azcon-Aguilar 1983), phosphate-solubilizing bacteria (Azcón et al. 1976; Ramasamy et al. 2011; Karthikeyan et al. 2008, 2009; Sakthivel and Karthikeyan 2015), and biocontrol agents (Abdel-Fattah and Mohamedin 2000; Barea et al. 1998) to enhance plant growth. Numerous authors (Mansfeld-Giese et al. 2002; Karthikeyan et al. 2007; Bharadwaj et al. 2008) reported the presence of Mycorrhiza-associated bacteria such as *Paenibacilli*, *Bacilli*, and *Pseudomonas* spp. identified based on culture-dependent methods. Culture-independent methods based

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B. Karthikeyan
Department of Microbiology, Faculty of Agriculture, Annamalai University,
Chidambaram, India

B. Abitha
School of Biosciences and Technology, VIT University, Vellore, India

A.J. Henry • M.M. Joe (✉)
Department of Microbiology, School of Life Sciences, VELS University,
Chennai 600117, India
e-mail: micromelvin@gmail.com

T. Sa
Department of Environmental and Biological Chemistry, Chungbuk National University,
Cheongju, South Korea

M.C. Pagano (ed.), *Recent Advances on Mycorrhizal Fungi*, Fungal Biology,
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on phospholipid fatty acid analysis (PLFA) (Rillig et al. 2005) and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Lioussanne et al. 2008) reported the presence of *Candidatus Glomeribacter gigasporarum* as Gram-non-cultivable bacteria-associated *Gigaspora margarita*. Additionally, legumes form tripartite symbiotic associations with nodule-inducing soil bacteria of the genera *Rhizobium*, *Bradyrhizobium*, or *Azorhizobium* (Caetano-Anolles and Gresshoff 1991; Hirsch 1992) and with VAM fungi (Bonfante-Fasolo 1987; Koide and Schreiner 1992).

Xie et al. (1995) reported that both rhizobial and fungal microsymbionts play a major role in improving the mineral nutrition of the host plant in exchange for assimilates produced by the plant. AM fungi are known to interact with bacteria either directly by providing niche and/or habitat or indirectly by modifying host physiology (Bianciotto et al. 2000; Walley and Germida 1997).

These interactions play a major role in stress agriculture, since plants are exposed to various biotic and abiotic stresses, resulting in reduction in the growth and yield of plants. Harsh climatic conditions such as drought, salinity, extreme temperatures (high and low), and heavy metal contamination significantly affect qualitative and quantitative crop production (Edmeades 2009; Zhu 2002; Lee et al. 2001). Both AM fungi and soil bacteria can adapt to specific environmental conditions and develop tolerance to stressful environments (Vivas et al. 2003a, b, c, d). The cooperation of bacteria and AM fungi in nutrient uptake by plants may be due to specific attributes of microorganisms and there is a growing interest in improving our understanding of their involvement not only in nutrient cycling but also in their effect on non-nutritional physiologic values that make the plant more tolerant to numerous environmental stresses and in particular drought stress (Vessey 2003; Barea et al. 2005). AMF–PGPR interactions can suppress pests and diseases in plants through induction of systemic resistance (Pineda et al. 2010). This type of resistance induced by it shares the characteristics of both pathogen-induced SAR and rhizobacterial ISR (Fritz et al. 2006). Many studies have shown a clear reduction on the detrimental effects by endoparasitic nematodes such as Pratylenchus and Meloidogyne in mycorrhizal plants (Pinochet et al. 1996; de la Pena et al. 2006; Li et al. 2006; Elsen et al. 2008; Vos et al. 2012). Interactions between AMF and PGPR with plants may enhance nutrient availability, biocontrol, abiotic stress tolerance, and phytoremediation in sustainable agriculture (Ramasamy et al. 2011).

Taking all this into account this chapter discuss the different aspects of interactions between AM and PGPR.

## 11.2 Physiological and Biochemical Changes in Plants as Influenced by AM–PGPR Interaction

Kohler et al. (2008) examined the effect of inoculation with *Pseudomonas mendocina* in combination with AM fungus, *G. intraradices* (Schenk and Smith), or *G. mosseae* on antioxidant enzyme activities in leaves of *Lactuca sativa* L. cv. Tafalla affected by water stress. These authors reported that at moderate drought, *P.*

*mendocina* and mycorrhizal *Glomus intraradices*, inoculation alone or in combination, stimulated nitrate reductase activity significantly. At severe drought, *P. mendocina* inoculation, alone or in combination with AM fungi, increased significantly phosphatase activity in lettuce roots and proline accumulation in leaves. Total peroxidase (POX) and catalase (CAT) activities increased in response to drought, whereas superoxide dismutase activity decreased.

Ruiz-Sánchez et al. (2010) reported that the biomass production in AM and AM+*Azospirillum* plants subjected to drought stress increased by 103 % compared to the corresponding uninoculated control. The increase in biomass production under well-watered conditions was over 500 %. Both results suggest that rice plants benefited not only from the AM symbiosis, but also from *A. brasilense* root colonization, regardless of the watering level. Additionally, the beneficial effects of *A. brasilense* on most of the physiological and biochemical traits of rice plants were only clearly visible when the plants were mycorrhized.

Barnawal et al. (2013) reported that the tripartite combination of *Bacillus subtilis* LDR2+*Rhizophagus irregularis* Ri+*Ensifer meliloti* acted synergistically to induce protective mechanisms against decreased soil water availability in *Trigonella* plants and improved plant weight by 56 % with lower ACC concentration (39 % less than stressed and non-inoculated plants) under severe drought conditions. Drought-induced changes in biochemical markers like reduced chlorophyll concentration, increased proline content, and higher lipid peroxidation were monitored and clearly indicated the protective effects of LDR2 under drought stress. Under drought conditions, apart from alleviating ethylene-induced damage, LDR2 enhanced nodulation and arbuscular mycorrhizal fungi (AMF) colonization in the plants resulted in improved nutrient uptake and plant growth.

Vafadar et al. (2014) transferred regenerated plantlets of stevia in tissue culture to pots in greenhouse and inoculated with plant growth-promoting rhizobacteria (PGPRs) (*Bacillus polymyxa, Pseudomonas putida*, and *Azotobacter chroococcum*) and arbuscular mycorrhizal fungus (AMF) (*G. intraradices*). The results on this experiment showed that in comparison to control, inoculation with a single microorganism significantly increased root and shoot biomass as well as stevioside, chlorophyll, and NPK content in plants. However, such increased effects were found to be further enhanced significantly due to dual compatible mixtures of inoculants resulting from their strong synergistic relationships among themselves.

Xun et al. (2015) based on pot culture experiments with oat plants (*Avena sativa*) with different amounts of oil, 5 and 10 g/kg, respectively, found that petroleum stress increased the accumulation of malondialdehyde (MDA) and free proline and the activities of the antioxidant enzyme such as superoxide dismutase, catalase, and peroxidase. Application of PGPR and AMF augmented the activities of three enzymes compared to their respective uninoculated controls, but decreased the MDA and free proline contents, indicating that PGPR and AMF could make the plants more tolerant to harmful hydrocarbon contaminants. It also improved the soil quality by increasing the activities of soil enzymes such as urease, sucrase, and dehydrogenase.

## 11.3 Mycorhizza Helper Bacteria

Mycorrhizal helper bacteria (MHBs) are bacteria associated with mycorrhizal roots and mycorrhizal fungi play a major role in the formation of Mycorrhizas (Garbaye 1994a, b). Two functions of MHBs are as follows: (1) MHBs stimulate the process of mycorrhiza formation and (2) MHBs can interact with the already present symbiosis and both the terms are used to represent both the groups (Frey-Klett et al. 2007).

MHBs isolated from different plant–fungal combinations of AMF have been identified in both Gram-negative and Gram-positive bacteria (Founoune et al. 2002). MHBs have been identified in many Gram-negative Proteobacteria such as *Agrobacterium, Azospirillum, Azotobacter, Burkholderia, Bradyrhizobium, Enterobacter, Pseudomonas, Klebsiella*, and *Rhizobium*; Gram-positive Firmicutes such as *Bacillus, Brevibacillus,* and *Paenibacillus*; and Gram-positive actinomycetes such as *Rhodococcus, Streptomyces,* and *Arthrobacter* (Rigamonte et al. 2010; Table 11.1).

Duponnois (1992) hypothesized that MHBs could soften the cell walls and the middle lamella of the root cortex by producing enzymes such as endoglucanase, cellobiose, hydrolase, pectate, lyase, and xylanase for easier fungal penetration and were detected in pure cultures of each of several MHBs from the Douglas fir *L. laccata* system. The attachment of bacteria to the root or the fungus, or both, can modify the cell-wall properties and they can facilitate the establishment of the symbiosis by providing a mechanical link between the two partners (Duponnois 1992). These authors used a poor medium to compare a number of bacterial isolates ranging from mycorrhization inhibitors to mycorrhization helpers toward *L. laccata*, as determined previously in co-inoculation experiments with Douglas fir seedlings under controlled conditions. Significant correlation was observed between the ability of the bacterial isolates to reduce or promote the mycelial growth of *L. laccata* and their effect on mycorrhiza formation was observed. Olivier and Mamoun (1998) suggested pH changes or the complexing of ions by siderophore-producing fluorescent *Pseudomonas* in tubers of *Melanosporum* ectomycorrhizas. Reid et al. (1984) and Szaniszlo et al. (1981) observed that chelating ligands contribute to mineral uptake by the root, especially hydroxamate siderophore. Fries (1987) reports stimulation of basidiospore germination by *Rhodotorula* species and bacteria, but the substances involved have not been identified for the possibility of MHB's role on spore germination. Azcón (1987) reported that rhizosphere bacteria and their culture filtrates enhanced the germination of *Glomus mosseae* chlamydospores.

Unknown gaseous compounds produced by MHB *Pseudomonas monteilii* increased *P. albus* growth rate, when inoculated in tryptic soy broth agar or on a minimal medium with trehalose (Duponnois and Kisa 2006). The hyphae in the soil substrate and arbuscular mycorrhizal colonization of roots with *Glomus fistulosum* were significantly higher when the fungus was co-inoculated with *P. putida* (Vosátka and Gryndler 1999).

**Table 11.1** Mycorrhiza helper bacteria (MHBs) with significant effects on mycorrhiza formation

| Mycorrizal fungi | Host plant | MHB isolates | Origin of the MHB isolates | MHB effect | References |
|---|---|---|---|---|---|
| *Pisolithus alba* | *Acacia holosericea* | *Pseudomonas monteilii*<br>*Pseudomonas resinovorans* | Rhizosphere | 2.2-fold increase in the ectomycorrhizal infection | Founoune et al. (2002) |
| *Pisolithus* sp. | *Acacia holosericea* | *Fluorescent pseudomonads* | Rhizosphere<br>mycorrhizosphere galls | 1.7–2.3-fold increase in the ectomycorrhizal infection | Founoune et al. (2002) |
| *Laccaria bicolor S238N* | *Pseudotsuga menziesii* | *Pseudomonas fluorescens BBc6R8* | Rhizosphere | Growth, morphology, and gene expression of its fungal associate | Frey-Klett et al. (1997) |
| *Amanita muscaria* | *Picea abies* | *Streptomyces* sp. AcH 505 | Rhizosphere | Mycelial growth | Riedlinger et al. (2006) |
| *Pinus thunbergii*<br>*Suillus granulates* | *Pinus thunbergii* | *Ralstonia* sp.<br>*Bacillus* sp. | Mycorrhizosphere | Improved growth of *P. Thunbergii* seedling | Kataoka and Futai (2009) |
| *Laccaria bicolor* | *Populus deltoides* | *Pseudomonas* sp. | Rhizosphere | *L. bicolor* growth and root colonization of *Populus* tree | Labbé et al. (2014) |

MHB *Streptomyces* sp. ACH 505 promotes mycelial extension in *Amanita muscaria*, reduces hyphal biomass/colony, which in turn reduces mycelial density (Schrey et al. 2005), and reduces the thickness of the fungal hyphae (Maier 2003). Deveau et al. (2007) reported that morphological modification in fungal mycelium was induced by the MHB strain in *P. fluorescens* BBc6R8–*L. bicolor* S238N pair. The expression level of the *A. muscaria* cyclophilin gene *AmCyp40*, which encodes a prolyl isomerase, is involved in cell growth, and the cell stress response is upregulated in *A. muscaria*–*Streptomyces* sp. AcH 505 under coculture conditions (Schrey et al. 2005). *Pseudomonas fluorescens* BBC6R8 had a positive influence on fungal biomass only when the soil was autoclaved before the bacterium and fungal inoculums were added (Brulé et al. 2001).

Increase in the number of lateral roots in *Bacillus* strain was reported by Bending et al. (2002), who reported the increased formation of only first-order ectomycorrhiza roots. Poole et al. (2001) reported *Burkholderia* and *Rhodococcus* strains' isolation by the increased formation of only second-order ectomycorrhiza roots in Scots pine (*Pinus sylvestris*). Auxins and ethylene also produce morphological changes in roots during mycorrhiza formation (Kaska et al. 1999), including the formation of lateral roots and dichotomous branching of short roots (Barker and Tagu 2000). MHBs also facilitate root colonization by mycorrhizal fungi, by inducing the release of plant flavonoids. The nodulation (Nod) factors produced by MHB *Bradyrhizobium japonicum* strain stimulated the production of flavonoids in soybean (*Glycine max*) seedlings and mycorrhiza formation (Xie et al. 1995). Among these microbial groups bacteria and arbuscular mycorrhizal (AM) fungi are ubiquitous in the soil, and there is abundant literature to support the idea that these rhizosphere microbes interact in rather specific ways to influence their relationship, which in turn affects the plant growth (Galleguillos et al. 2000; Marulanda et al. 2006). Plant growth-promoting rhizobacteria (PGPR) play an important role in modifying the soil fertility and facilitates plant establishment and development (Caravaca et al. 2002a, b).

## 11.4 Spore-Associated Bacteria

AMF spores can host bacteria in their cytoplasm in unique (Lumini et al. 2007) and intracellular structures similar to bacteria-like organisms, and they have been observed inside AMF spores with transmission electronic microscope (Bonfante et al. 1994; Cruz 2004). Most AMF carry bacteria and this relationship can be dated back to the time when AMF established symbioses with land plants (Bonfante 2003).

Bacteria which remained protected within the spore walls were detected only when the spores were placed on appropriate media. Mugnier and Mosse (1987) reported that *G. mosseae* spores germinated under in vitro conditions only in the presence of *Streptomyces orientalis*. Walley and Germida (1995) reported that exposure of spores of *Glomus clarum* NT4 to solutions of chloramine-T (2.5–10 %w/v) for 10–120 min failed to fully decontaminate all spores. Scanning electron microscopy did not show the presence of contaminants on treated spores, but transmission electron microscopy revealed bacterial cells embedded within the outer spore wall layer. Carpenter-Boggs et al. (1995) demonstrated a positive correlation between higher germination rate and the amount of production of geosmin, $CO_2$, and 2-methylisoborneol by the actinomycetes. Bacteria can colonize the surface of AMF spores and hyphae (Xavier and Germida 2003), and they can be located inside the spore walls (Walley and Germida 1995). In addition, the bacteria affect spore germination, hyphal growth, and root colonization (Horii and Ishii 2006; Horii et al. 2008). Hildebrandt et al. (2006) reported that *Paenibacillus validus* DSM ID617 and ID618 stimulated growth of *G. intraradices* Sy167 by the formation of fertile spores, which recolonize carrot roots.

Bharadwaj et al. (2008) reported that AM fungal root colonization in potato plants increased 6–9-fold in the presence of *Pseudomonas* FWC70, *Stenotrophomonas* FWC94, and *Arthrobacter* FWC110 isolates. In another study, Bharadwaj et al. (2008) reported that root exudates of *G. irregulare* stimulated growth of AM fungi-associated bacteria (AMB) isolates tested under microtiter plate conditions. AMB growth stimulation was observed also during co-cultivation of three of these AMB with *G. irregulare* in the hyphal compartment.

## 11.5 Culture-Dependent Techniques

Artursson and Jansson (2003) used 16S rRNA gene sequence information and a selective medium to isolate the *Bacillus cereus* strain VA1 by using thymidine analogue, bromodeoxyuridine (BrdU) method. Furthermore, these authors reported that by tagging with green fluorescent protein by using confocal microscopy, this bacterium was shown to attach clearly to arbuscular mycorrhizal hyphae.

Cruz and Ishii (2011) reported the isolation of three bacterial strains: *Bacillus* sp. (KTCIGM01), *Bacillus thuringiensis* (KTCIGM02), and *Paenibacillus rhizosphaerae* (KTCIGM03) from *G. margarita* using a hypodermic needle (diameter, 200 μm). Panneerselvam et al. (2012) reported AM-associated bacteria from *G. mosseae* spores from different guava cropping systems in southern India. Among seven isolates, these authors selected four bacteria based on their efficiency in stimulation of AM root colonization and spore production and identified them as *P. putida* (HM590706), *Pseudomonas aeruginosa* (HM590705), *Brevibacillus* sp. (HM590700), and *B. subtilis* (HM590703) by polymerase chain reaction amplification of 16S rRNA.

Bharadwaj et al. (2008) analyzed 385 AM fungal spore-associated bacteria (AMB) using fatty acid methyl ester (FAME) profile analysis and identified using 16S rRNA gene sequence analysis. Based on these analyses AMB were most abundant in the genera *Arthrobacter* and *Pseudomonas* and in a cluster of unidentified isolates related to *Stenotrophomonas*.

## 11.6 Cutivation-Independent Techniques to Study Bacteria Associated with Spores

Jargeat et al. (2004) reported that *Candidatus* sp. and *Glomeribacter gigasporarum* obtained from *Gigaspora* could not be grown independently of the host and apparently has lost their nitrogenase genes. Banciotto et al. (1996) identified a stable cytoplasmic component associated with *G. margarita* based on 16S rRNA gene sequences as genus *Burkholderia*. Ruiz-Lozano and Bonfante (2000) identified that *Candidatus* sp. and *Glomeribacter* were not able to grow under in vitro conditions. Long et al. (2009) using PCR-denaturing gradient gel electrophoresis analysis sequenced two bacterial 16S rRNA gene V3 region sequences, 7A and 7B, associated with spores of the AMF *G. margarita*. DNA sequencing and phylogenetic analysis revealed that 7B was mostly related to the endosymbiotic bacterium *Candidatus Glomeribacter gigasporarum*; however, strain 7A could not be confidently assigned to any known taxon.

Bianciotto et al. (2000) reported that with the exception of *Gigaspora rosea*, Gigasporaceae family harbored bacteria, and their DNA could be PCR amplified with universal bacterial primers specific for the endosymbiotic bacteria of BEG 34. These primers were successfully used as probes for in situ hybridization of endobacteria in *G. margarita* spores. Neighbor-joining analysis of the 16S ribosomal DNA sequences obtained from isolates of *Scutellospora persica*, *Scutellospora castanea*, and *G. margarita* revealed a single, strongly supported branch nested in the genus *Burkholderia*.

Roesti et al. (2005) based on his PCR-denaturing gradient gel electrophoresis analysis on the spores of the AMF *Glomus geosporum* and *Glomus constrictum* harvested from single-spore-derived pot cultures of *Plantago lanceolata* or *Hieracium pilosella* revealed that the bacterial communities depend more on AMF than host plant. These authors reported that the composition of the bacterial populations linked to the spores could be predominantly influenced by a specific spore wall composition or AMF exudates rather than by specific root exudates. The majority of the bacterial sequences were common to *G. geosporum*, and *G. constrictum* spores were affiliated with taxonomic groups known to degrade biopolymers (*Cellvibrio*, *Chondromyces*, *Flexibacter*, *Lysobacter*, and *Pseudomonas*). The homogeneity among replicates of DGGE patterns of bacterial communities associated with AMF spores was higher in cultures on *H. pilosella* than in those on *P. lanceolata* (Table 11.2).

**Table 11.2** Effect of spore-associated bacteria on plant growth

| AM fungi | Associated bacteria | Beneficial effect exerted on plant | References |
|---|---|---|---|
| *G. intraradices* | *Pseudomonas*, *Arthrobacter*, and *Burkholderia* | Cucumber plants (*Cucumis sativus*. Large differences in bacterial community structure were observed | Mansfeld-Giese et al. (2002) |
| *Glomus clarum* NT4 | *Bacillus pabuli*LA3, *Bacillus chitinosporus* LA6a and NT4 | Enhanced the shoot growth, AMF colonization, shoot N content, and P use efficiency | Xavier and Germida (2003) |
| Arbuscular mycorrhizal (AM) fungi | *Pseudomonas* FWC70, *Stenotrophomonas* FWC94, and *Arthrobacter* FWC110 | AMB are likely to contribute to the often described ability of AM fungi to inhibit pathogens, acquire mineral nutrients, and modify plant root growth. | Bharadwaj et al. (2008) |
| *Glomus irregulare* | *Stenotrophomonas maltophilia* FWC14, FWC94, FwC101, LWC2, LWC39 | Inhibition of *Rhizoctonia solani*, *Verticillium dahliae*, and *Pectobacterium carotovorum* ssp. *carotovorum* growth was evident in the presence of the AMB isolates tested together with the *G. irregulare* exudates. | Bharadwaj et al. (2012) |
| *Glomus irregulare* | *Stenotrophomonas maltophilia* FWC14, FWC94, FwC101, LWC2, LWC39 | Inhibition of *Rhizoctonia solani*, *Verticillium dahliae*, and *Pectobacterium carotovorum* ssp. *carotovorum* growth was evident in the presence of the AMB isolates tested together with the *G. irregulare* exudates. | Bharadwaj et al. (2012) |

## 11.7 AM Fungi and PGPR for Management of Abiotic and Biotic Stress in Crop Plants

Abiotic stress tolerance in soil microorganisms has been studied to provide a better understanding of the adaptation and survival of living microorganisms in extreme environments. Among these microbial groups, bacteria and arbuscular mycorrhizal (AM) fungi are ubiquitous in the soil, and there is abundant literature to support the idea that these rhizosphere microbes interact in rather specific ways to influence their relationship with and their effect on plant growth (Galleguillos et al. 2000; Marulanda et al. 2006). PGPR play an important role as modifiers of soil fertility and as facilitators of plant establishment and development (Caravaca et al. 2002a, b).

## 11.8 Role of AM Fungi and PGPR Interaction in Stress Agriculture

### *11.8.1 Salt Stress Tolerance*

The use of plant growth-promoting bacteria (PGPR) and symbiotic microorganisms, especially arbuscular mycorrhizal (AM) fungi, might be useful in developing strategies to facilitate plant growth in saline soils. Glick et al. (1998) reported that under different stresses, the use of PGPR such as *Pseudomonas fluorescence* can alleviate the adverse effects of stress on plant growth. It has been indicated that some PGPR are able to produce polysaccharide products, thereby binding $Na^+$ in the root zone and hence alleviating the effects of salt stress on plants and enhancing microbial growth and activities (Tank and Saraf 2010). Many studies (Sharifi et al. 2007; Azcón et al. 2013) have demonstrated that inoculation with AM fungi improves growth of plants under salt stress.

Rabie and Almadini (2005) reported that the tolerance index to salinity in AM plants increased in the presence of nitrogen-fixing bacteria (NFB). These authors reported that the tolerance index of salinity in AM plants with and without the NFB was 3.8 and 3.1-fold higher than that of non-AM plants, respectively, at 6.0 $dSm^{-1}$ level of salinity. The average values of tolerance index for AM plants in the absence and the presence of NFB were 155 and 181 %, respectively, while for non-AM plants the values were 81 and 35 %, respectively, in the absence and the presence of NFB. This result indicated that the presence of NFB is more evident for AM than non-AM plants, which suggests that the dual inoculation of AM fungi and NFB may play an important role in increasing salinity tolerance of faba plants under salinity stress. The potentiality of AM fungi to minimize the inhibitory effect of salinity on nitrogen fixation by faba plants was promoted by the presence of nitrogen-fixing *A. brasilense*. In the presence of NFB, nodulus formation, nitrogen level, protein content, and nitrogenase activity of AM faba plants were measured as 4.0-, 1.4-, 3.8-, and 2.9-fold higher than that in non-AM plants, respectively, at a level of salinity of 6.0 $dSm^{-1}$.

The dual inoculation of AMF and *B. subtilis* showed greater plant height, higher number of branches, fresh and dry weight, essential oil % and yield, as well as N, P, K% and lower Na% over control (Rahman et al. 2011). In addition to that *Bacillus substilis* increased the stability of the cell membrane and improved the plant vigor and photosynthesis under salt stress by increasing the net photosynthetic rate and the stomatic conductance (Fig. 11.1).

The mechanism of stress endurance in AM fungi is through hyphal effect or exudation of specific compounds; soil biota alter root physiology by acting on different biochemical and molecular mechanisms that can essentially improve water and nutrient acquisition and exclude toxic ions from the rhizosphere (Dodd and Pérez-Alfocea 2012). These authors reported that the altered root physiology modifies root-to-shoot communication and contributes to water, ionic, and hormonal homeostasis in the shoot, alleviating the effect of salt-

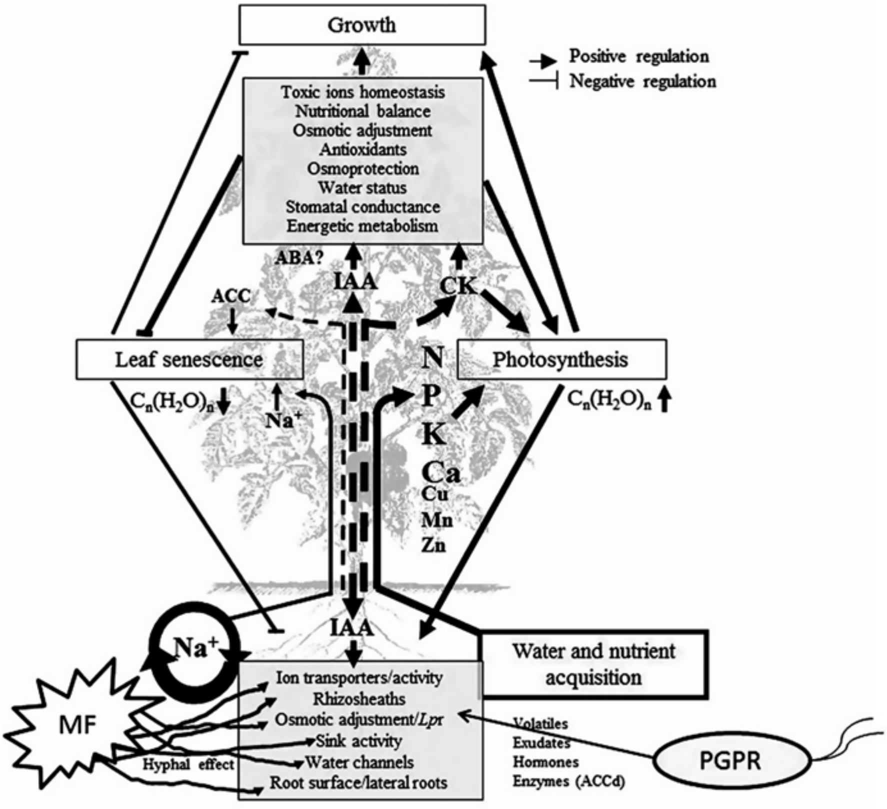

**Fig. 11.1** Salt stress alleviation by mycorrhizal fungi (MF) and plant growth-promoting rhizobacteria (PGPR) (From: Dodd and Pérez-Alfocea 2012)

induced growth impairment during the osmotic phase of salinity and delaying the toxic effect on leaf senescence. Microbes modify the expression and activity of ion and water transporters and increase the effective root surface area via fungal hyphae or enhancement of lateral root formation by altering auxin (IAA) transport and signaling. Some exudates (polysaccharides) sequester toxic ions (Na+), while other metabolites (amino acids, trehalose) and hormones (cytokinins and auxins) can be transported to the shoot to protect growth and photosynthetic machinery.

Yonesi and Moradi (2014) reported that the contribution of the PGPR and AM fungi to plant salt tolerance was closely related to salinity avoidance mechanisms, including increased water uptake and restricted Na uptake. The protection of mycorrhizal plants against moderate salt stress was also related to the effect that the added endophytes had on increasing P uptake. In addition to this induction of antioxidant enzyme activities it was involved in the ability of the PGPR to increase the tolerance of lettuce grown under severe salt stress.

### *11.8.2 Drought Stress*

Water stress may occur because of either excess water or water deficit, and flooding is the example for excess of water; water deficit otherwise known as drought stress is a most common stress. Drought stress causes the dehydration of cells and osmotic imbalance (Mahajan and Tuteja 2005). Vivas et al. (2003a, b, c, d) reported that co-inoculation with *G. mosseae*, *G. intraradices*, and bacteria *Bacillus* sp. enhanced fungal development and metabolism. These authors also emphasizes that stressed plants increased drought resistance by maintaining high levels of proline, photosynthetic activity, and water use efficiency. Marulanda et al. (2006) reported that the relative water uptake in plants colonized by *G. intraradices* was improved by 106.4 %, over the control, whereas plant colonized by *G. intraradices* improved by 113.9 % over the control.

Under drought stress, plants inoculated with mycorrhiza (Augé 2001) or PGPR (Creus et al. 2004) often showed enhanced osmotic adjustment. Augé (2001) reported that the mycorrhizal maintenance of root turgor during drought was not related to osmotic adjustment, despite fungal alteration of concentrations of several key solutes (Augé et al. 1992), but changed apoplastic/symplastic water partitioning (Augé and Stodola 1990).

Both mycorrhizae and PGPR can affect root hydraulic conductance (*L*); for instance; *G. intraradices* BEG 123 infected bean (*Phaseolus vulgaris*) plants had a greater osmotic root hydraulic conductance under saline (3.1 $dSm^{-1}$) stress (Aroca et al. 2007) than uninoculated plants. It was proposed that mycorrhizal roots increased active solute transport as a mechanism to sustain water flow across the root. Although regulation of root hydraulic properties by AM symbiosis was strongly correlated with the regulation of aquaporin (PvPIP2) protein abundance and phosphorylation state (Aroca et al. 2007), different PIP genes were regulated differentially under salinity stress.

Interactions of ABA with other hormones [such as auxins, cytokinins, jasmonic acid (JA), and ethylene] were found to be altered by the stress (Luo et al. 2009). Although these changes in root hydraulic properties will assist the maintenance of plant water status, salt generally decreases plant relative water content, water uptake, and transpiration rate (Jahromi et al. 2008). Mycorrhizal symbiosis can have a negative, positive, or no effect on transpiration rate depending on the timing and type of stress applied (Aroca et al. 2008; Jahromi et al. 2008), which is perhaps not surprising given that mycorrhizal symbiosis can affect numerous phytohormones involved in root-to-shoot signaling processes that regulate leaf gas exchange (Dodd et al. 2010).

There is ample literature (Kohler et al. 2007; Marulanda et al. 2009; Ruiz-Sánchez et al. 2011; Valdenegro et al. 2001) showing positive effect on plant drought tolerance when AMF and PGPR are inoculated together. Mechanisms related to this effect can be several, but improvements in plant root development and water uptake capacity, alteration of plant hormonal balance, and protection against the oxidative stress generated by drought seem to be involved.

Drought is a major limitation for crop production in rain-fed ecosystems that lowers yield potential (Jongdee et al. 2002). Synergistic effect has been reported on co-inoculated bacteria and AMF helping in restoring plant growth under drought conditions (Marulanda et al. 2008, 2009). The use of indigenous drought-tolerant *G. intraradices* strain along with native bacterium reduced 42 % water requirement in the production of *Retama sphaerocarpa* (Marulanda et al. 2006).

Positive interactions between *P. putida* or *Bacillus megaterium* and AMF in stimulating plant growth and drought tolerance have been reported by Marulanda et al. (2009). Under well-watered conditions, AMF plants showed 500 % increase in shoot fresh weight (SFW) compared to uninoculated control plants. Interestingly, these AMF plants co-inoculated with *Azospirillum* showed 12 % in SFW. In drought-stressed conditions, combined inoculation of AMF and *Azospirillum* increased SFW by 103 % compared to the uninoculated control (Ruiz-Sánchez et al. 2011). Similar results were also observed by Franzini et al. (2010) in co-inoculation of AMF with *Rhizobium* in *P. vulgaris* under drought-stressed conditions.

ACC is the precursor for ethylene synthesis in plant; bacterial ACC deaminase cleaves the ACC to ammonia and α-ketobutyrate, thereby lowering ethylene levels in plant (Glick et al. 1998). The plant hormone ethylene regulates several phases of plant growth (i.e., fruit ripening, flower senescence) and is mainly involved in plant responses to biotic and abiotic stresses (Abeles et al. 1992). Lowering of plant ethylene levels is essential during early stages of plant development and when exposed to environmental stresses like drought and salinity (Glick 2004). Combined inoculation of ACC deaminase-positive *Psudomonas putida* and *G. rosea* showed increased plant growth and improved root architecture (Gamalero et al. 2008).

The growth effect of PGPR may be mediated by IAA, salicylic acid, and gibberellin signaling pathways (Bent et al. 2001; James et al. 2002). It is also known that an increase in plant growth caused by AM fungi is mediated in part by an alteration in plant hormone levels (Ruiz-Lozano et al. 2001; Marulanda et al. 2006). The manipulation of these microorganisms is important with regard to sustainability issues (Bowen and Rovira 1999; Medina et al. 2003; Vivas et al. 2003a, b, c, d). However, positive results depend on a proper understanding of the system applied and the suitable selection of microbes (Galleguillos et al. 2000; Valdenegro et al. 2001; Marulanda et al. 2003; Vivas et al. 2006a, b). Marulanda et al. (2006) reported the effect of drought-tolerant AM fungus and *Bacillus* sp. in improving plant tolerance to drought stress and water transport system in *R. sphaerocarpa*. Possible mechanisms involved in the effectiveness of microbial inoculation were analyzed and it was found that both AM fungi and soil bacteria can be adapted to specific environmental conditions, and a higher tolerance to stress of indigenous microorganisms in comparison to those non-autochthonous AM fungi from non-stressed sites (Vivas et al. 2003a, b, c, d; Marulanda et al. 2006).

Bowen and Rovira (1999) used AM fungi and the inoculated bacterial strains from Mediterranean arid soil as inocula under natural soil conditions in microcosms. These authors reported that these microorganisms were able to survive and multiply to reach a sufficient population to express their activities in the natural soil through time (four harvests at least). Paleg et al. (1984) confirmed in axenic culture that *B. megaterium* exhibited the highest tolerance to water deficit caused by osmotic stresses (PEG or NaCl), with an increase in proline content and IAA production as much as the increase in the osmotic stress in the growing medium. The increasing proline accumulation in axenic culture of the native bacteria *B. megaterium* under increasing stress conditions could induce the adjustment of cell osmotic potential, indicative of osmotic cellular adaptation. This is a mechanism by which microbial cells can cope with drought stress. According to our results, inoculation with *B. megaterium* increased shoot and root biomass and water content in plants. The highest IAA production by these bacteria under stressful conditions may explain its effectiveness in promoting shoot and root growth by 86 % and water content, respectively. This PGPR capacity and the proline content may enhance its competitive advantage under dry environments and was most effective in interactions with three of the four AM fungi assayed, resulting in particular effects on plant performance depending on the AM fungus involved. When inoculated in dry environments we observed that the highest IAA and proline production by *B. megaterium* in axenic culture are directly correlated with the greatest root growth.

Marulanda et al. (2003) reported that rhizosphere bacterium isolated from arid soil identified as *Bacillus* sp. positively influenced the development and activity of two *Glomus* sp., stimulating the activity of intraradical mycelium from *Glomus* sp. and supported the development of extraradical mycelium from *G. intraradices*. In a recent (unpublished) study, we found that *B. megaterium* increased the amount of photosynthetic pigments in *G. intraradices*-colonized plants in which there is an increase in total chlorophyll and carotenoid content. Marulanda et al. (2008) reported that the effects of specific interactions between the bacterium and each *Glomus* sp. on plant physiology and metabolism were independent of drought stress.

Gray and Smith (2005) reported that PGPR associated with plant roots (inside and/or outside) directly or indirectly stimulates plant growth, but there is a gradient of root proximity and intimacy depending on the niche. There are bacteria living in the soil near the roots, bacteria colonizing the rhizoplane (root surface), and bacteria residing in root tissue (inside cortical cells). These aspects are important for intimacy with the associated plant, from almost casual to extremely regulated and housed in specialized structures. In general, for an effective growth stimulation a close interaction between microorganisms and host plants is a prerequisite for utilization of plant assimilates and microbial metabolites, respectively, by the partners (Grayston et al. 1996). Particular and specific interactions between plants and microbial groups need to be compatible at a physiologic level (Marulanda et al. 2006). Combined inoculation of bacterial strains with AM fungi produced growth-stimulating effects that surpassed those of individual inoculations (Galleguillos et al. 2000).

Marulanda et al. (2008) reported that the *B. megaterium* strain was effective in co-inoculation with *Glomus coronatum*, the same autochthonous strain used here, but it did not improve plant growth when it was associated with *G. coronatum*. Physiologic and biochemical traits of these AM fungi–bacteria associations were discussed. We cannot rule out the changes in IAA content as affected by each of the bacterial inoculants under stress conditions; however, this may be relevant in the growth effect observed.

### *11.8.3 Heavy Metal Stress*

Wu et al. (2006) reported that plant inoculated with *Brassica juncea* did not greatly alter the metal concentration but effectively promoted the plant growth from growth inhibition; authors also documented that the Pb and Zn concentrations in plant tissues and Cu and Cd concentrations in shoots were increased. Plant *Trifolium repens* L inoculated with AMF and *Brevibacillus brevis* under nickel supplemented soil showed significant growth improvement, mycorrhizal colonization increased essential nutrients in plants, and plants had a decreased amount on Ni absorption (Vivas et al. 2006a). In Zn-polluted soil, mycorrhizal colonization improved superior nodule formation; nodulation by *Rhizobium* becomes sensitive to Zn contamination than AM symbiosis. Evidence suggests that these microorganisms can be efficiently resistant to increasing Zn concentrations (Vivas et al. 2006a, b).

Phytoremediations are technologies that use plants (and their associated microorganisms) to remove, transfer, stabilize, decrease, and/or decompose pollutants in the environment (Denton 2007); mycorrhizal fungi assist in phytoremediation in the rhizosphere (Wenzel et al. 2008). Soil microorganisms are often well adapted to survival in the presence of heavy metals and they can interact and transform them by changing their oxidation state (Van Hullebusch et al. 2005). Thus, the interactions between plant roots and microbes in the rhizosphere may have a great influence both on the increase in nutrient uptake and on the decrease in metal toxicity (Azcón et al. 2009; Rajkumar et al. 2008; Azcón et al. 2013)

It is possible to improve the phytoremediation capabilities by inoculating the plant with appropriate AMF and PGPR. *Brevibacillus* sp., one of the most Cd-tolerant bacterial strain, in symbiosis with AMF enhanced nodulation and N and P uptake and improved Cd stress tolerance in *T. repens*. Further studies using Ni-tolerant *B. brevis* strain along with AMF reduced nickel toxicity in plant (Vivas et al. 2003a, b, c, d). Vivas et al. (2006a, b) used *B. brevis*, *Rhizobium trifolii*, and *G. mosseae* as a single and co-inoculation with different Ni concentrations 30 (Ni I), 90 (Ni II), and 270 (Ni III) mg $NiSO_4$ $kg^{-1}$ of soil. Co-inoculation of these microorganisms reduced plant Ni concentrations by 4.9 (Ni I), 6.4 (Ni II), and 6.0 (Ni III) fold compared with non-treated control plants. The microbial activity changed depending on the available Ni in soil and these treatments show increased P uptake in *Trifolium* plants by more than ten times (Ni I and Ni II). Vivas et al. (2006b) reported that *Brevibacillus* sp. along with co-inoculation of indigenous AMF isolated from Zn-contaminated soil reduced Zn uptake and promoted growth in *T. repens* plant. *Brevibacterium* sp. isolated from Ni-, Cd-, and Zn-contaminated sites showed higher PGPR activity and also acted as MHB. AMF provided an adaptable environment in the mycorrhizosphere for *Brevibacterium* sp. by secreting a stimulant and involved in the modification of root exudates composition for its better survivability in root zone.

## 11.9 Biotic Stress

### *11.9.1 Biocontrol*

Maximum reduction in galling and nematode multiplication causing root rot in chick pea was observed with combined inoculation of *G. intraradices* with the biocontrol agents *Pseudomonas striata* and *Rhizobium* sp. (Akhtar and Siddiqui 2008) and dual inoculation of *G. mosseae* with *P. fluorescens* (Siddiqui and Mahmood 1998). Several studies have demonstrated that microbial antagonists to pathogens, either fungi or PGPR, do not exert any negative effect against AM fungi (Barea et al. 2005). Mycorrhization helper bacteria (MHBs), defined by (Garbaye 1994a, b) as bacteria which consistently promote mycorrhizal development, would even increase AM impact on pathogens. Rhizobacteria and conditions of stimulation of mycorrhizal symbiosis have been listed by (Frey-Klett et al. 2007).

Synergism between PGPR and AMF in the mycorrhizosphere also plays a major role in the activation of plant defense mechanisms (Akköprü and Demir 2005; Linderman 1994). Sayeed and Siddiqui (2008) reported that AM fungus can coexist along with root nodule bacterium without exhibiting adverse effects on each other. Furthermore, they reported that it could be used as a biocontrol agent to control most of the soil-borne diseases. Combined use of *Rhizobium*, *G. intraradices*, and *P. striata* strains is reported to control root rot disease of chickpea. Combined inoculated plants showed largest reduction in nematode (*M. incognita*) population than single inoculations.

Co-inoculation of PGPRs along with AM fungi protecting the plants better than AM fungus alone against root pathogens has been reported by many workers (Dhillion 1992; Singh and Kapoor 1998). Wilt of the medicinal plant *Coleus forskohlii* caused by *Fusarium chlamydosporum* is very serious in India. Inoculation with AM fungus plus *Trichoderma viride* was found to increase root yield and root forskolin concentration and reduce the severity of the disease significantly under field conditions (Singh et al. 2012).

Most of the AM–root pathogen interaction studies have been conducted in crop plants important in agriculture and horticulture. But the information available on forest tree species is scanty. Mycorrhizal technology can thus play an important role in the production of low-cost quality seedlings. Like most instances of biological control, AM fungi cannot offer complete immunity against the infestation by plant pathogens. They could only impart a degree of resistance against soil-borne plant pathogens. However, the possibility of biologically controlling soil-borne plant pathogens looks promising.

Interestingly, four strains of *Paenibacillus* isolated directly from the external mycelium of the AM fungus *G. intraradices* showed *Pythium* biocontrol properties that may be involved in the observed biocontrol property of mycelium of *G. intraradices* in root-free soil (Larsen et al. 2003). Similar to our results, Budi et al. (1999) showed that strains of *Paenibacillus* obtained from a mycorrhiza root organ culture were antagonistic against various root pathogens. Also, the ectomycorrhiza helper bacteria *Paenibacillus* EJP 73 isolated from *Lactarius rufus–P. sylvestris* ectomycorrhizal roots (Poole et al. 2001) demonstrated *Pythium* biocontrol features, which may also be relevant in the biocontrol of root pathogens in forest nurseries, where *Pythium* also causes severe problems (Salerno et al. 2000; Bin et al. 2007).

The effects of *G. intraradices*, *Rhizobium* sp., and *P. striata* on the root rot disease complex of chickpea caused by *Meloidogyne incognita* and *Macrophomina phaseolina* were observed. Inoculation of *G. intraradices*, *P. striata*, and *Rhizobium* caused a significant increase in plant growth, number of pods, chlorophyll, nitrogen, phosphorus, and potassium contents of pathogen-inoculated plants. Inoculation of *Rhizobium* caused a greater increase in plant growth, number of pods, chlorophyll, nitrogen, phosphorus, and potassium contents of pathogen-inoculated plants than caused by *P. striata* or *G. intraradices*. Combined inoculation of *G. intraradices* with *P. striata* plus *Rhizobium* to pathogen-inoculated plants caused greater increase in plant growth, number of pods, chlorophyll, nitrogen, phosphorus, and potassium contents than by inoculation of *G. intraradices* plus *Rhizobium* or *G. intraradices* plus *P. striata*. The numbers of nodules per root system were significantly higher in plants inoculated with *Rhizobium* compared with uninoculated ones. Inoculation of *Rhizobium* with *P. striata G. intraradices* further increases nodulation per root system over plants inoculated with *Rhizobium* alone. Root colonization by *G. intraradices* was high in plants inoculated alone. In the presence of *P. striata* and *Rhizobium*, root colonization by *G. intraradices* was increased, while inoculation of pathogens reduced colonization by *G. intraradices*. Inoculation of *Rhizobium* caused higher reduction in galling and nematode multiplication, followed by *P. striata* and *G. intraradices*. Maximum reduction in galling and nematode multiplication was observed when G. intraradices was inoculated with both bacteria. Biocontrol of root rot disease complex of chickpea may be achieved by the combined use of *Rhizobium*, *G. intraradices*, and *P. striata* or use of *Rhizobium* plus *P. striata* (Sayeed and Siddiqui 2008).

Similarly, combined use of *Rhizobium* and *G. intraradices* also increased the availability of N and P, which may have an adverse effect on pathogens. Moreover, combined use of these biocontrol agents also improves root growth, which may result in increased plant growth (Azcon-Aguilar and Barea 1996). *Pseudomonas* spp. also promote and stimulate colonization of AM fungi and are called “Mycorrhiza helper bacteria” (Barea et al. 1998) and also stimulate the germination of AM spore and mycelial development (Meyer and Linderman 1986). Combined application of the AM fungus, *Pseudomonas* and *Rhizobium*, resulted in greater root colonization and nodulation than individual application, which may be a reason for better plant growth. Reduced disease intensity in combined application of *G. intraradices* with rhizobacteria observed in the present study has also been reported earlier because they inhibited pathogens more efficiently than when inoculated individually (Budi et al. 1999).

AM fungus, a root nodule bacterium and a plant growth-promoting rhizobacterium, can coexist without exhibiting adverse effects on each other. These biocontrol agents may be used concomitantly for the biocontrol of diseases. Moreover, it is concluded that suitable combinations of these biocontrol agents may increase plant growth and resistance to pathogens (Sayeed and Siddiqui 2008).

Serfoji and Rajeshkumar (2010) observed that the application of vermicompost + *Glomus aggregatum* + *Bacillus coagulans* increased plant growth characters and reduced root knot index, nematode reproduction rate, number of galls, and egg masses on tomato CV Pusa Ruby in sandy loam acidic soils.

Liu et al. (2012) reported that nematode penetration of roots, nematode reproduction, and nematode-incited disease were decreased by dual inoculation with AM fungi and PGPR than the single inoculations. This may be due to the interactive effect of AM fungus and PGPR's in the induction of systemic resistances in plant toward nematode.

## 11.10 Conclusion

AM fungi are known to associate with different plant species and their association increases the nutrient absorptive root surface area, by the external mycelium, which in turn increases the access of nutrients and water to the host plant. Additionally they are known to associate with different microorganisms in the mycorrhizosphere. These interactions have provided numerous benefits to the associated plants in terms of growth development and stress endurance ability. For the best utilization of these interactions in improving plant growth, a better understanding is necessary. Though this chapter tries to provide a brief outline of numerous aspects of AMF–PGPR interactions, still many questions remain unanswered, which needs further research to explore the molecular and biophysical factors underlying these interactions.

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