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Recent Advances on Mycorrhizal Fungi

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Chapter 8
Mycorrhizas in Agroecosystems

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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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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 ≤ 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.

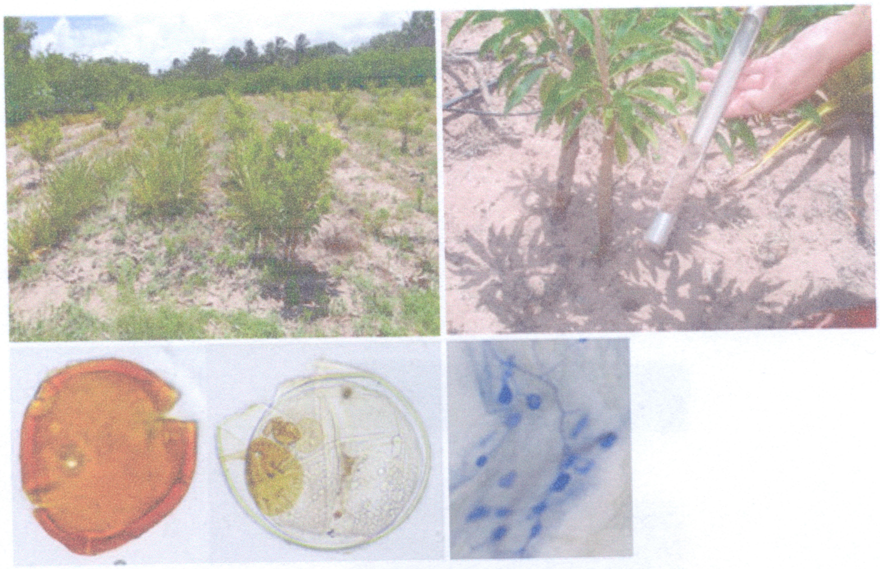

Fig. 8.1 Cultivated areas in Ceará, Brazil. Clockwise, from *upper left*: pineapple and sapota tree cv. BRS 228, colonized roots of *Cocos nucifera* and AMF spores of *Scutellospora* and *Glomus* isolated from soils (Photo-credit: B. Dantas and M. Pagano)

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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