Chapter 17
Mycorrhizas in South American Anthropic Environments

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17.1 Introduction

The agricultural expansion has leaded to increase the irrigated cropland area and the use of fertilizers, resulting in water degradation, increased energy use, and common pollution (Foley et al. 2011). Of particular concern is the increased interest to reduce the environmental impacts of high quantities of water dedicated to irrigation by agricultural activities (Foley et al. 2011).

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We are now truly recognizing the importance of sustainable measures in agriculture such as conservation of the vegetation cover and management approach to understand surface and deep soil responses to global change (Chaparro et al. 2012). For example, promising plant species can be tested to engineer the cultivable soil microbiome (Ellouze et al. 2013). The new alternatives for the agro-ecosystem management, such as inter-cropping, tillage and organic amendments, affect soils physical and chemical properties, modifying the abundance, diversity and activity of the mycorrhizal communities (Cardoso and Kuyper 2006; Pagano et al. 2011). Thus, the agroecology management based on key processes from natural ecosystems can help to solve some agricultural difficulties. For example, cultural practices (rotation, intercropping and fungal inoculation) that mimic the natural processes can reinforce the mycorrhizal potential in degraded ecosystems (Wahbi et al. 2016).

Increasing studies on the Arbuscular mycorrhizal fungi (AMF) has showed their importance for soil ecology (Bradford 2014) and studies on their biodiversity have spread in some agro-ecosystems such as corn and soybean monocultures (Carrenho et al. 2001; Gomes et al. 2015; Pontes et al. 2017) and coffee plantations (Cogo et al. 2017). Therefore, it is needed to deeply study the mycorrhizal functions under global change. In this chapter, we examine the major developments and advances on mycorrhizal fungi based on recent research from South American countries. New reports on the occurrence of mycorrhizas in Amazonian dark earth, as well as the inoculum production of arbuscular mycorrhizal fungi native of soils under native forest covers (dos Santos et al. 2017), have resulted in a more detailed understanding of the soil biology from South America.

Reports from Amazonian dark earth or "Terra preta do índio" soil has stimulated the use of biochar worldwide as a soil conditioner (Glaser 2007) that can add value to non-harvested agricultural products (Major et al. 2005) and promote plant growth. Few reports from Brazil showed that the addition of inorganic fertilizer, compost and chicken manure resulted in increases in plant cover and plant species richness (Major et al. 2005). In this sense, the biochar/mycorrhizae interactions also can be prioritized for sequestration of carbon in soils to contribute to climate change mitigation (Warnock et al. 2007).

17.2   The Mycorrhizal Symbioses in Agro-Ecosystems

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 noticed, as of 100,000 known fungal species more than one million are predictable to exist (Schueffler and Anke 2014). Among soil fungi, AMF are of special interest for agriculture and increasing investigation from South America is continuously reported (Stürmer and Siqueira 2006; Pagano and Covacevich 2011; Castillo et al. 2016).

More information on indigenous AMF occurrence in agro-ecosystems as well as enough understanding of inoculum persistence, and cover crops that favor the

indigenous arbuscular mycorrhizal fungi (AMF) by means of active roots (Douds et al. 2005) is needed. In general, compared to grasslands, conventionally managed fields can present low AMF diversity and low sporulation capacity (Thougnon Islas et al. 2016). Moreover, fruit plants (pineapple, Sapota trees) under organic management systems can also reduce the AMF species richness and abundance in relation to natural vegetation areas (Dantas et al. 2015).

Increasing interest in plant species for forest use as commercial plantations in Brazil has led to studies of response to inoculation of seedlings with AMF at different doses of P, such as for the Australian red cedar (*Toona ciliata* M. Roem var. *australis*) which presented high mycorrhizal root colonization, and thus, high quality seedlings (da Silva et al. 2017). Moreover, other researchers investigated the diversity of mycorrhizal fungi in planted forest in Northeast Brazil (Weber et al. unpublished) providing indication of Brazilian trees for reforestation in the tropical region.

AMF density and distribution vary both spatially and temporally with soil types and with host plant species diversity. Important economic plant species worldwide are being examined for AMF symbioses. Some agroecosystems have high economic interest such as coffee, vineyards and olive plantations, which are in the focus of interest from new technologies for their cultivation including their associated microbiota. Olive trees are mycotrophic species (Roldan-Fajardo and Barea 1986) associated with a high number of AMF species in the rhizosphere of plants growing in Morocco (Chliyeh et al. 2016) and Spain (Porras Soriano et al. 2002). The AMF diversity was studied for sustainable management of vineyards, showing low values in France (Bouffaud et al. 2016) and high values in vineyards from Germany under permanent vegetation cover or not (Oehl and Koch 2018). In Brazil, few studies such as from Rosa et al. (2016) investigated the application of AMF to reduce copper toxicity in young rootstock grapevines, pointing out some fungal species as promoters of great benefit. In the wine-growing regions of Southern Brazil a high humidity increases the susceptibility to foliar pathogens and thus, successive applications of copper fungicides are commonly used.

Much interest is nowadays dedicated in the preparation of inocula suitable for use in nurseries as this symbiosis improve plant performance and resistance to pathogens and water stress after transplantation. In Argentina, robust plants for field cultivation were obtained under greenhouse and nursery conditions by the coinoculation of two AMF strains at the beginning of plant propagation (Bompadre et al. 2014). It is known that the addition of organic amendments to the substrate can improve sporulation avoiding the replacing of nutrient solutions, vermicompost being commonly utilized. In Brazil, inoculated corn presented high number of infective propagules and biomass when inoculated with AMF and amended with vermicompost (Coelho et al. 2014). Peanut also responds positively when inoculated with different AMF species; however, the dependence on phosphorus (P) modified the plant responses (Hippler and Moreira 2013).

With regard to biochar, most reports are from Europe and the USA, and few reports from south American researchers, most from International Conferences, mention its interaction with AMF. One of them showed that biochar from *Eucalyptus*

at high temperatures (700 °C) improved plant growth and AMF root colonization of sorghum, besides a higher spore germination (Dela Piccolla et al. 2016). Reports from Chile showed the early effect of the application on wheat in an Andisol and Ultisol improving root colonization by native AMF and glomalin content besides soil properties, thus encouraging implementation of sustainable systems. Biochar also improved sustainable barley grain production in field trials in the Araucanía Region of southern Chile (Curaqueo et al. 2014a, b).

In Brazil, investigating field samples in economic tree plantations and cassava in crop rotation, Pereira et al. (2014) found higher AMF species richness (30 taxa) in rhizospheric soil samples. However, Oehl et al. (2005) stressed that deep soil layers should be included in studies to better know the AMF diversity, especially in agro-ecosystems, where soil stirring is frequent. At present, research on crops, especially corn (Gomes et al. 2015), have increased and new reports compiled new information on AMF (Table 17.1). Weber (2014) also compiled the importance of biofertilizers and AMF in agriculture (Fig. 17.1).

In Chile, reports compiled during the last 10 years form the Southern-Central zone showed a total of 21 genera (represented by 57 species of AMF) that have been recognized, equivalent to 21% of all AMF species described worldwide (Castillo et al. 2016). Twenty-four AMF species were associated with different cultivars of *Triticum aestivum* and, differently, *Acaulospora* and *Scutellospora* predominate. In that study, AM fungal community structure differed along wheat cultivars: 'Porfiado' and 'Invento', with 19 species in relation to 'Otto' cultivar (15 species) (Aguilera et al. 2014). Castillo et al. (2006) studied the effects of tillage on AMF propagules. They found little differences in spore numbers, however a high root colonization in no-tillage treatments. Moreover, *Scutellospora* was common under no-tillage.

In Argentina, earlier studies have found less management of AMF 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. To avoid decreases in the grassland productivity, which leads to decline livestock production, new studies including AMF ecology and on the impact of agricultural practices on AMF symbiosis pointed to a selective decrease of viable spore number with glyphosate applications in native grasslands (in the Flooding Pampa), resulting in altered AMF community structure. However, the use of sublethal doses of the herbicide was more useful contributing to project more sustainable land management agro-ecosystems (Druille et al. 2015). In this regard, undisturbed (pristine) soils could be considered a reserve pool of diversity of native AMF, showing that spore and large number of propagules (hyphae) can be the main source of inoculum. Thus, low or no relationship between spore number with the root colonization and/or glomalin content can be found (Thougnon Islas et al. 2016).

Investigating the richness of AMF in soybean fields in Argentina (Fig. 17.2), Faggioli et al. (2019) found 95 AMF virtual taxa (VT) belonging to 8 families: Acaulosporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, Glomeraceae (57), Pacisporaceae, and Paraglomeraceae. Among

Table 17.1 Total number of identified species in some agro-ecosystems/anthropic environments from South America

Country/state	Biome/region	Crops/ Vegetation type	AMF species/ genera	Indicator/dominant species	Root colonization by AMF/ECM	Reference
Brazil	Amazonia	Cowpea	Inoculation (13 isolates)	ND	1–82%	Silva et al. (2018)
Brazil	Amazonia	Secondary vegetation	12	ND	NE	Pagano et al. (2016)
Brazil	Amazonia	Secondary vegetation	24	ND	NE	Leal et al. (2009)
Brazil	Amazonia	Babassu palm	16	ND	NE	Nobre et al. (2018)
Brazil, Pernambuco	Atlantic rain forest	Sapodilla, rubber tree, mahogany, eucalyptus plantation and cassava	24–30	Detected	NE	Pereira et al. (2014)
Brazil	Atlantic rain forest/ Caatinga ecotone	Forest trees	15–20	Detected	15–57% AMF / 12–29% ECM	Weber et al. (unpublished)
Brazil	Cerrado (14 sites)	Coffee	70	ND	13–40%	Cogo et al. (2017)[†]
Brazil	Cerrado	Maize	10 genera	ND	NE	Gomes et al. 2015)
Brazil	Cerrado	Grassland (*Brachiaria brizantha*)	11	ND	40–62%	Ferreira et al. (2012)
Brazil	Atlantic rain forest/ Cerrado ecotone	Native and exotic trees	16	D	NE	Correa et al. (unpublished)
Brazil	Cerrado	Soybean	15–18	D	NE	Pontes et al. (2017)
Brazil / Sao Paulo	Sugarcane cropping region	Sugarcane	22	ND	30–52%	Azevedo et al. (2014)

(continued)

Table 17.1 (continued)

Country/state	Biome/region	Crops/ Vegetation type	AMF species/ genera	Indicator/dominant species	Root colonization by AMF/ECM	Reference
Brazil / Sao Paulo		Leguminous green manure and sunflower in rotation with sugarcane	NE	ND	49–74%	Ambrosano et al. (2010)
Brazil / Londrina, Paraná		Soybean and cotton	*Rhizophagus clarus* inoculation		~20–70%	Cely et al. (2016)
Brazil	Pampa	Various species	NM	NM	Presence	König et al. (2014)[†]
Brazil	Santa Catarina state/ Experimental Station	Cassava	*Rhizophagus clarus* inoculation	4–9	13–20%	Heberle et al. (2015)
Chile	Agroecosystems of the southern-central zone	Horticultural, wheat managed grasslands, wheat rotation, other crops	5–24	*Glomus* spp.	NM	Castillo et al. (2016)[†]
Argentina	Pampa	Wheat	NE	ND	NM	Schalamuk et al. (2013)
Argentina	Pampa	Wheat	Inoculation of *Glomus mosseae*	ND	~40%	Schalamuk et al. (2011)
Argentina	Pampa (126 sites)	Soybean	37 species	*Glomus fuegianum* (long term agriculture)		Faggioli (2016)
Argentina	Rainforest of Misiones	*Ilex paraguariensis* (traditional / high technology fertilized crops)	NE	ND	~25–50%	Velázquez et al. (2018)

AMF (spores): species (N° min – N° max); Indicator species (the most characteristic of a site): D (detected) or ND (not detected); NE (not evaluated); NM (not mentioned); †Checklist or review
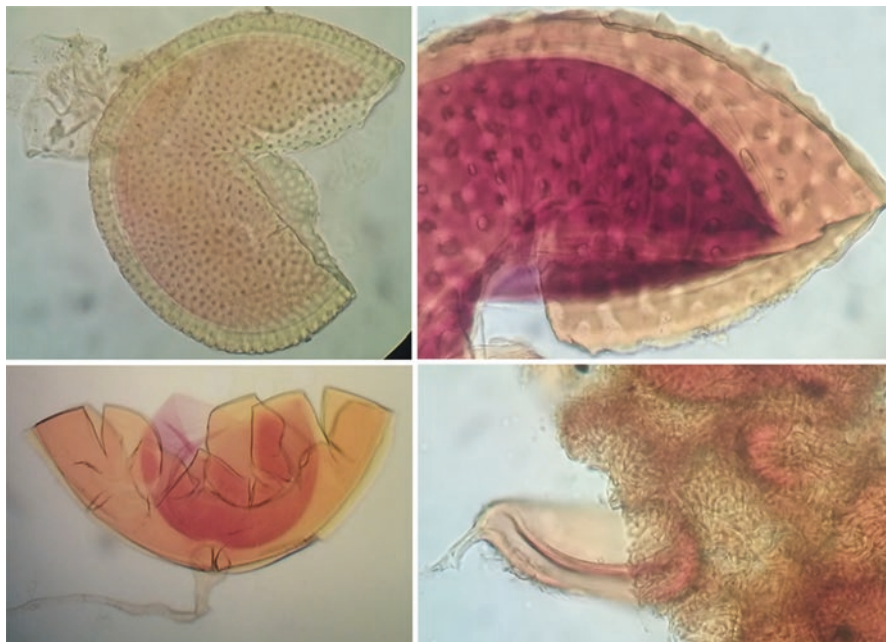
Fig. 17.1 Some AMF spores from cultivated areas in Brazil. Clockwise, from upper left: AMF spores of *Acaulospora* spp., *Glomus* and *Gigasporales* representant isolated from Northeast region (Photo-credit: M. Pagano)

them, Diversisporaceae was the most sensitive to long term Agricultural practices (Fig. 17.3). VT richness per sample did not differ between historical land uses and it could be attributed to the widespread use of no-tillage practices associated with soybean cultivation. This conservative soil management has been well documented as positive in the maintenance of AMF richness (Colombo et al. 2014). Soil textural components (i.e. clay and sand content) appeared as significant determiners of AMF richness (Fig. 17.4). Coarser soils were related to high VT richness in soil but low VT richness in roots. This probably was consequence of different textural preferences of AMF species (Lekberg et al. 2007). However, it is worth to highlight here that sandy soils were located in the driest area. Hence, the effect of drought on plant growth could also negatively affect key stages of AMF colonization resulting in the diminution of VT richness in roots of Livestock sites.

Among crop variables, only plant density was significantly correlated with VT richness (Fig. 17.5). Larger density of plant roots might improve resource availability for AMF because more carbohydrates would be available to support the symbiosis (Lekberg et al. 2010). In addition, roots and the associated fungal network might explore higher soil volume and contact propagules of rare and infrequent AMF species which may result in increases of VT richness. Therefore, our results reveal that appropriate plant density is a promising agronomical parameter for the maintenance of AMF species in agroecosystems.

Fig. 17.2 Historical land uses (HLU) currently cultivated with soybean in Pampas Region (Cordoba, Argentina): (**a**) agricultural, (**b**) Livestock-Agricultural, (**c**) Agricultural after recent deforestation of shrub land area. Each location was approximately 100 km from another one. Ten sampling sites were selected in each situation (Faggioli et al. 2019) (Photo-credit: V Faggioli)
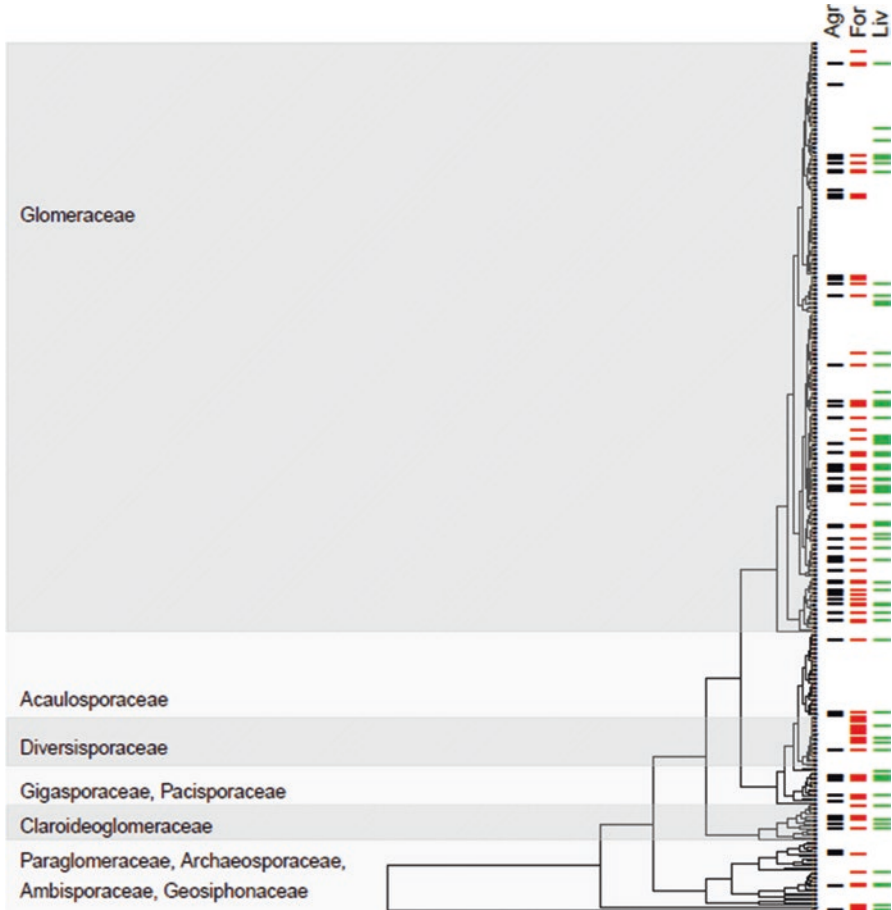
Fig. 17.3 Glomeromycota phylogenetic tree with virtual taxa (VT) recorded in different historical land use (HLU). The tree contains type SSU rRNA gene sequences of VT from the Maarj*AM* database (Öpik et al. 2010). Coloured lines indicate the presence of VT in HLU: Agricultural (Agr., black lines), Forest (For., red lines) and Livestock (Liv., green lines). Molecular study performed by 454 pyrosequencing and taxonomic assignment of sequences against MaarjAM database according to Faggioli et al. (2019)

In South America, the impact of different agricultural practices on AMF in arable fields is still poorly understood. Wheat phenology improved AMF biodiversity during grain filling; however, tilling and fertilization did not decrease spore biodiversity (Schalamuk et al. 2006). Spore populations of AMF communities in arable fields of wheat crop can vary between from just one to 4 spores g−1 soil in conventional tillage, from 3 to 5 in no-tillage (Schalamuk et al. 2013) but it also depends on plant phenological stages. Rarely more than 26 AMF species were reported in field studies (Schalamuk and Cabello 2010a, b). Pioneer studies on propagules in soils (propagule bank) from Argentina showed that different environmental
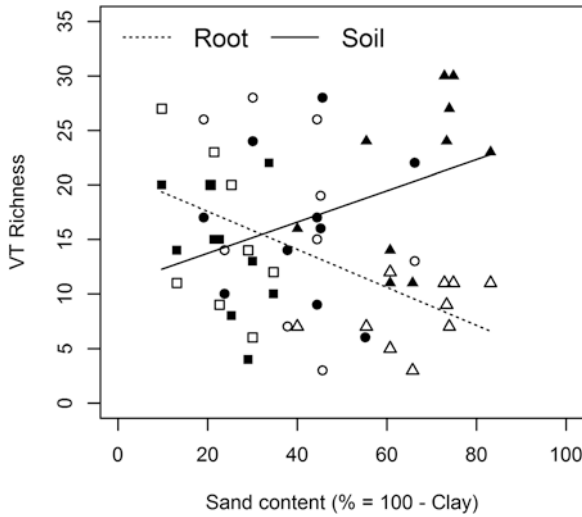
Fig. 17.4 Correlations between soil particles (%) and AMF Virtual Taxa (VT) richness from soybean fields with contrasting HLU: Agricultural (squares), Forest (circles) and Livestock (triangles); solid or empty symbols represent soil or root samples, respectively. Correlations are statistically significant (Spearman Test $p < 0.001$). Molecular study performed by 454 pyrosequencing and taxonomic assignment of sequences against MaarjAM database according to Faggioli et al. (2019)

Fig. 17.5 Correlation between plant density (number or plant per square meter) and VT richness in soil samples in soybean fields with contrasting HLU (p-value < 0.01, Spearman coefficient 0.47). Molecular study performed by 454 pyrosequencing and taxonomic assignment of sequences against MaarjAM database according to Faggioli et al. (2019)
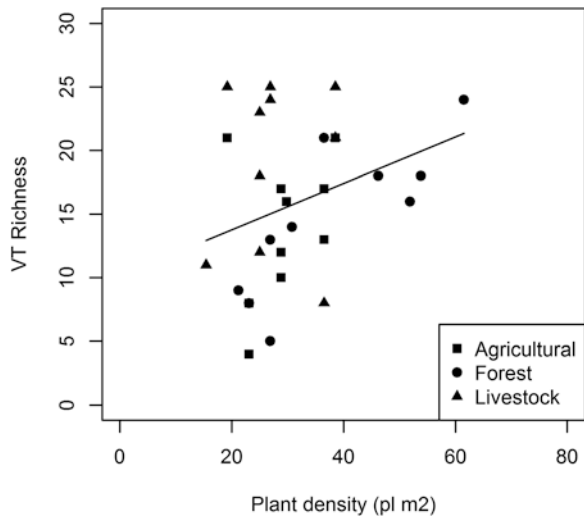
conditions and the effects of tillage and no-tillage modify both the composition of the AMF soil propagule bank and the diversity (Schalamuk and Cabello 2010a, b). Generally, Acaulosporaceae, Gigasporaceae, Glomeraceae can be found in agricultural fields; however, *Glomus* predominate (Schalamuk and Cabello 2010a, b). This can lead to think in different types of AMF inocula based on the proportions of their AMF families (Acaulosporaceae, Gigasporaceae, Glomeraceae) between field and trap cultures. For instance, in the forest garden, 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 against residues from the forestry industry (pine bark and pulp sludge).

In the trap cultures from agro-ecosystems more than 90% of AM species belong to Glomeraceae (Schalamuk and Cabello 2010a, b). *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 roots fragment (Hart and Reader 2002). Thus, in the trap cultures prepared from crop systems generally *Glomus* or *Acaulospora* species are recovered.

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 Blueberries 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 fruits plants (pineapple, Sapota trees) under organic management, detecting *Glomus* spores in all the areas, and corroborated the fact that soil management in organic cropping systems reduce 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 (Correa et al. unpublished) under different plant covers. High diversity and abundance were related to adjacent native forest, with 16 AMF species; however, grassland and maize field presented lower values.

Lastly, another anthropic environment is the man-made anthrosoils conformed by Amazonian Dark earth, also called Terra preta do índio (TPI), a highly fertile soil whose processes of formation has not yet been resolved (Hofwegen et al. 2009). In this regard, more recent reports (Tsai et al. 2009; Pagano et al. 2016) on the microbial communities of TPI have pointed to the presence of AMF of varied families/order unlike trends for cultivated field soils, with dominance of Glomeraceae. Black carbon prevalence and its unique physical and chemical characteristics, point it as the chief component conforming recalcitrant biochar with unique microbial communities (Tsai et al. 2009). For example, in TPI samples at different depths: from 0–20 to 100 cm, from Amazonas State, Brazil, Pagano et al. (2016) identified 12 AMF species (*Acaulospora bireticulata, A. mellea, A. rhemii, A. scrobiculata, A. spinosa, Ambispora appendicula, Claroideoglomus etunicatum, Scutellospora calospora, Racocetra castanea, Funneliformes geosporus, Glomus tortuosum, Pacispora franciscana*) and 6 were *Glomus* like species. Glomeromycota were dominated by Diversisporales, followed by Glomerales and Gigasporales.

As seen in previous observations in other soil types most of the AMF species richness and diversity (Shannon index) were concentrated in the topmost soil horizons. The *Scutellospora* species was found only in the deeper strata, in agreement with some previous reports (Oehl et al. 2005). *Scutellospora calospora* was also found in the control soil only at subsurface layer (0–20 cm) in contrast to its occurrence at 60–100 m in TPI soil samples.

With regard to the control adjacent soil samples (oxisol and ultisols), similar AMF species were detected, with 8 species identified and 3 unidentified. *Racocetra castanea* found only in the control soil at subsurface layer (0–20 cm) together with *Glomus tortuosum* (20–40 cm depth) occurred exclusively in adjacent soils and most species (11) were in common between the TPI and adjacent soils. This microbiological analysis showed that the abundance of AMF was greater in TPI than in control soils. AMF richness decreases only at great depth; however, diversity remained similar.

17.3   The Soil Conditioners in Agro-Ecosystems

Similar to methods to potentialize the mycorrhizal fungal inoculation of roots using soil amendments (Smith and Read 2008), no-tillage methods used to apply biochar into the root zone of crop soils and the mycorrhizal responses to biochar addition were amongst the pioneering works in biochar research. Another anthropic environment originated from South America is the ancient man-made anthrosoils conformed by Amazonian Dark earth, also called *Terra preta do índio* (TPI) (Fig. 17.5), a highly fertile soil whose processes of formation has not yet been resolved (Hofwegen et al. 2009). In Brazil, the Amazonian Dark Earth "Terra Preta" is dated about 7000 years being common at the Amazon basin (Falcão et al. 2003; Glaser 2007) and it is a promising subject to help sustainable agriculture, soil C sequestration and thus, climatic change mitigation. The climate at these areas is Koeppen's Af tropical rainforest with an annual average temperature between 25 °C and 35 °C. At the time of sampling the vegetation cover is usually secondary forest. In this regard, more recent reports (Tsai et al. 2009; Pagano et al. 2016) on the microbial communities of TPI have pointed to the presence of AMF of varied families/order unlike trends for cultivated field soils, with dominance of Glomeraceae. Black carbon prevalence and its unique physical and chemical characteristics, point it as the chief component conforming recalcitrant biochar with unique microbial communities (Tsai et al. 2009). For example, in TPI samples at different depths: from 0–20 to 100 cm, from Amazonas State, Brazil, Pagano et al. (2016) identified 11 AMF species (*Acaulospora bireticulata, A. mellea, A. rhemii, A. scrobiculata, A. spinosa, Ambispora appendicula, Claroideoglomus etunicatum, Scutellospora calospora, Racocetra castanea, Funneliformes geosporus, Glomus tortuosum, Pacispora franciscana*) and 6 were *Glomus* like species. Glomeromycota were dominated by Diversisporales, followed by Glomerales and Gigasporales.

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Lastly, there is more nuances in the study of TPI, the Terra preta (very dark, with broken potsherds and highly nutrient content) form under sites of home inhabitation, and the Terra mulata (light brown and with less nutrient content), which is less well documented. Thus, Amazonian dark earths are subdivided into: terra preta and terra mulata (black earths and brown earths respectively) (Kern and Kämpf 1989; Arroyo-Kalin 2008) that associates with respectively, past settlement areas and cultivated fields (Arroyo-Kalin 2010). TPI usually exhibit highly elevated levels of phosphorus (P), calcium (Ca) and other essential minerals for plants (Figs. 17.6 and 17.7). Terra mulata present less nutrient content, light brown, being adjacent to TPI

Fig. 17.6 Sites of Terra Preta de Índio in the Jiquitaia Farm (Lat 2° 37′S, Long 59° 40′W). The vegetation is secondary forest capoeira type, with approximately 40 years of age. Soil samples are usually collected from the 0–20 cm and 20–40 cm depth layers. Clockwise, from upper left: Overview of the area with Latossolo Amarelo with A anthropic horizon (Terra Preta de Indio) at Rio Preto da Eva, AM; Representative profil (Photo-credit: NPS Falcão) and spores of AMF retrieved from soil samples (Photo-credit: M Pagano)
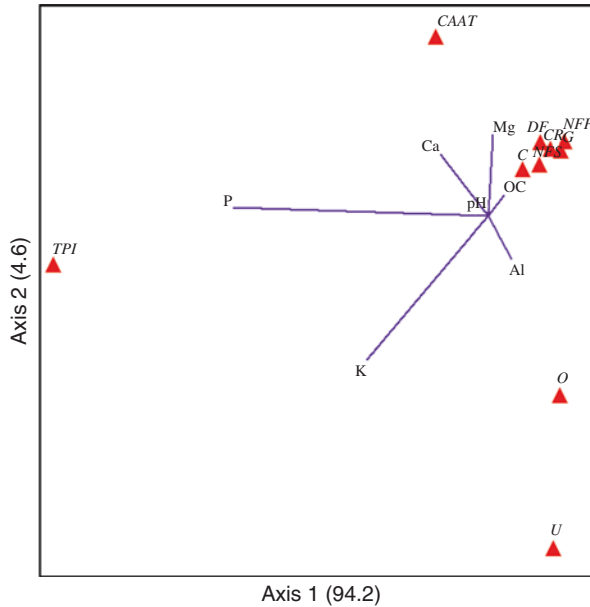
Fig. 17.7 Environmental similarity among some different soil samples (forest, TPI, control soils and cultivated sites) studied in Brazil. The similarity among the geographic areas represented as a nonmetric multidimensional scaling (NMDS). Distance and placement is indicative of similarity among areas TPI = "Terra preta do Indio" soil sample; U = ultisol and O = oxisol (control soils from the Amazon region), NFS = soil sample from Atlantic forest at Minas Gerais state; G = cultivated grassland at Minas Gerais state; NFP = soil sample from a Atlantic forest at Minas Gerais state, DF = soil from a dry forest at Ceará state; CR = soil from dry vegetation type and C = cultivated site

sites. It is believed that it was formed through intensive agriculture involving burning and mulching under low oxygen (Hecht 2003, Fraser et al. 2011).

17.4 AM Inoculation for Agro-Ecosystems

The mycorrhizal inoculation technologies or to manage native arbuscular mycorrhizal fungus communities can serve to replace or reinforce the mycorrhizal potential in degraded ecosystems (Wahbi et al. 2016). For example, to manage AMF soil infectivity in agrosystems it was proposed reductionist and holistic schemes that could be combined: the reductionist pattern aims to improve plant performance in disturbed soils by adding specialized AMF inocula adapted to the environmental conditions and to the target crop. Still, the objectives of the holistic pattern are to preserve and restore the composition of native AMF communities (Wahbi et al. 2016). However, benefits can be obtained from the integration of AMF in agricultural practices through the combination of the "reductionist" and "holistic" approaches (Wahbi et al. 2016).

The management of AMF in the rhizosphere provides an alternative to high inputs of fertilizers and pesticides in sustainable plant production systems (Reviewed by 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 a difficult multiplication on artificial growth media without a host (Sieverding 1991).

Some researchers suggested a careful choice of compatible host/mycorrhiza/substrate combination for crop success (Azcón-Aguilar and Barea 1997). Many methods are used to handle AMF, inoculating them on host plants, and replicating large amounts of inoculum. In vivo cultures of AMF species from different regions are preserved in ex-situ collections (Giovannetti and Avio 2002).

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 (Giovannetti and Avio 2002). But these inoculation procedures are highly expensive and only utilized in agriculture of high value products.

An alternative source of inocula is to use roadsides around crop fields as a repository for the conservation of AMF diversity affected by Land use (Dai et al. 2013). 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.

In South America, several works showed the feasibility and importance of AM inoculation in a large number of economic value and fruit plants. The applications of mycorrhizas in agriculture and environmental issues are still incipient. AMF inoculant for farm application requires large-scale multiplication fungi. The expensive technology of inoculum production comprises formation of single cultures of AMF. A cheaper method is the "on farm" system (farmers can produce their inoculum) (Douds et al. 2008, 2010), native AMF being more efficient due to local adaptation to the environment (Sreenivassa 1992). Infective propagules of AMF (spores, hypha and colonized roots) can be used as inoculum (Sieverding 1991).

In fertile soils from Argentina (Pampa Ondulada region), the effects of agronomic practices on the AMF communities, was reported by using pyrosequencing or a morphological approach (Colombo et al. 2014) showing that soil management has a negative effect on AMF community biodiversity. This study greatly improved the knowledge about AM fungi in South America where the molecular diversity of AM fungi was practically unknown.

Maize crop in Argentina is, after soybeans, the second most important crop (with the highest planted area, followed by wheat, citrus, sugarcane, and sunflower (Boix and Zinck 2008). However, non-tillage and contemporary hybrids with high yield that accumulation of 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 inoculum to compensate Zn deficiency in maize. The inoculum of indigenous AMF from sites presenting different levels of P and Zn resulted in changes in

Table 17.2 Some book or reviews dealing with AMF and ecological restoration in South America

Reports on AMF and plant restoration	Biome/ Country	References
Restored environments	Argentina, Brazil	Pagano (2012)
Riparian forest	Brazil	Braghirolli et al. (2012)
Arbuscular mycorrhizas in degraded land restoration	Brazil	Soares and Carneiro (2010)
Native species for restoration and conservation of biodiversity in South America	Argentina, Brazil	Pagano et al. (2012), Pagano (2016)

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 lowest efficient. Thus, to compare 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 2014).

Interestingly, in Colombia, the edaphic factors such as Soil pH had a direct relationship with species richness and with the diversity index, but, height above sea level can also affect the AMF community composition. Thus, a heterogeneous distribution in patches with little influence of the type of crop management (mono or polyculture) can be found. This highlight the constraints of developing specific biofertilizers for crops that contain AMF and not including natural adaptations to the different characteristics of the varied agriculture soil types (Mahecha-Vásquez and Sierra 2017). We lack the field studies that are needed to understand with confidence how to do an effective AMF inoculation.

With regard to the ecological restoration of species-rich grasslands that are of priority for conservation of biodiversity, reports have showed many options for that task in South America (Table 17.2). Torrez et al. (2016) determined if plant species recolonization of degraded nutrient-poor grasslands could be increased by adding a local source of AMF inoculum at different distances from intact remnant grasslands. There are effects by the well-dispersed generalist plant species, particularly at 20 m from the intact patches, the role of below-ground processes being crucial for restoration success that can be improved by AMF additions in the short term and at relatively close distances to intact grassland patches (Teste 2016). In Fig. 17.8 we show a protocol to add AM fungi to disturbed ecosystems.
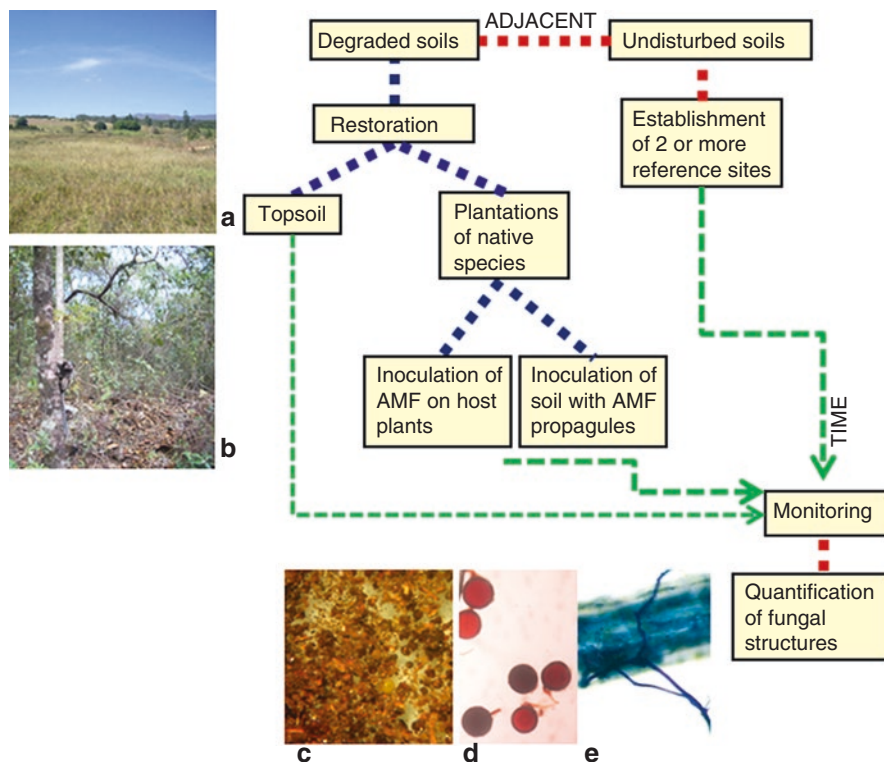
Fig. 17.8 Protocol to add AM fungi to disturbed ecosystems. After evaluation of diagnostic of degraded site (**a**) and establishment of 2 or more undisturbed sites (**b**), restoration can be achieved by introduction of topsoil or plantations. AMF inoculation can be performed on host plants or by inoculation of health soil (**c**). Monitoring the restored sites: determination of infective propagules including spores recovered from rhizospheric soils (**d**) and roots of plants growing in the degraded and reference soils stained for AM colonization (**e**) (Photos by M. Pagano)

17.5 Conclusion

In this chapter, the examination and use of arbuscular mycorrhizas in different crop systems has been mentioned and the needs for more information to understand agro-ecosystems and soils under different management have been highlighted. Throughout the chapter, the study of the occurrence of mycorrhizas in agriculture in South America were showed as still incipient. Morphological identification procedure of AMF continues to be important, although the specific training and experience. Moreover, better technology for commercial mycorrhizal inoculum is needed. Finally, this chapter argues that agro-ecosystems generally present low AMF diversity; however, organically managed fields are more similar to natural ecosystems, Amazonian dark earth being a model of highly fertile soils. Consequently, further

research is necessary on this field, especially regarding the applications of mycorrhizas.

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