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ARBUSCULAR MYCORRHIZAL PARAMETERS AND INDICATORS OF SOIL HEALTH AND FUNCTIONING: APPLICATIONS IN AGRICULTURAL AND AGROFORESTAL SYSTEMS

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ABSTRACT

Increasing recognition that agricultural intensification and soil pollution can adversely affect soil quality, modifying the number, diversity and activity of the soil microbiota, including symbiotic fungal populations, is being worldwide accepted. Soil is a living system, and this is a fact nowadays more accepted among researchers as well as farmers. Among the soil microbiota, the plant symbiotic arbuscular mycorrhizal fungi, which associate with terrestrial and also with some aquatic plants are usefully being studied in agriculture, agroforestry, restoration of degraded lands and in relation to endangered vegetal species. Nevertheless, arbuscular mycorrhizal fungi (AMF) are affected by disturbs in the ecosystems, like global change, pollution, fertilization, among others. AM fungi belong to Glomeromycota phylum, and form symbiotic associations with most plant species (80%). The presence and activity of arbuscular mycorrhizas can be measured throughout different methodologies, both in the soil or in plants, which inform us about the biomass, activity, diversity and their interaction with plants. An increased use of the AMF potential to counter the challenges of ecosystem restoration and food production is being nowadays recognized. The purpose of this chapter is to provide evidence for decision-makers and researchers of the significant links between the health of ecosystems and human well-being, based on case studies over world. Relevant findings are emphasized, such as the current information on the occurrence of symbioses in ecosystems, drawing on results of research on cultivated, forested and natural sites in Argentina and Brazil. AMF diversity is illustrated, and research directions that are needed to increase understanding of microbial associations aiming to the wise management of ecosystem services are pointed.

Keywords : Soil mycobiota – Mycorrhizal symbioses – Ecosystem services – Conservation

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INTRODUCTION

The development of the concept of soil quality involving the ability of the soil to maintain an appropriate productivity reducing the effect on the environment and contributing to human health has modified society's expectations of science, and there is an urgent need to improve the communication among researchers from multidisciplinary groups. Moreover, the interaction of scientists with decision makers is an issue of extreme importance for conservation of biodiversity to be used in future developments in sustainable agriculture.

Past research in arbuscular mycorrhizas (AM) has shown that reviews (e.g. Kling and Jakobsen 1998) have explained various methods for the assessment of mycorrhizas and have stressed the importance to include these fungi into the soil quality assessment. Nowadays, AM association is considered an important component in sustainable agriculture (Jeffries and Barea 2001); however, to the best of our knowledge only one review on arbuscular mycorrhizal fungi (AMF) and soil health (Jeffries et al. 2003) has been reported. Those authors focused on the antagonistic activity against soil-borne pathogens of AMF that implies their useful for maintaining healthy soils.

In the report by Francis and Read (2006) the needs to recognize the role of the mycorrhizal mycelium, by scientists involved in research on AM, extending their vision beyond the limited horizons which are currently defined by considerations of the phosphorus nutrition of individual host plants, were highlighted. In another recent review, Rillig and Mummey (2006) described the needs of consider feedbacks between soil structure and AMF, as well as pointed the little research dedicated to this topic. Pioneer research with AMF and soil compaction (due to the use of modern machinery in fields) by Miransari et al. (2007, 2009) showed that AMF inoculation can enhance corn growth in greenhouse. In addition, studies focusing on AMF propagules bank in soils (Schalamuk and Cabello 2009, 2010) have showed variations in AMF species composition in wheat crops under different tillage systems.

As stated by Allan et al. (1995) and Karlen et al. (1997) the soil quality can be defined as follows: "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation". However, among several definitions that given by Doran and Parkin (1994): "The capacity of the soil to interact with the ecosystem in order to maintain biological productivity, environmental quality and to promote animal and plant health" is preferred. The parameters for the evaluation of soil quality can be subdivided into: physical, chemical and biological; their integration being crucial to improve the actual knowledge on this topic. However, biological parameters gained particular importance because organisms respond more rapidly than most chemical and physical parameters to changes in land use, environmental condition or contamination (Burns et al. 2006 and references therein). Due to the important role of soil organisms in many processes that underpin soil quality, the abundance of fungi has also been used as indicator of soil quality. The aim of the present chapter is to outline the knowledge regarding AMF and soil health.

This includes topics on mycorrhizal effects on soil quality, AMF responses to several environmental stresses, findings about the natural occurrence of AMF in different cultivated soils and some ecological aspects of this specific association. Trends for future investigations are also outlined, which must elucidate the real benefits of mycorrhizae to ecosystem services. This chapter will review the current methods, and their advantages, for studying the AM contribution in soil health.

Arbuscular mycorrizal fungi

Mycorrhizal fungi associate with the roots of higher plants, indeed over 80% of plant species, including forest trees, wild grasses, some aquatic plants and most crops (Smith and Read 2008, Miranda 2008). Among the six mycorrhizal types (according to Read 2003), for the remainder of this chapter we focus on AM fungi, which belong the phylum Glomeromycota (Schussler et al. 2001). Those fungi present aseptate and coenocytic hyphae, with hundreds of nuclei sharing the same cytoplasm, that improves the rate of transport of nutrients (Smith and Read 2008). In all types of mycorrhizas a bidirectional movement of nutrients takes place, where carbon flows to the fungus and inorganic nutrients moves toward the plant (Smith and Read 2008).

Arbuscular mycorrhizas form obligate symbiotic associations between plants and fungi that colonize the cortical tissue in the roots during periods of active growth, being the most frequent association in nature and widely distributed geographically (Harley 1989, 1991).

The interaction between the fungus and its host plant mainly consists of nutrient transfer (the plant provides the AMF with carbon compounds while the fungus delivers nutrients to the plant). The increased nutrient uptake from the soil, particularly of phosphorus and nitrogen, is the main benefit attributed to mycorrhizal symbiosis (Smith and Read 2008, Govindarajulu et al. 2005). However, other benefits consist in enhancement of resistance to root parasites (Borowicz 2001), improvement of drought tolerance (Augé et al. 2001) and mitigation of impacts of environmental stresses such as salinity (Ruiz-Lozano et al. 1996). Other important role by improving soil stability which can diminish erosion is also attributed to AMF (Cuenca et al. 1998, Rillig et al. 2002, Allen et al. 2003, Rillig 2004). These fungi are grouped into 260 species described to date (Schussler et al. 2010; http://www.amf-phylogeny.com), and the effects that they have on their host plants differ greatly between fungal strains or species (O´Bannon et al. 1980, Miller et al. 1985, Bethlenfalvay et al. 1989, Modjo and Hendrix 1986).

In a recent review, Gianinazzi et al. (2010) stressed the key role of AM in ecosystem services (for agrosystems), listing the main roles that the AM symbiosis can play as an ecosystem service provider. Moreover, Bothe et al. (2010) emphasize the potential role of AMF in protecting endangered plants and habitats, pointing that AMF population specific of endangered species has not been investigated, probably supporting unique AMF.

METHODS TO EVALUATE SOIL HEALTH USING MICROORGANISMS

In previous review, Schoenholtz et al. (2000) listed some chemical and physical properties as indicators of soil quality of forest ecosystems. Those authors summarized the chemical properties separating the physical ones into two types: static (easily and routinely measured) and dynamic (more laborious and costly), including soil organic carbon (C), nutrient availability, soil acidity and salinity. Those authors also separated the physical ones into two types: static (soil texture, soil depth or topsoil depth, soil bulk density, available water holding capacity, soil roughness, saturated hydraulic conductivity, soil loss, soil strength, porosity, aggregate stability and size distribution, soil tilth) and dynamic indicators (least limiting water range, trafficability, leaching and erosion potential). However, they stated that soil quality indicator would be accurate to soil type. For a historical view of the evolution of the concept of soil quality in agriculture and forestry see Schoenholtz et al. (2000) and for a review of the concepts and terms of the soil quality management see Schjønning et al. (2004).

Recently, more attention has been paid to the biological parameters and their interrelation with the chemical and physical properties of soils (Jastrow et al. 2007) and the health of the soil microbial community, due to their critical role in soil C sequestration. For example, the size distribution and tortuosity of pores (which is related to aggregate size distribution) and in particular pore neck size, controls the distribution, movement and activity of decomposers and soil food web dynamics, affect the physical protection of soil organic C (Jastrow et al. 2007). Notably, several researchers over the world have recognised that minimizing disturbance of the soil by tillage (see Pagano in press and references therein) or erosion (Herrera et al. 1993), can enhance the fungal communities and the (bio)chemistry associated with soil humification.

After a meeting on the evaluation of soil quality, in Germany, the problems related to sampling, storage and pre-incubation of soil samples for microbiological analyses were discussed and the choice of the most efficient methods and indicators (Bloem and Breure 2003) was proposed. The methods can be divided into four groups, depending on the information they can provide: 1) Soil microbial biomass and number; 2) Soil microbial activity; 3) Soil microbial diversity and community structure; 4. Plant–microbe interactions (Benedetti and Dilly 2006).

Possibilities and restriction of AM as soil management indicators

While many kinds of microorganisms are considered of interest as indicators of soil management, AM fungi and their interactions with plants show distinctive characteristics which can be of importance in their potential use as indicators. Many traits of AMF can make them useful as indicators of soil management considering their high sensitiveness to disturbance.

The fact that Glomeromycota relies on the host constitutes a major difference with other microorganisms. The mycorrhizal communities are dependant on the C from the plant, and therefore they reflect the activity of the plants in the site. Besides, Glomeromycota have limited spread and viability in the absence of a live host, and this fact is of great interest for their use as indicators. For example, the poor ability of Glomeromycota fungi to spread in the space comparing to other microorganisms open the possibility of comparing contiguous sites or agricultural plots presenting different soil management. Moreover, the apparent lack or lower specificity compared to other symbiotic microorganisms, may be another interesting trait for using AMF as indicators of soil management, as it will be a more generalised model, in addition to the few diversity attributed to those fungi.

On the other hand, since mycorrhizal biomass comprises a great part of the fungal biomass in the soil (30%) (Rillig et al. 1999, Olsson 1999) changes of soil management (e.g. tillage, fertilization, disturbance) can reduce their presence.

Therefore, the accumulation of AM biomass in an undisturbed plot can greatly contrast with other neighbouring plots subjected to soil disturbance. Since different species of AMF, the vegetal community and environment factors are closely interrelated, these fungi are highly sensitive to soil management changes, and their use for monitoring the agriculture intensification and impacts for crop system functioning may be of great interest.

In previous work, Aon et al. (2001) showed that AMF are candidate to be "sensors" from the plant nutrition, and from soil physico-chemical conditions and disturbance. As Dick (1994) pointed that early indicators of ecosystem stress could play a role of "sensors" whose perturbation may sensitively inform us about soil degradation as compared to other slowly changing soil properties (soil organic matter), Aon et al. (2001) pointed the possibility to use AMF as soil indicators.

It was highlighted that mycorrhizal propagules can be severely influenced by damage to vegetation and soils resulting from human intervention, as well as intense fires, topsoil removal and flooding (Brundrett 2002). The number of AMF surviving propagules in soils decline with time in the absence of host plants (Brundrett 2002). Therefore, the abundance of AM fungi can be used as an indicador of the above mentioned degradation in the soil, and also for comparing soils with different agricultural practices. To restore the inoculum potencial of AMF in eroded soils, bioaugmentation (inoculating soils with AMF or by using transplanted seedlings that already have the appropriate AMF in their roots) is an alternative (Jeffries et al. 2003). Alternatively, indigenous but depleted populations of AMF may be restored by the use of a mycotrophic cover crop that stimulates the development of inoculum such that subsequent crops or plant communities gain the benefits (Dodd et al. 1990). On the contrary, the extraradical hyphal network, which is often the most important source of AMF propagules, as we explained above, can be easily damaged (for instance, mechanically through tillage), and can be reduced when a non-mycotrophic crop is cultivated (e.g. Brassicaceae or Chenopodiaceae).

Thus, we can assume that AMF communities reflect the past activity of the plant host which had been growing in the site, and then they could indicate several events which had occurred there, such as tillage systems used in the plots, fires, contamination, clearing, grazing, long fallow, herbicide application or any cause to eliminate vegetation, and this could reflect in AMF biomass. This is due to the direct relationship of AMF with host plants.

Different ways to evaluate mycorrhizae and soil quality, and the parameters and methods to adopt, have been discussed in previous papers (Kling and Jakobsen 1998 and references therein, Rillig 2004, Kahiluoto et al. 2009) (Table 1), however, little has been stated with regard to indicators and monitoring systems to report on trends for the future and to evaluate the effects of soil management, which are needed by Policy makers, as well as land users (Benedetti and Dilly 2006). The AM were included in one (Plant–microbe interactions) of four groups, according to the classification into groups of microbiological, biochemical and molecular methods (Benedetti and Dilly 2006).

The methods can be divided into four groups, depending on the information they can provide:

I. Soil microbial biomass and number.
II. Soil microbial activity.
III. Soil microbial diversity and community structure.
IV. Plant–microbe interactions.

Following the methodology proposed by Benedetti and Dilly (2006) in this chapter we attempt to order the methods for measuring Glomeromycota in the plant and soil into the groups above mentioned:

I: Estimations of Arbuscular mycorrhizal biomass and number

The estimations of AM biomass in the soil are problematic, taking into account the difficulties of differentiating the hyphae which belong to mycorrhizal fungi from those of other fungi. Direct extraction of the hyphae from the soil is also a difficult and time consuming process. Therefore, besides the extraction and quantification of AMF hyphae, biochemical methods using specific markers and other indirect methods have been developed to estimate mycorrhizal structures in soil. Since AMF spores (Fig. 3) have been easier than hyphae for extracting from the soil, the spore number have been a valuable and direct indicator of the abundance of AMF in soils. However, spore number show some limitations which will be discussed later in this chapter. The bioassay developed by Plenchette et al. (1989) is a practical method to evaluate the inoculum potential of mycorrhizas, and we will mention it below in this section.

Methods using direct extraction of hyphae from soils

AMF biomass in soils can be estimated with measurements of hyphal length, trough an aqueous extraction of soil (Abbot et al. 1984) or by adding 20% H_2O_2, and processed (see Herrera et al. 1986) finally obtaining a subsample (0.01g) which is mixed with 2 drops of glycerin on a microscope slide, then the number of coenocytic hyphae typical of AMF is counted at x100 magnification using a compound microscope. Thus, the length of AMF hyphae in soil or litter can be calculated (Aristizábal et al. 2004). Nevertheless, other modifications of this method can be found in research papers (e.g. Egerton-Warbunton et al. 2003). This method may underestimate hyphal length in soils with high OM or clay content, and moreover this method does not distinguish between dead and living hyphae (Kling and Jakobsen 1998). Therefore, vital staining techniques are available (e.g. succinate dehydrogenase), which can be used for to evaluate the active mycelium, both inside and outside the roots (Kough et al. 1987).

Studies on external hyphae of AMF are scarce. Past research in grasslands (Miller et al. 1995) has shown that external hyphae of AMF were identified and quantified by the gridline intercept method. Notably, those authors mentioned many AMF spores and auxiliary cells associated with hyphae. However, most of the studies carried out on AM lack measurements of external hyphae, especially field studies (Miller et al. 1995). The extraradical mycelium (large portion of the AM mycelium outside the root) grow dispersedly and hyphae of small diameter (<5 µm) are difficult to study quantitatively, as their longevity assumed to be short (5 to 6 days), which represent a high turnover rate (Staddon et al. 2003).

Lastly, Manns et al. (2007) evaluated the fungal hyphal number and length in a microcosm study, with growing and dried ground cover. They enumerated fungi by counting the hyphal fragments in soil solution,following this method: a soil solution is made by blending 1 g fresh soil in 100 ml distilled water at high speed and filtering at 0.5 mm to remove heavy sediment. Then, samples of a mixture of 1 ml of filtrate and two drops of 5% ink/vinegar stain are viewed in a grid-lined Petri dish at 20x magnification with a stereoscopic microscope. The hyphal fragments are visually distinct and therefore counted separately as large diameter (>5 µm, which appeared darkly stained) or small diameter (<5 µm, which are barely visible as lightly stained) hyphae. The size distinction of 5 µm can be confirmed with microscope pictures at 650x magnification.

As a diameter of 5–10 µm was specified for AMF hyphae (Read and Boyd 1986), Schreiner and Bethlenfalvay (2003) assumed that hyphae >5 µm diameter are primarily mycorrhizal and hyphae <5 µm diameter mainly saprophytic fungi, but recent research indicates AM can vary in diameter within the mycelium, according to their location in branching (Rillig et al. 2002).

Given current knowledge, diverse AMF communities can produce a more extensive mycelium, which could be related with a more efficient exploitation of nutrients from soils to plants (Hart and Klironomos 2003). Past research have shown hyphal anastomose for *Glomus* (Giovanetti et al. 1999). However, De La Providencia et al. (2005) pointed that AMF showed distinct patterns of anastomosis formation between *Glomeraceae* (that is, the family of AMF with the smallest spores) and *Gigasporaceae* (that is, the family of AMF with higher spore size) mainly in the number of anastomosis per hyphal length. Additionally, *Glomeraceae* presented anastomosis between different hyphae, whereas *Gigasporaceae* more often showed bridges in the same hyphae. These differences, which indicate functional complementarities, are also important, with the goal being to ecological studies of AMF, and specially in the implications for agriculture innovation.

Estimations of AMF biomass using biochemical markers

The utilisation of lipid markers, such as the fatty acid 16:1ω5, which is found especially in *Glomus* has been of particular interest (Paul and Clark 1996, Gaspar et al. 2001, Gryndler et al. 2006). However the resolution of this method is low (that is, it can not be used at species level) and moreover, the lipid composition of a mycelium can change with time and environmental factors, therefore this method is suitable for use in combination with others.

There are also enzymatic methods, such as the fluorescein diacetate (FDA) hydrolytic activity, which may be used as a rapid, cheap, and reliable estimator of fungal biomass (Gaspar et al. 2001). Owing to the high relationship exhibited by FDA hydrolysis with organic carbon and total nitrogen content of soil (soybean and wheat fields), the enzymatic activity was correlated with the microbial biomass estimated through marker lipids or plate counts. It is known that there are also methods involving compartmentation and tracers (Kling and Jakobsen 1998).

The role of the AMF mycelium in the soil aggregation (Figure 1) is well documented (for a more exhaustive review, see Rillig 2004). Additionaly, AMF produce a very stable hydrophobic glycoprotein, glomalin. There are four common measurements of glomalin: Bradford reactive soil protein (BRSP), easily extractable BRSP (EE-BRSP), immunoreactive soil protein (IRSP), and easily extractable IRSP (EE-IRSP). They are determined by extraction process (easily extractable vs. total glomalin) and detection method (Bradford protein vs. enzyme-linked immunosorbent assay [ELISA]) (review by Treseder and Turner 2007). Although the antibody approach appears to be more specific, polyphenols from the leaf litter (soils with high concentrations of organic matter) may overestimate glomalin content in the Bradford, and underestimate in the ELISA assay.

Treseder and Turner (2007) stated that glomalin is deposited on the outer hyphal wall and, as the AM hyphae senesce, they are thought to leave a residue of glomalin in the soil. Hyphal stocks, hyphal glomalin content, and hyphal turnover rate seems to determine the rates at which glomalin is deposited in the soil. With regard to lifespans of AM hyphae, reports for laboratory studies indicate that they might survive on the order of a few days to a few months, however no data is available for natural systems. Given current knowledge, glomalin concentrations in soil are positively related to net primary productivity, are augmented under elevated CO_2, and are often greater in the presence of AM host plants that maintain relatively high AM colonization rates (Treseder and Turner 2007).

Bioassay for measurement of mycorrhiza formation

The bioassays for measurement of mycorrhiza formation provide information about the ability of AM inocula present in the soil to colonize roots. Estimations of inoculum potential based on spore counts or root colonization have been found unreliable since propagules of AMF (spores, sporocarps, vesicles, colonized root fragments) (Figure 1, 2 and 3) are difficult to quantify (Smith and Read 2008), and their viability varies with many factors, such as the climatic conditions or the C concentration in soil (Lima et al. 2007). Therefore, bioassays using diluted soils and mycotrophic species, such as the method developed by Plenchette et al. (1989) allow to compare the ability of different soils to induce colonization in plants, depending on the activity of all the types of propagules in soil. Although these methods provide valuable information, they cannot allow to distinguish the relative contributions of the different types of propagules to the colonization of root systems (Smith and Read 2008).

The AMF propagule levels can be determined by a bioassay based on Plenchette et al. (1989), by dilute soil e.g. 100, 30, 10 and 3 % with the same autoclaved soil to provide a logarithmic scale of concentration. Then, Leek (*Allium porrum* L.) or seeds of other highly mycotrophic species are surface sterilized and grown in sterilized substrate. Later ten plantlets are planted in each pot containing soil dilutions of each treatment. Leek plants are colonized depending on the inoculum level and four weeks after planting, they are harvested and root colonization evaluated (Phillips and Hayman 1970). The mycorrhizal soil infectivity is calculated using regression analysis (Plenchette et al. 1989). The rate of initiation of primary colonization from propagules in soil is influenced by the availability and density of inoculum. At the first crop stages the percentage of colonization depends in a great extent on the propagule density of the soil (Sieverding 1991).

Evaluation of AMF spores from soils

Spores of AMF are normally formed terminally on absorting hyphae (Bago et al. 1998); however, some AMF species such as *Glomus irregulare* and *Glomus intraradices* can sporulate inside roots or in the soil (Błaszkowski et al. 2008). Spore abundance is evaluated as spore density (number of spores / ml soil) or spore number, usually using 100g of sampled soil. Spore number is the most common tool used as rough indicator to infer the Glomeromycota occurrence, biomass and the reproductive capability of the AMF species present in soils. It is well known that spores can survive in soil for several years (Sieverding 1991). Their survival depends on morphological traits, mainly determined by the specie of Glomeromycota, as well as biotic and abiotic conditions.

Although spore numbers should be considered as useful indicators for AMF activity in a soil system, the presence of AMF spores does not always imply recent activity of the fungal symbionts (Schalamuk and Cabello 2010). Spores are included in the AMF soil propagule bank. In undisturbed soil, it is expected that new infection units arise primarily from extraradical hyphae, spores being less important (Jasper et al. 1989) due to their dormancy.

In 1983, Tommerup related the erratic germination of spores, which are dormant when first formed, but after storage dormancy is overcome under appropriate conditions of moisture and temperature. The role of the different types of propagules (Figure 1) in the colonization of plant host roots in field, is difficult to distinguish and, moreover, the rate of initiation of primary root colonization from propagules in soil is influenced by the availability and density of inoculum (Smith and Read 2008). Additionally, large spores can contain more resources to support multiple germination and hyphal growth in the period while the AMF is searching for a host (De Souza et al. 2005).

The number of spores reflects both the sporulation and the action of many factors that affect their survival and accumulation in the soil. Therefore, its number or density is the result of a complex balance, and while the sporulation probably is related to the recent activity of the AMF, the number of spores in the soil may not be conclusive information because the measurements include spores formed earlier in the season or in the previous seasons (the number of spores in soils, includes recent formed structures, as well as spores formed earlier). Therefore, the survival of the spores in the soil has great impact on the variations that occur between different moments of sampling (Lee and Koske 1994a). Experiments carried out in pots showed the production of spores depends mainly on the growth of the host plant, the fertilizer application and the intensity of light (Furlan and Fortin 1977, Douds and Schenck 1990). Experiments under field conditions, pointed that the increase in spore number can be associated to the root growth (Hayman 1970) and with the maturity or senescence of the host (Hayman 1970, Koske and Halvorson 1981, Giovannetti 1985, Gemma et al. 1989). In addition, increases in the number of spores have been related to the progress of the fungal colonization in the roots (Gazey et al. 1992). However, the relationship between the number of these structures and the root colonization is unclear (Smith and Read 2008), and some authors have found positive correlations between these two variables (Hayman 1970, Barron and Sutton 1972, Giovannetti 1985, Jakobsen and Nielsen 1983), while others found no association (Powell 1977, Daniels and Bloom 1986) or even inverse relationship were observed (Louis and Lim 1987).

The death of spores is one of the main factors determining the variations in the number or density of these structures in the soil. Despite its importance, the literature on spore survival is little compared to that about sporulation (Lee and Koske 1994a). In natural ecosystems, decreases in the number of these structures have been attributed to their germination, the activity of macro and micro fauna, and their destruction by other soil fungi and parasites (Gerdemann and Trappe 1974, McIlveen and Cole 1976, Ross and Ruttencutter 1977, Ross and Daniels 1982, Rabatin and Stinner 1985, 1988). AMF are commonly infected with other fungi (Daniels and Menge 1980, Lee and Koske 1994a, Rousseau et al. 1996) or by actinomycetes (Lee and Koske 1994b). The environmental conditions have an influence on these processes (Janos 1980, Koske 1988), as well as agronomic practices can also generate decreases in the density of spores in the soil from agroecosystems. For instance, inversion of the soil, in conventional tillage, dilute soil rich in propagules with other poor in propagules, affecting the presence of spores in the soil (Crovetto 1985, Sieverding 1991).

The AMF spore biovolume (spores present in the soil) can also be calculated by using the following equations: $V = 1/6\pi D^3$ (D = spore diameter) for species with spherical spores, or $V= 1/6\pi D_1 D_2^2$ (D_1= larger dimension; D_2 = smaller dimension) for species with elongated spores (Wolf et al. 2003). The dimensions used for biovolume calculations are, thus, represented by the mean for each morphotype measuring 50 spores of each morphotype by using i.e. the Quantimet 500 image analysis software (Leica, Milan, Italy) (Bedini et al. 2007).

Lastly, AMF are affected by disturbs in the ecosystems, like heavy metal pollution (Meharg and Cairney 2000); however, these fungi can accumulate metals from soil components (Gadd 2005). Morphological studies of small structures are possible using energy-dispersive spectrometers and wavelength-dispersive spectrometers (WDS) coupled to a scanning electron microscope (SEM); however, these methods do not detect minor and trace elements (Przybylowicz et al. 2004). Not only is the Energy-dispersive X-ray spectrometry (EDS) technique limited to the detection of elements with an ordinal number between 10 and 25, such as aluminum (Al), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and sulfur (S) (Leapman and Hunt 1991), but also peak values of micronutrients and lighter elements, such as nitrogen (N), cannot be clearly distinguished from the background (Bücking et al. 1998). There are increasing reports of metal-tolerant AMF (Weiersbye et al. 1999, Orlowska et al. 2002, Hildebrandt et al. 2007, Soares and Siqueira 2008, Pagano et al. 2010); however, some of them did not identify isolated AMF species. Moreover, Cruz (2004) highlighted that quantitative light element microanalysis of AMF spores employing EDS is a technique still little explored and which may inform the chemical spectrum of AMF spores and point the differences among species. In Brazil, Pagano et al. (2010) reported that nickel, which at higher concentrations can lead to poisoning (heavy metal), detected in only one spore type (*Scutellospora reticulata*) frequent in restored riparian site of Velhas River, Minas Gerais State was related to riverine soil pollution.

II. Estimations of the Arbuscular mycorrhizal activity

Since spores accumulate in soil during several years, they are not direct indicators of actual fungal activity. Thus, the density of viable spores can be measured, separating them from soil by wet sieving, incubated in iodonitrotetrazolium chloride solution (10 g L^{-1} according to Walley and Germida 1995) in Petri dishes, and counted; those stained with this colorimetric assay are considered viable. Another technique is the incubation in 3-(4,5-dimethillthiazol-yl) -2,5-diphenil-2Htetrazoilium bromide (MTT) (An and Hendrix 1988). Petri dishes are left at room temperature for about five days, therefore, viable spores (those that stain red) are counted. Spores without citoplasmic contain, and non stained ones, are considered non viable. Notably, the spore extraction can be carried out about a year after sample collection. For example, Lima et al. (2007) found that 2.58% of spores were viable propagules in tropical dry forest converted into areas used for subsistence agriculture or woody production in Brazil.

III. *Arbuscular mycorrhizal diversity*

AM fungal spores are commonly isolated and identified using morphological characters. Spores can be extracted from soil by wet sieving procedures (Gerdemann and Nicolson 1963), decanting and sucrose centrifugation (Walker et al. 1982), counted (just healthy spores) and the analyzed data are usually expressed as number of spores/100 grams of dry soil. Each spore type is mounted in PVLG (polyvinyl alcohol-lactic acid glycerol), and a mixture of PVLG and Melzer's reagent for identification, as well as to obtain permanent voucher specimens. Morphological properties and subcellular structures are observed under light microscopy at 100x magnification. Identification is based on spore colour, size, surface ornamentation and wall structure, with reference to the descriptions provided in a manual by Schenck and Pérez (1988), the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, West Virginia, USA; www.invam.caf.wvu.edu), Błaszkowski, http://www.agro.ar.szczecin.pl/~jbłaszkowski/, and the original species descriptions, which are available or interchanged among researchers. In general*,* spore numbers are square root transformed and statistically analyzed. Already Stürmer and Siqueira (2006) in his review encouraged micorrhizobiologist from Brazil to develop a germplasm bank of Glomerales due to its relevance for scientific research. As Pagano and Covacevich (in press) compiled taxonomical and biodiversity studies of AM are restrained by the lack of considerable experience of both culturing and spore morphotyping by researchers. Moreover, changes in taxonomy of AMF need a continuous actualization of terms, for example the recent designation of the new genus *Racocetra* is nowadays more accepted.

The problems associated with measuring microbial diversity in soil have been reviewed (Kirk et al. 2004), molecular techniques showing up as overestimate AMF diversity. Moreover, a high proportion of undescribed AMF species is nowadays increasingly recorded in the studied ecosystems (Pagano et al. 2008, 2009, 2010; Stürmer and Siqueira 2006, 2010) as well as, sampling effort and strategy can affect detection of AMF community structure (Whitcomb and Stutz 2007).

Therefore, there are traditional and molecular methods to characterize fungal communities. For example, as we discuss above, approximately 260 species of AMF have been described by traditional-taxonomical studies. However, the morphological diversity of AMF spores may not reflect their physiological and genetic plasticity, which suggests that morphospecies may not be sufficient or appropriate for ecological studies (Clapp et al. 2001). For that reason biochemical and molecular techniques based on DNA analysis (e.g. isozyme profiles or sequencing of ribosomal genes) have been used to identify the genetic heterogeneity of AMF (see Pagano and Covacevich, in press). Recent advances in DNA identification and designed primers (reviewed by Covacevich 2010) could probably modify AMF species delimitation in the future (for instance there are spore and root identification). In the study of AMF, the use of molecular techniques avoid problems associated with morphological identification. The polymerase chain reaction (PCR) can target specific AMF DNA sequences, the majority being ribosomal RNA (rDNA) genes, and sequence variation can be visualized with electrophoresis (see Clapp et al. 2002 for a review).

Molecular techniques aided the characterization of AMF to enhance our understanding of their ecology (Helgason et al. 1998, Liang et al. 2008), evolution (Sanders 2002) and phylogeny (Schussler et al. 2001); however, there are many AMF genotypes present in field systems that cannot be cultured (Clapp et al. 2003).

Simon et al. (1992) initiated molecular characterization of AMF, and nowadays different PCR-based methods have been applied to AMF, denaturing gradient gel electrophoresis (DGGE) (Kowalchuk et al. 2002, Opik et al. 2003, Ma et al. 2005), and temperature gradient gel electrophoresis (TGGE) (Cornejo et al. 2004).

Trap cultures

To obtain species which did not form spores in the site at the moment of collection in the field, we can establish trap plants in pots under greenhouse conditions.

Several papers indicated that "trap cultures" using diluted soil have helped to find species that had not been observed in field samples (Miller et al. 1985, An et al. 1990, Stutz and Morton, 1996, Oehl et al. 2004, Wang et al. 2008). However, this can occur or not, and some studies found fewer AMF species in the trap cultures than in fields (Tchabi et al. 2008, Schalamuk and Cabello 2010).

For trap cultures using soil, Sieverding (1991) has detailed the procedure: a part of field soil (250 g or less) (commonly containing the three types of AMF propagules: spores, mycelium and colonized roots) collected from each soil treatment is mixed with a tindalized substrate composed of perlite-vermiculite (1:1 v/v), and placed in 2 L pots. A gramineae and a legume as *Sorghum vulgare* L and *Medicago sativa* L, respectively, are planted to host AMF in the cultures. Then, seedlings (germinated and grown in sterile sand), are transplanted into these pots. Thus in the study of AMF communities at field sites, a long-term strategy may be employed: a preliminary thorough sampling of the study site is conducted (spores are isolated, segregated into species type groups, and pot cultures initiated). Trap cultures, using soil and roots collected from the field, must be monitored regularly for spore production. Then, spores are isolated, identified, and used to produce monoespecific cultures. Species richness of the site then can be calculated. When a familiarity with the AMF species of the site is achieved, the identification of spores directly from field soil in response to management practices can be attained. Dominance, diversity and biovolume indices may be calculated to describe the sporulating community (Douds and Millner 1999).

The indirect culture strategy (trap culture) is time consuming and biases are often introduced by plant preference for AMF species, different growth conditions, and other environmental factors, which disfavor its suitability for characterization of AMF communities (Oehl et al. 2003); however some AMF species can be successfully isolated and propagated.

A complete description of the AMF community of a soil would include the identity of fungi present as spores, extraradical and intraradical hyphae and vesicles as well as information on the relative abundance of each species in each component; however that exhaustive description is impossible at present.

Molecular techniques exist to identify hyphae in roots and soil of a few of the described AMF species. Though these methods show great promise, currently they have utility only to trace the persistence of an introduced isolate for which one has a probe, or interactions among several isolates under controlled conditions. Quantification of the effects of agricultural management practices upon communities of AMF presently requires a compromise. Nowadays we quantify the total hyphal length in soil and total mycorrhization of plant roots. The identification of AMF species at a site as well as the quantification of diversity and dominance is limited to the sporulating species. Non-sporulating species can be detected via trap cultures, which give no indication of the relative abundance of AMF species in the field sample. Thus, description of the community based on spore counts and identification probably evidence inaccurately the total contributed biomass of each species to the community. Furthermore, we have scarce information about which species are primary contributors of the extraradical mycelium which enhance nutrient uptake of roots and produce glomalin, thus having a significant role in soil aggregation (Douds and Millner 1999).

Functional diversity of AMF

Variations in function of fungal structures provide information about functional diversity in relation to the diversity of AMF; however, as van der Heijden (2003) stated, the studies of mycorrhizal functional diversity are in their infancy. Our ability to generalize about interactions between AMF and plants is currently weak, as a result of the limited amount of work in this area. Wolfe and Klironomos (2005) enumerated the techniques for assessing the functional diversity of soil microbial communities.

With regard to AMF, it is known that there are differences among families (Hart et al. 2001, De Souza et al. 2005). However, to establish those differences is difficult because various methodologies are used to estimate them. Some authors used the index of diversity to inform about the biodiversity of a site or to compare the diversity among different treatments or sites in the same biome; however, it is difficult to use them as indicators.

A great number of scientific papers, mainly reviews, pointed out interesting reflections or assumptions on soil microbial biodiversity and ecosystem function (i.e. Turco et al. 1994, Beare et al. 1995, Kennedy and Smith 1995, Bengtsson 1996, 1998, Wolters 1997, Giller et al. 1997, Sparling 1997, Bowman 1998, Andrén and Balandreau 1999, Wardle et al. 1999a,b). However, taken together, the majority of information given here does not allow the conclusion to be drawn that soil biodiversity regulates soil fertility. On the contrary, the majority of authors advocate an opinion which was provocatively expressed by Bengtsson (1996) *"there is no (direct) mechanistic relationship between diversity and ecosystem function. To think that one single number – species richness or a diversity index - can capture the complex relationships between many species and the functional roles of these interactions is ..naive .. and negates most ecological research since the 1960s"*. More basic research is needed since facts are missing. Indeed there is an absolute discrepancy between the number of discussion and experimental papers on this topic, with a very low number of the latter.

There still exists a fundamental lack of knowledge concerning the functional capabilities of AMF assemblages in the field (Gamper et al. 2010). AMF are functionally diverse at several levels of systematic integration and vary widely across a range of characters (resistance to root parasites (Borowicz 2001), improvement of drought tolerance (Augé et al. 2001) and mitigation of impacts of environmental stresses such as salinity (Ruiz-Lozano et al. 1996).

Recent review by Treseder and Turner (2007) compiled the different ecological traits of *Glomus:* less investment in extraradical hyphae and more in intraradical root structures than *Gigaspora*, *Acaulospora*, and *Scutellospora.* Those authors linked the fact that *Glomus* often dominates the AM community following N additions, when host plants are thought to reduce the investment of C in AM fungi; and, conversely, *Glomus* frequently declines under atmospheric CO_2 enrichment, when plants should be allocating more C to their symbionts, suggesting that *Glomus* is particularly suited to situations in which host plant C is relatively limiting. This suite of ecological traits is consistent with the intrinsic tendency of *Glomus* to produce less glomalin per unit biomass, since glomalin requires a notable investment of C.

As regards species composition Egerton-Warbunton and Allen (2000) found three species of *Glomus*, which could be useful indicators of eutrophization (nitrogen enrichment) in coastal sage scrub in southern California. Studies have revealed that Glomeromycota taxa may vary in their colonization strategies, with regard to the use of different propagule types by the major AM families (Tommerup and Abbott 1981, Biermann and Linderman 1983, Braunberger et al. 1996, Brundrett et al. 1999, Klironomos and Hart 2002, Hart and Reader 2002, 2004, Schalamuk and Cabello 2010). However, contrasting evidence exists on the ability of each Glomeromycota family to use each propagule type. According to Hart and Reader (2004) *Gigasporaceae* is less sensitive to soil disturbance than *Glomeraceae*, based on differences in their colonization strategies. *Gigasporaceae* colonize primarily from spores whereas *Glomeraceae* can colonize from hyphae (Biermann and Lindermann 1983, Morton 1993, Tommerup and Abbot 1981). As hyphae are more sensitive than spores to soil disturbance subsequent colonization of additional roots is more affected. According to De Souza (2005) life history strategy of members of *Gigasporaceae* are “K” strategists in contrast to *Glomus*.

Selosse et al. (2004) pointed the needs to increase studies in more realistic environments, such as microcosms or field plots, thus in such conditions we can ensure that a given microbial strain can persist. Moreover, in a recent review by Velázquez and Cabello (2010) the mycobization (inoculation of agronomically important crops with different functional groups of fungi) has been pointed as a biotechnological tool less costly that does not have a negative impact for systems of intensive use such as organic orchards.

IV. *Plant-AMF interactions*

Since a single root can be simultaneously colonized by various AMF species, root colonization is mediated by interspecific fungal interactions, such as competition, antagonism and dominance (Allen et al. 2003).

During AM development there is a presymbiotic phase, which is characterized by continued hyphal growth, increased physiological activity and profuse branching of hyphae.

Multiple, successive rounds of spore germination and retraction of nuclei and cytoplasm as an exploratory hyphal development changes in the presence of plant-derived signals. The stimulatory effect of plant root exudates on AM fungal hyphae named ‘branching factors’ are attributed to strigolactones (responsible for the induction of branching and alterations in fungal physiology and mitochondrial activity). Strigolactones are ephemeral compounds, which also stimulate spore germination in some AM fungi, being short-lived in the rhizosphere (Parniske 2008). Although the role of the root exudates stimulating the formation of mycorrhizal associations is very important, the primary colonization and number of entry points seems to highly depend on the density of AMF propagules in soil (Smith and Walker 1981, Carling et al. 1979).

A single root can be colonized simultaneously by various, commonly 5 to 6, AM species in the rhizospheric soil (Miranda 2008). Root colonization indicates the magnitude of benefits from improved management of AMF. Most of the host plant benefits obtained by AM symbiosis, mainly phosphorus acquisition, depend on the early colonization of roots. The rapid colonization is related to AMF propagule density and composition, *i.e.* the so-called propagule bank. A graph of the percentage of the root length colonized against time has a sigmoid form showing three phases: lag phase, linear phase and a plateau (Sieverding 1991). A higher AMF propagule density often reduces the length of the lag phase, and thereby accelerates the process of mycorrhizal colonization (Smith and Read 2008).

Arbuscules play an important role in nutrient transfer between the symbionts (Smith and Smith 1989). Arbuscules are relatively short lived (less than 15 days). Other structures produced by some AM fungi include vesicles, auxiliary cells, and asexual spores. Vesicles are thin-walled, lipid-filled structures that usually form in intercellular spaces. Their primary function is thought to be for storage; however, vesicles can also serve as reproductive propagules for the fungus. The increase in the number of vesicles is coincident with the last stage of wheat culture where plants become senescent. Vesicles are resting structures (Bonfante-Fasolo 1984); their number is increased in old or dead roots.

The AMF genera *Gigaspora* and *Scutellospora* produce only arbuscules and inter- and intracellular hyphae, whereas *Glomus*, *Entrophospora* and *Acaulospora* also produce vesicles (hence the frequently used term vesicular-arbuscular mycorrhizal [VAM] fungi used in the past), which are terminal, globose, lipid rich structures in intracellular areas of the root cortex (Strullu et al. 1983).

Biochemical and molecular techniques can be confidant tools for the identification and quantification of AMF in roots. Nevertheless, they are time consuming and costly resulting in not recommended for routine use. The staining of the roots and the counting of the stained fungal structures in the root by routine light microscopy is still the standard technique (Vierheilig et al. 2005).

A detailed review of the state of the art of methods for the detection and visualization of arbuscular mycorrhizal fungi in roots is given by Vierheilig et al. (2005). A number of methods (destructive–non-destructive; vital–non-vital) on how to visualize AMF in roots have been published. Those authors provide an overview on present techniques used to AMF in roots and gives recommendations on their use. We hope that the present review will help the readers to choose an appropriate method to visualize AMF in roots for their specific experimental set-up. In Brazil, techniques for staining of AMF in roots of agricultural crop and fruit trees were recently compiled (Miranda 2008).

Staining (e.g. trypan bue) reflects the presence of mycelium into roots; however; we cannot affirm that is a living mycelium. Therefore, the succinate dehydrogenase analysis can be used for to determine the active mycelium. For this evaluation the the root system of harvested plants can be divided into two portions to record the following: mycorrhizal root length and mycorrhizal fungus succinate dehydrogenase (SDH) activity detected in the fungus mycelium by the reduction of tetrazolium salts at the expense of added succinate (Kough et al. 1987). Thus, the hyphal SDH activity, which is observed histochemically, can be used as an index of AMF metabolic activity. As reported by Gaspar et al. (2002) the active mycelium can follow the same pattern of the percentage colonization, and, moreover, less SDH activity could be observed in colonized roots isolated from polluted substrates (phenanthrene) than in roots obtained from non-polluted substrates.

In studies on AMF there is not a model plant species (such as *Arabidopsis*, which is non-mycorrhizal). Thus, a potential index for use in detection of AMF propagules in different soils could be planting host plants which can be colonized by a broad spectrum of AMF species (e.g. *Plantago lanceolata* or *Zea mays*) (Table 2). For example, if the rate of colonization in the roots of these host plants is measured in different environments, an index can be carried out taking into account the phenological stage of these “model”\ species. As commented by Velázquez and Cabello (2010) some crops, such as sweet potato, soybean, maize, sorghum, barley, sugarcane, tobacco, cotton, and cacao, frequently exhibit high colonization rates under natural conditions. However, wheat, beans, coffee and tomato (Table 1) can have more moderate colonization rates. In addition, some small intraspecific differences can be observed in the colonization percentages between different ecotypes, cultivars or clones of the same crop. By way of *Sorghum sudanense* is being confirmed to form AMF with the highest number of species (20 of *Glomus*, 11 of *Acaulospora*, 2 of *Entrophospora*, 5 of

Gigaspora and 12 of *Scutellospora*) (Smith and Read 2008), therefore, their use should be indicated for tropical regions.

Both for temperate or tropical regions, an economic method to be used as indicator of soil health could be the planting of *Plantago lanceolata*, and posteriorly, the determination of the colonization level, and the sporulation after 4 months. Such methodology could be used to compare disturbed and undisturbed soils.

Recently, Yanqing et al. (2010) considered that AM fungi can be used as a biological indicator, and the fuzzy optimization model can be used to evaluate the soil conditions (Soil moisture, pH, available nitrogen, available phosphorus, organic matter, proteinase, urease, hyphal colonization, vesicular colonization, arbuscular colonization, total colonization and spore density) as the main indicators. They used the analysis of soil quality assessment in a fuzzy optimization system model (He and Hou 2008).

AGROFORESTRY AND SOIL HEALTH

The objective of agroforestry is to produce systems that exhibit an ecological structure more similar to that of natural forests, thus woody perennials are deliberately used on a land management unit as agricultural crops and/or animals, or as tree cover with a multipurpose species. Afforestation of mixed plantation provides wood supply for local communities thus minimizing exploratory actions in biological reserves. In agroforestry, most studies have also often disregard soil biological properties such as mycorrhizal occurrence and interactions between plants, fungi, and the environment. Mycorrhizas are multifunctional and their management through agroforestry is of increasing interest in recent years (Cardoso and Kuyper 2006).

Studies by Cardoso et al. (2003) showed that greater numbers of AMF spores were found in the deep soil layers of agroforestry systems (shaded) than in monocultural coffee plantation (unshaded) soil in Brazil. Coffee systems appear as the best studied crop in Brazil; however a high proportion of undescribed AMF species was recorded in these systems (Stürmer and Siqueira 2006). Undescribed species were also found in agroforestry systems in Minas Gerais, Brazil (Marcela Pagano, unpublished work). Research on native agroforestry trees in Brazil (Pagano et al. 2010a), mostly noduliferous legumes, intercropped with *Eucalyptus* spp. showed the AM benefits for the plant species when inoculation with rhizobia (nitrogen fixing bacteria that associate with most legumes) and AM, which are the most frequent fungi supported by legumes trees, enhancing the uptake of phosphorus, nitrogen and other nutrients, was carried out. Exotic species as *Eucalyptus camaldulensis* (a fast growing tree which can tolerate extended dry seasons) and *Eucalyptus grandis* presented about 50% colonization by AM, was more dependent on ectomycorrhizas (symbiosis that improve water balance of host plants, reduce impacts on trees from root pathogens, and mobilize essential plant nutrients directly from the soil), respectively. Mixture of AM-dependent species *(Plathymenia reticulata*, *Schinopsis brasiliensis* and *Handroanthus heptaphyllus*) (Figure 2), with *E. camaldulensis* was indicated, when there is a need for minimizing the impacts of eucalypt monoculture and to preserve the Brazilian Dry Forest by using native species of this biome; thus restoration programs should take mycorrhizae into account. Those authors emphasize the need to consider the symbiotic fungi in agroforestry management practices, which show great implication in the persistence of AMF species, the choice of agroforestry tree species having great implication in the manipulation and conservation of them. Morover, highly dependent tree hosts should be selected over non-mycorrhizal hosts, and the ability of native AMF to colonize plants in natural conditions and the loss of these fungi with disturbance needs more studies. Moreover, the importance of leaving vegetal slash (mostly crown and specially the bark) on the site in order to decrease the loss of tree productivity was stressed.

CONCLUSIONS

In the introduction to this chapter, we briefly described that various methods for the study of mycorrhizas into the soil quality assessment were discussed; however, few reviews on AMF and soil health has been reported. Moreover, little research was dedicated to feedbacks between soil structure and AMF. Pioneer research with AMF and soil compaction as well as focusing on AMF propagules bank in soils have showed variations in AMF in corn and wheat crops under different tillage systems, checking that AMF provide a wide range of significant benefits to their plant hosts in agricultural systems.

Throughout the chapter, we have showed that authors worldwide focused on different aspects of AM symbiosis that implies their useful for maintaining healthy soils. This review has highlighted the practical tools to study AMF related to soil health despite the intensification of conventional agriculture.

While there are significant gaps in our knowledge about AMF, it is concluded that actual research on soil health providing a benefic role of AMF, is vital to increase, restore or manage soil fertility. Mixing cropping and the symbiotic plant association with AM holds great promise for furthering our knowledge of soil health, and for informing suitable management.

Additionally, the development of practical methods or indicators must be specifically adapted for each region according to its biological, social, and economic characteristics, in order to achieve the wise management of ecosystem services, restraining a deepening of poverty. Molecular and biochemical tools can be complicated and expensive, and so they will not carried out in underdeveloped countries, which require cheaper methods to evaluate indicators of soil health.

The choice of agroforestry tree species would have great implication in the manipulation of AMF species, and highly dependent plant hosts should be selected over mycorrhizal-independent ones. The ability of native AMF to colonize plants in agricultural conditions and the loss of them with disturbance require more studies.

Finally, this chapter point that in order to increase studies of soil health further research is crucial, especially regarding AMF functionality, soil characteristics and nutrient dynamics.

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Figure legends

Figure 1. Mechanisms by which AMF affect ecosystem services. AM plant hosts select their microbial interactions and also increase plant health. AMF affects propagules in soil through its effects on altered micorrhization. AMF may directly affect key traits (for example, the soil aggregation and glomalin content) in ecosystem processes. AMF may alter the soil quality and therefore the soil health. Examples of the related structures include (a) AM colonized roots (b) arbuscules of AMF inside fine roots, (c,d) spores of AMF and (e) dry soil aggregates (photos by M. Pagano).

Figure 2. (a) Agroforestry system in Minas Gerais, Brazil; (b) a physiognomic type of semiarid vegetation (dry forest) in the rainy season; (c) cultivated site; (d,e) AM colonization in fine roots; (f) EM colonization in roots of eucalypt (photos by M. Pagano).

Figure 3. Some functional aspects of AMF in semiarid ecosystems in Brazil. Spores of AMF found in Minas Gerais, Brazil: (a-c) species of *Scutellospora*, (d) spore of *Gigaspora*, (e,f) spores of *Acaulospora*, (g,h) spores of *Glomus* (photos by M. Pagano).

Figures

Fig. 1

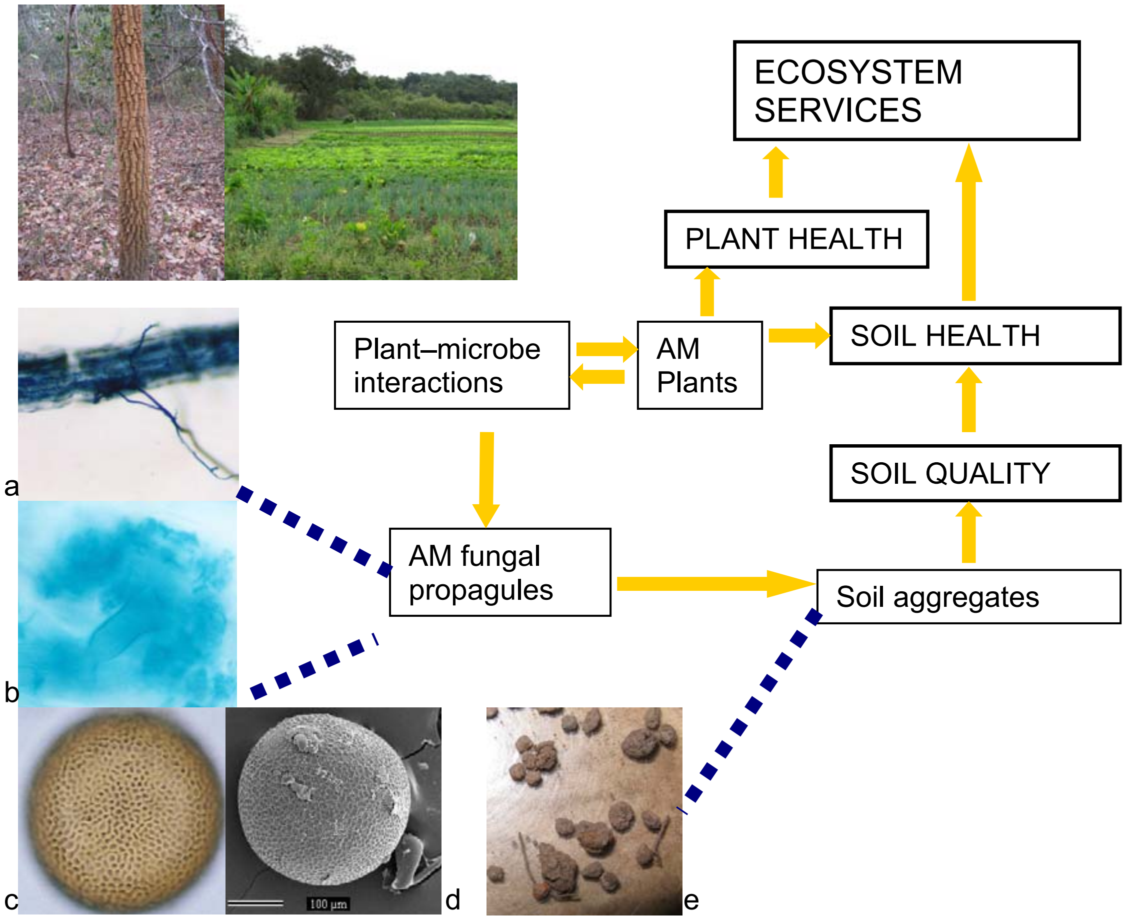


Fig. 2

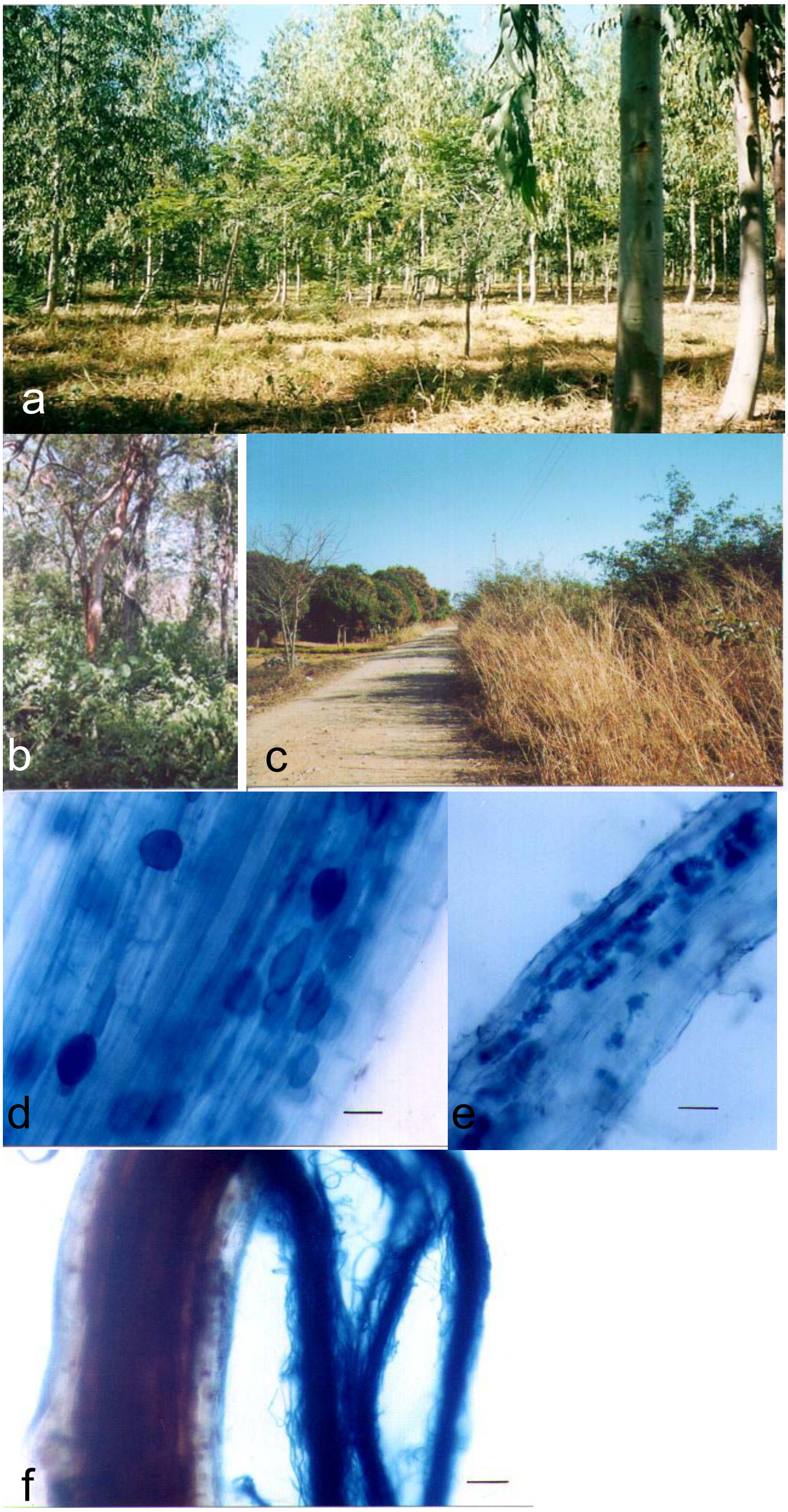
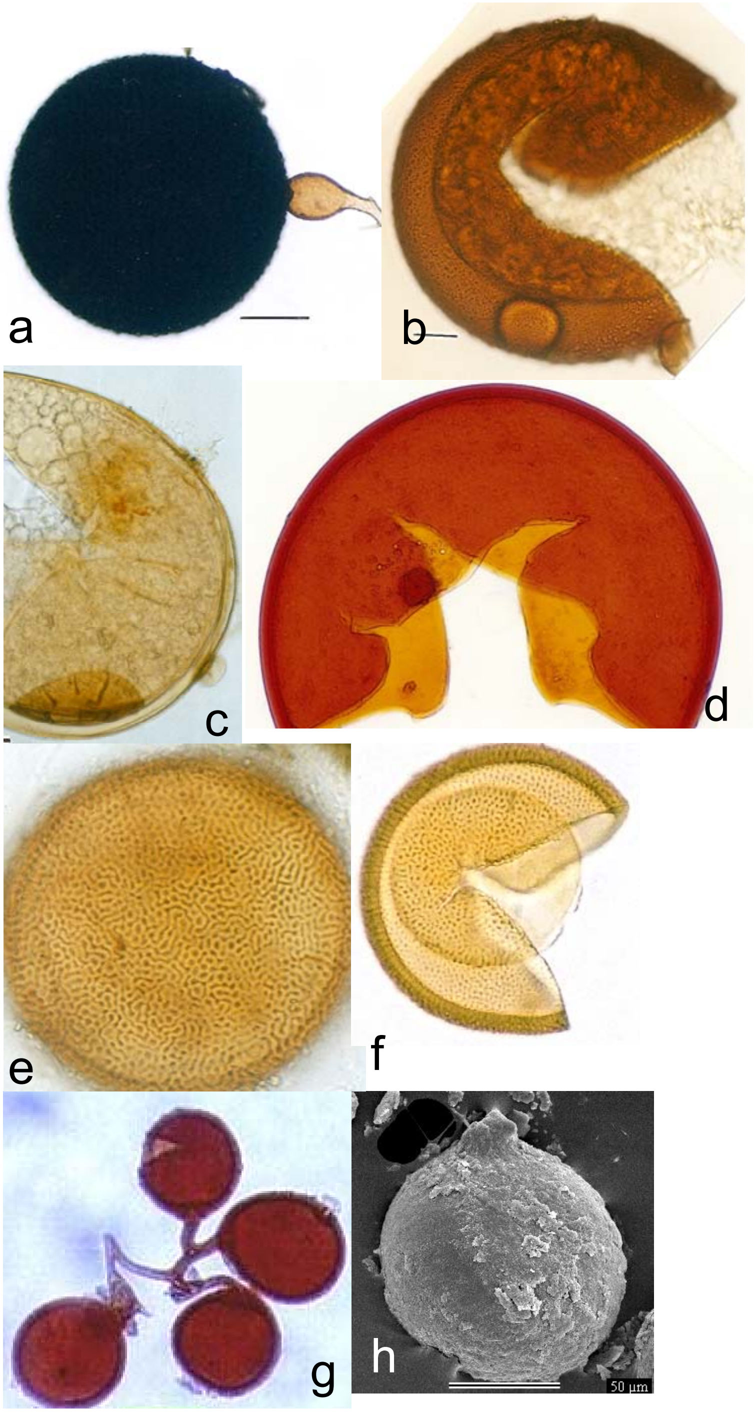


Fig. 3



Tables

Table 1 Summary of evidence on AM and soil health/quality

Source	Ecosystem type	Crops	Focus on
Velázquez and Cabello (2010)‡	Agroecosystems	Sweet potato, soybean, maize, sorghum, barley, sugarcane, tobacco, cotton, and cacao, wheat, beans, coffee and tomato	Mycobization
Schalamuk and Cabello (2010a)‡	Agroecosystems (agricultural experimental station)	Wheat	Tillage, Propagule Bank
Gianinazzi et al. (2010) ‡	Agroecosystems	Various	AM ecosystem services, plant quality
Pagano (in press) ‡	Agroecosystems, agroforestry	Various	Cover crop, tillage, soil compaction
Kahiluoto et al. (2009)	Agroecosystems Conventional / low-input cropping	Barley, rye, oat, potato	Ecosystem services
Siddiqui et al. (2008) ‡	Agroecosystems, agroforestry	Various	AM, EM
Bedini et al. (2007)	Monoculture	Maize	Glomalin-related soil protein, spore biovolumes
Cardoso and Kuyper (2006)‡	Agroecosystems, agroforestry	Maize, soybean, coffee, banana	Inoculation, soil compaction nutrient uptake, AM, EM
Jeffries et al. (2003)‡	Agroecosystems, restored ecosystems	Various	Horticulture, soil health, microorganism interactions
Siqueira et al. (2002) ‡	Agroecosystems	Citrus, maize, coffee, papaya, pineapple, tomato	Inoculation
Rillig (2004)‡	Agroecosystems, natural ecosystems	Maize, wheat, Sorghum fields	glomalin-related soil protein, aggregate water stability, extraradical mycelium, habitat engineering capability
Kling and Jakobsen (1998) ‡	Agricultural managed soils	Sugar beets, oil-seed rape	Pesticide effects

‡Review, book chapter or book; AM = Arbuscular mycorrhizae, EM = Ectomycorrhizas.

Table 2 Summary of actual evidence on major trap plants

Source	Trap plant	RC†	Dominant or total AM species number	Glomeraceae dominant	Total AM species number	NMP of AMF
Schalamuk and Cabello (2010a)	*Sorghum vulgare* *Medicago sativa*		*Glomus aggregatum, Glomus etunicatum, G. clarum* and *G. claroideum*	+	21	NI
Smith and Read (2008)	*Plantago lanceolata*	NI	*Acaulospora* (6 species); *Gigaspora* (3); *Glomus* (10) and *Scutellospora* (2)	+	21	NI
	Zea mays	NI	*Gigaspora* (2); *Glomus* (5) and *Scutellospora* (2)	+	9	NI
	Sorghum sudanense	NI	*Acaulospora* (11 species); *Gigaspora* (5); *Glomus* (20) and *Scutellospora* (12)	+	48	NI
Blaszkowski et al. (2009)	*Plantago lanceolata*		*Glomus perpusillum*	-	NI	NI
Miranda (2008)	Bean	76	67-97 spores 50g-1 soil	NI	NI	NI
	Sorghum	92	1028 spores 50g-1 soil	NI	NI	NI
Ahulu et al. (2006)	white clover	26-60	32-97 spores 50g-1 soil	NI	NI	119
Carrenho et al. (2002)	*Arachis hypogaea*	24.5	*Entrophospora colombiana*	-	NI	NI
	Sorghum bicolor	15.9	*Glomus geosporum*	+	NI	NI
	Zea mays	19.7	*Acaulospora longula*	-	NI	NI

†% maximal AM root colonization; NI = Not informed; NMP of AMF = the most probable number of AMF propagules.