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Review

Arbuscular Mycorrhizas and Wastes for Healthy Soils

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Abstract: Composting of wastes, biochar and vermicompost, shows different morphological aspects and functionalities, being used to mitigate the increasing CO_2 levels related to climatic change and soil carbon stability, biochar presents long chemical permanency due to incomplete combustion of biomass, is increasingly utilized worldwide for soil amelioration, heating and cooking. The agricultural expansion and increasing soil contamination adversely affect soil quality, modifying the number, diversity and activity of the soil microbiota, including symbiotic fungal populations. Soil is a living system presenting its soil microbiota, besides plant symbiotic arbuscular mycorrhizal fungi (AMF) associated with terrestrial and some aquatic plants which are the target of studies in agriculture, agroforestry, restoration of degraded lands and endangered vegetation types; however, AMF is affected by disturbances in the ecosystems, like global change, pollution, or excessive fertilization. AMF belongs to the phylum Glomeromycota, forming symbiotic associations with 70% plant species. The presence and activity of AMF can be measured through different methodologies, both in the soil and in plants, which inform us about their biomass, activity, diversity and their interaction with plants. The need for an increased use of the AMF to counter the challenges of ecosystem restoration and food production is nowadays admitted. We intend to provide evidence for researchers, farmers and decision-makers of the significant links between healthy ecosystems and human well-being, based on current studies on soil health. In the present work, relevant AMF diversity and microbial associations for the wise management of ecosystems are studied.

Keywords: soil mycobiota; mycorrhizal symbioses; soil quality; soil amendments; soil health

1. Introduction

Soil quality involves the ability of the soil to maintain appropriate productivity, reducing environmental impacts and contributing to human health, being studied by multidisciplinary groups. Previous reports on arbuscular mycorrhizas (AMF) have explained the assessment of mycorrhizas and indicated these fungi for soil quality valuation as mycorrhizal association is an important component of sustainable agriculture. AMF forms ubiquitous symbiosis in all terrestrial ecosystems and on most plant species, including agricultural crops, being manipulated, intentionally or not, through the plant, soil and AMF species of the tripartite symbiosis [1]. Among the literature on AMF, Jeffries et al. [2] focused on the antagonistic activity of AMF against soil-borne pathogens, for maintaining healthy soils. Advances in hyphosphere research showed emerging roles of AMF associated microbes in soil functions [3]. It also highlighted the role of the mycorrhizal mycelium, besides phosphorus nutrition of host plants [4] and the relationship between soil structure and AMF was extensively investigated by Rillig and Mummey [5]. Additionally, AMF can decrease soil compaction enhancing corn growth by AMF inoculation [6]. Studies focusing on AMF

propagule bank in soils under wheat with different tillage systems pointed out variations between AMF species [7,8]. Soil quality is the capacity of a specific soil, within natural or managed ecosystems, to sustain plant and animal yields, maintaining water and air quality, supporting human health and habitation [9]. The parameters for the evaluation of soil quality are physical, chemical and biological, with their integration being recommended. However, biological parameters have gained importance because organisms respond promptly to changes in land use, environmental conditions or contamination compared to most chemical and physical parameters [10]. Due to the important role of soil organisms in soil processes, the abundance of fungi has been used as an indicator of soil quality. The aim of this work was to compile the important role of AMF in soil health, and ecological aspects of the mycorrhizal association which participate in soil health, to elucidate the benefits of mycorrhizae as ecosystem services. The goal of this research was to provide an illustration based on current reports on soil quality, with a focus on arbuscular mycorrhizas.

2. Arbuscular Mycorrhizal Fungi

AM fungi (AMF) form symbiotic associations with 70% plant species [11], they associate with the roots of forest trees, wild grasses, aquatic plants and most crops, among 72% of vascular plants in terrestrial and aquatic environments [12], including the existing six mycorrhizal types [11], AMF, belong to the phylum Glomeromycota presenting three classes, six orders, sixteen families and ~50 genera [13]. These fungi present aseptate and coenocytic hyphae, sharing the same cytoplasm, which improves the rate of transport of nutrients. All types of mycorrhizas present a bidirectional movement of nutrients, where carbon flows to the fungus and inorganic nutrients move toward the plant. They can also transport water. Arbuscular mycorrhizas, the most frequent association in nature form symbiotic associations colonizing the cortical tissue of roots. The interaction with its host plant consists of nutrient transfer (the plant provides carbon while the fungus delivers nutrients to the plant). Increased nutrient uptake from the soil, particularly of phosphorus and nitrogen, is its main benefit [14] together with, higher resistance to root parasites [15], among improvement of drought tolerance [16] and mitigation of environmental stress, such as salinity. Other important roles attributed to AMF are improving soil stability and reducing erosion [5]. AMF are grouped into more than 260 described species, with different effects on the host. Glomeraceae is the family with the highest species richness (>150) [12] among sixteen families and 50 genera. The role of AMF as agroecosystem service providers was highlighted by Gianinazzi et al. [17], and its potential role in protecting endangered plants and habitats was also brought to light [18]. Arbuscular mycorrhizas are the most important microbial symbiosis for the majority of plants and, under conditions of P-limitation, influence plant communities, nutrient uptake, water relations and above-ground productivity [2]. They also act as bioprotectants against pathogens and toxic stresses. We discuss here the benefits of AMF for improving soil health in the rhizosphere of crop and native plants and methods to measure them, with attention dedicated to the management and conservation of AMF diversity.

2.1 Indicators of Soil Quality and Health

Chemical and physical properties, which can be static (easily measured) or dynamic (more laborious to evaluate), including soil organic carbon, nutrient availability, soil acidity and salinity, were pointed as indicators of soil quality of ecosystems. The physical properties can be static (soil texture, soil depth, topsoil depth, bulk density, available water holding capacity, saturated hydraulic conductivity, soil loss, soil strength, porosity, aggregate stability and size distribution), and dynamic indicators (least limiting water range, trafficability, leaching and erosion vulnerability) soil quality indicators would be precise to each soil type [19]. Biological parameters and their interrelation with the chemical and physical properties of soil are considered of most importance together with the soil microbial community, due to their critical role in soil carbon sequestration. The size distribution and tortuosity of soil pores, related to aggregate size distribution, in particular pore neck size, controls the activity of decomposers and soil food web dynamics, and affects the physical protection of soil organic C [20]. Notably, several researchers worldwide have recognized that minimizing soil disturbances by tillage or erosion can enhance the fungal communities and the biochemistry associated with soil humification. The choice of indicators [21] from four groups of methods were proposed 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 [22].

2.2 Indicators of Soil Management

While many kinds of microorganisms are considered of interest for indicating the soil management employed, AMF, which relies on their plant host, being dependent on the plant C, and therefore reflecting the activity of the plant, present many traits as high sensibility to disturbances, limited spread and viability in the absence of a live host, which are useful to consider them as indicators. For example, the poor ability to spread in space compared to other microorganisms in contrasting contiguous sites or agricultural plots presenting different soil management. Moreover, their apparent lack or lower specificity compared to other symbiotic microorganisms may be another interesting trait for using AMF, since mycorrhizas comprise 30% of the fungal biomass in the soil [23], changes of soil management (tillage, fertilization, and disturbance) can reduce its presence. Therefore, AMF biomass in undisturbed sites contrasts with adjacent plots subjected to disturbance. Since different species of AMF, the vegetal community and environmental factors are closely interrelated, these fungi are highly sensitive to soil management, thus, their use for monitoring agriculture intensification is of great interest as AMF are “sensors” of plant nutrition, soil physicochemical conditions and disturbance [24]. Early indicators of ecosystem stress measure soil degradation, compared to other slowly changing soil properties such as soil organic matter), thus the use of AMF as soil indicators, besides its slowness is more recognized. Mycorrhizal propagules are influenced by damage to vegetation and soils resulting from human intervention, as well as intense fires, topsoil removal and flooding. Additionally, AMF surviving propagules in soils decline with time and in the absence of host plants [25]. Therefore, the abundance of AMF is as an indicator of soil degradation, and different agricultural practices. To restore the inoculum potential of AMF in eroded soils, bioaugmentation, inoculating soils with AMF, or transplanted seedlings with AMF in their roots) are among sustainable solutions [2]. The

indigenous community of AMF may also be restored using mycotrophic cover crops which stimulates the development of inoculum in subsequent crops. The extraradical hyphal network, the most important source of AMF propagules, is damaged through tillage, and can be reduced when a non-mycotrophic crop (Brassicaceae or Chenopodiaceae) is cultivated. Thus, AMF communities reflect the past activity of the plant host growing in the site, and they could indicate past events, such as tillage, fires, contamination, clearing, grazing, long fallow, and herbicide application, reflected in AMF biomass, due to the direct relationship of AMF with host plants. Different parameters to evaluate mycorrhizae and soil quality [1] (Table 1) fail to indicate monitoring systems for the future and to evaluate the effects of soil management, which are needed by Policy makers, and land farmers [26]. The AMF was included within the Plant–microbe interactions group in the classification of microbiological, biochemical and molecular methods, proposed depending on the information they can provide, as follows: 1. Soil microbial biomass and number; 2. Soil microbial activity; 3. Soil microbial diversity and community structure; 4. Plant–microbe interactions. The estimations of AMF biomass in the soil, differentiating the hyphae which belong to mycorrhizal fungi from those of other fungi by direct extraction of hyphae from soil is difficult and time consuming. Therefore, besides the extraction and quantification of AMF hyphae, biochemical methods using specific markers and other indirect methods estimate mycorrhizal structures in soil. Since AMF spores have been easier to extract from the soil than hyphae, the spore number has been a valuable and direct indicator of the abundance of AMF in soils. However, the spore number shows some limitations. Additionally, the bioassay of Plenchette et al. [27] evaluates the inoculum potential of mycorrhizas despite other methods.

Table 1. Some studies on AMF and soil health.

Source	Ecosystem Type	Crop /Vegetation	Focus
[28] ‡	Agroforestry	Mixed plantations	Forest, AM Inoculation
[29] ‡	Agroecosystems	Sweet potato, soybean, maize, sorghum, barley, sugarcane, tobacco, cotton, and cacao, wheat, beans, coffee and tomato	Mycobization
[8]‡	Agroecosystems (agricultural experimental station)	Wheat	Tillage, Propagule Bank
[17]‡	Agroecosystems	Various	AM ecosystem services, plant quality
[1]	Agroecosystems Conventional/low-input cropping	Barley, rye, oat, potato	Ecosystem services
[30]‡	Agroecosystems, agroforestry	Various	AM, EM
[31]	Monoculture	Maize	Glomalin Protein, spore biovolume

[32]‡	Agroecosystems, agroforestry	Maize, soybean, coffee, banana	Inoculation, soil Compaction nutrient uptake, AM, EM
[33]‡	Agroecosystems, natural ecosystems	Maize, wheat, Sorghum fields	Glomalin, aggregate water stability, extraradical mycelium, habitat engineering capability
[3]‡	Agroecosystems, restored ecosystems	Various	Horticulture, soil health, microorganism interactions
[34]‡	Agroecosystems	Citrus, maize, coffee, papaya, pineapple, tomato	Inoculation
[35]‡	Agricultural managed soils	Sugar beets, oil-seed rape	Pesticide effects

‡Review; AM = Arbuscular mycorrhizae, EM = Ectomycorrhizas; Glomalin: Glomalin-related soil protein.

2.3 Direct Extraction of Hyphae from Soils

AMF biomass in soils can be estimated with measurements of hyphal length, through an aqueous extraction of soil or by adding 20% H_2O_2, and the processed samples obtaining a subsample (0.01g) which is mixed with 2 drops of glycerin on a microscope slide. The number of coenocytic hyphae typical of AMF is then counted at ×100 magnification using a compound microscope. Thus, the length of AMF hyphae in soil or litter can be calculated [36]. Nevertheless, modifications of this method [37] may underestimate hyphal length in soils with high OM or clay content; despite not distinguishing living hyphae. Most studies, especially field studies lack measurements of external dead and living hyphae. Vital staining techniques (e.g. succinate dehydrogenase), are used to evaluate the active mycelium, both inside and outside the roots. The extraradical mycelium (a large portion of the AM mycelium outside the root) grows dispersedly and hyphae of small diameter (< 5 μm) are difficult to study quantitatively, as their short longevity and elevated turnover rate **(**~ 6 days) [38]. External hyphae are identified and quantified by the gridline intercept method. AMF spores and auxiliary cells usually are visualized associated with hyphae, quantified by counting the hyphal fragments in soil solution according to the following method. A soil solution with 1 g fresh soil in 100 mL distilled water is mixed and filtered at 0.5 mm to remove heavy sediment. Then, 1 ml of filtrate and two drops of 5% ink/vinegar stain are inspected in a grid-lined Petri dish at 20× 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, lightly stained). The size of 5 μm can be confirmed with a microscope at 650× magnification [39]. As a diameter of 5–10 μm was specified for AMF hyphae, Schreiner and Bethlenfalvay [40] assumed that hyphae >5 μm diameter are primarily mycorrhizal and hyphae <5 μm diameter mainly saprophytic hyphae; however, AMF can vary in diameter within the mycelium, according to their location in branching [41]. Diverse AMF communities can produce a more extensive mycelium, related to more efficient exploitation of nutrients from soils [42,43]. Different patterns of hyphal anastomosis are present in *Glomeraceae* [44]. Moreover, in *Gigasporaceae* the number of anastomoses per hyphal length vary. Additionally, *Glomeraceae* presents

anastomosis between different hyphae, whereas *Gigasporaceae* presents bridges in the same hyphae. These differences, which indicate functional complementarities, are important, for ecological studies of AMF [45]. Additionally, a rapid methodology (Digital gridline intersection method) was proposed to estimate the length of external AMF hyphae in soil by Shen et al. [46], where images of the stained hyphae are observed by using a digital photomicrography technique to avoid the use of the microscope.

2.4 Estimations of AMF Biomass Using Biochemical Markers

The utilization of lipid markers, such as the fatty acid 16:1ω5, which is found especially in *Glomus* has been of particular interest [47,48]; however, the resolution of this method is low (it cannot be used at species level). Also, the lipid composition of a mycelium can change with time and environmental factors. This method is hence 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. The role of the AMF mycelium in soil aggregation (Figure 1) is well documented [49]. Additionally, AMF produces a very stable hydrophobic glycoprotein, glomalin, which is estimated by four common measurements: Bradford reactive soil protein (BRSP), easily extractable BRSP (EE-BRSP), immunoreactive soil protein (IRSP), and easily extractable IRSP (EE-IRSP). They are determined by the extraction process (easily extractable vs. total glomalin) and detection method (Bradford protein vs. enzyme-linked assay (ELISA) [50]. Although the antibody approach is more specific, polyphenols from the leaf litter (soils with high concentrations of organic matter) may overestimate the glomalin content in the Bradford and underestimate it in the ELISA assay. Treseder and Turner [50] also stated that glomalin is deposited on the outer hyphal wall and, as the AM hyphae senesce, they leave a residue of glomalin in the soil. Hyphal stocks, hyphal glomalin content, and hyphal turnover rate seem to determine the rates at which glomalin is deposited in the soil. Regarding lifespans of AM hyphae, reports for laboratory studies indicate that they might survive a few days or months; however, no data is available for natural systems. According to current knowledge, glomalin concentrations in soil are positively related to net primary productivity, and augmented under elevated CO_2, and are often greater in the presence of AM host plants presenting high AM colonization rates [50].

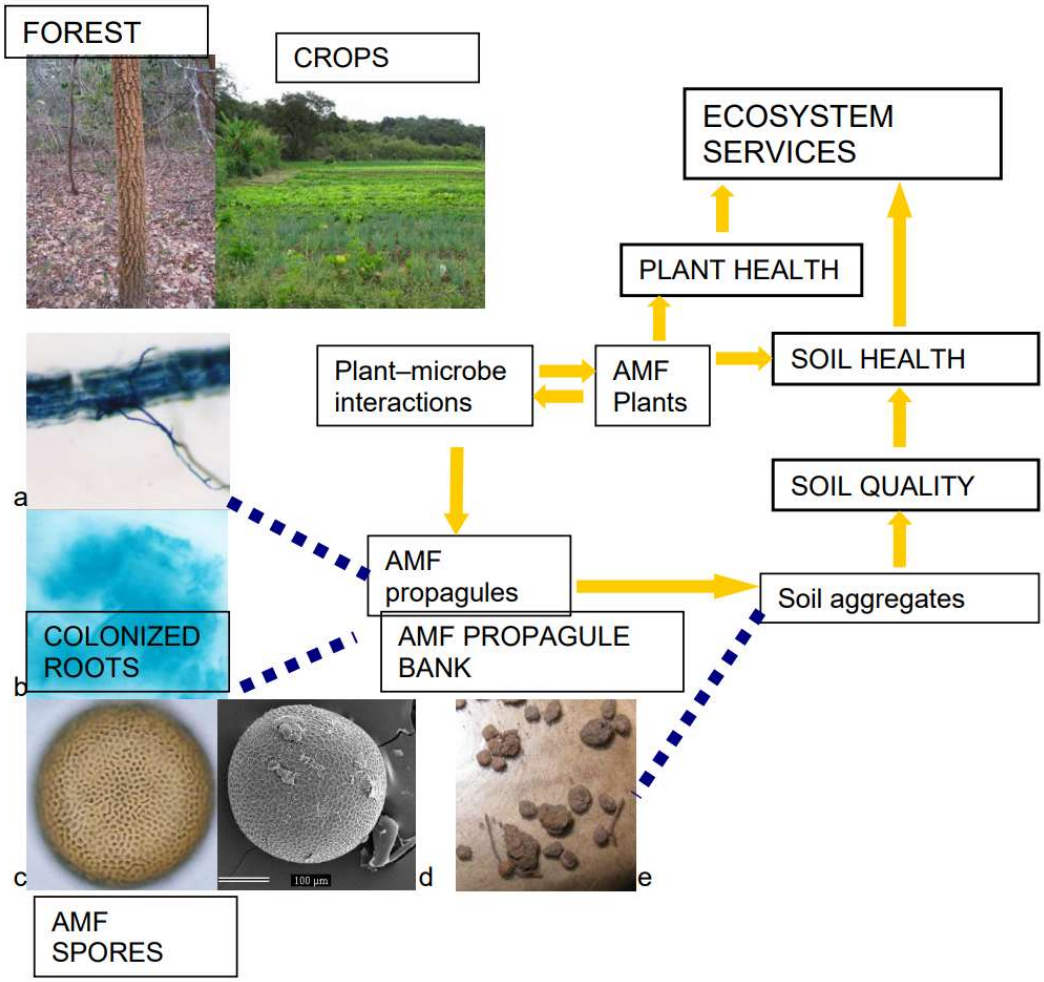

Figure 1. Mechanisms by which AMF provides ecosystem services. AM plant hosts select their microbial interactions and increase plant health. AMF affects propagules in soil through its effects on mycorrhization. AMF may directly affect the soil aggregation and glomalin content) in ecosystem processes. AMF alters soil quality and therefore, 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.

2.5 Bioassay for Measurement of Mycorrhizal Formation

The bioassays for the measurement of mycorrhiza formation provide information about the ability of AMF inoculum 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 1–3) are difficult to quantify [51], and their viability varies with many factors, such as the climatic conditions or the C concentration in soil [13]. Therefore, bioassays were proposed using diluted soils and mycotrophic species, such as the method developed by Plenchette et al. [27], which allows 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 distinguish the relative contributions of the different types of propagules to the colonization of root systems [51]. The AMF propagule levels can be determined

by a bioassay based on Plenchette et al. [27], by dilute soil e.g. 100, 30, 10 and 3 % with the same autoclaved soil to provide a logarithmic scale of concentration. Seeds of highly mycotrophic species are surface sterilized and grown in sterilized substrates. After that ten plantlets are placed in each pot containing soil dilutions of each treatment. As plants are colonized depending on the inoculum level. Four weeks after planting, they are harvested and root colonization evaluated. The mycorrhizal soil infectivity is calculated using regression analysis. 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 to a great extent on the propagule density of the soil [52].

2.6 Evaluation of AMF Spores from Soils

Spores of AMF are normally formed terminally on absorting hyphae; however, some AMF species such as *Glomus irregulare* and *Glomus intraradices* can sporulate inside the roots or in the soil [53]. Spore abundance is evaluated as spore density (number of spores /mL soil) or spore number, usually using 100g of sampled soil. Spore number is most used as a rough indicator of 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 [52]. Their survival depends on morphological traits, mainly determined by the species 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 [8]. Spores are included in the AMF soil propagule bank. In undisturbed soil, it is expected that new infection units (IU) arise primarily from extraradical hyphae, spores being less important due to their dormancy [54]. In this sense, there is a positive linear relationship between IU density and inoculum concentration. The role of the different types of propagules (Figure 1) in the colonization of plant host roots at field conditions is difficult to distinguish, as the rate of initiation of primary root colonization from propagules in the soil is influenced by the availability and density of inoculum and erratic germination of spores, which are dormant when first formed, but germinate under appropriate conditions of moisture and temperature. Additionally, large spores can contain more resources to support multiple germination and hyphal growth during the period when the AMF is searching for hosts [55]. The number of spores reflects both the sporulation and the action of many factors that affect their survival and accumulation in the soil. Therefore, the spore number or density is the result of a complex balance between sporulation, probably related to the recent activity of AMF, and spores formed earlier in the season and in previous seasons (the number of spores in soils includes recent formed structures, as well as spores formed earlier). Experiments have shown that the production of spores depends mainly on the growth of the host plant, fertilizer application and light intensity. Experiments under field conditions pointed out that the increase in spore number can be associated with the root growth and with the maturity or senescence of the host. In addition, increases in the number of spores have been related to the progress of fungal colonization in the roots. The death of spores is one of the main factors determining the variations in the number or density of these structures in the soil. In natural ecosystems, decreases in the number of spores have

been attributed to their germination, activity of macro and micro fauna, and their damage by other soil fungi and parasites as AMF are commonly infected by other fungi or by actinomycetes. Environmental conditions influence these processes, as well as agronomic practices, which can also decrease the density of spores in the soil. For instance, inversion of the soil, in conventional tillage, reduces propagules, affecting the presence of spores in the soil [52]. 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. The dimensions used for biovolume calculations are, thus, represented by the mean for each morphotype measuring 50 spores of each one [31]. AMF is affected by disturbances in the ecosystems, like heavy metal pollution [56]; however, these fungi can accumulate metals from soil [57]. Morphological studies of small structures are possible using energy-dispersive and wavelength-dispersive spectrometers (WDS) coupled to a scanning electron microscope (SEM). These methods, however, do not detect minor and trace elements [58]. Few reports of metal-tolerant AMF [59–62], identifying isolated AMF species. Cruz [63] pointed out that microanalysis of AMF spores may inform the chemical spectrum of AMF spores and show differences among species. In Brazil, nickel, which at high concentrations can lead to heavy metal poisoning was detected in one spore type (*Scutellospora reticulata*) frequent in restored riparian sites of Velhas River, Minas Gerais State, subjected to river pollution [62,64].

2.7 Estimations of Arbuscular Mycorrhizal Activity

Since spores accumulate in soil for several years, they are not direct indicators of recent fungal activity. For that, the density of viable spores can be measured, by separating them from soil by wet sieving, incubating in iodonitrotetrazolium chloride solution (10 $g \cdot L^{-1}$ in Petri dishes, and counting them). Those spores stained in this colorimetric assay are considered viable. and are counted. Spores without cytoplasmic contain, and non-stained ones, are considered nonviable. Spore extraction can be carried out about a year after sample collection. For example, Lima et al. [65] found that 2.6% spores were viable in tropical dry forest in Brazil.

2.8 Estimations of Arbuscular Mycorrhizal Diversity

AM fungal spores or glomerospores being commonly isolated and identified using morphological characters, can be extracted from soil by wet sieving, decanting and sucrose centrifugation. Healthy spores are then counted and analyzed as the number of spores/100 g^{-1} 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, and to obtain permanent voucher specimens. Morphological properties and subcellular structures observed under light microscopy at 100× magnification serve for morphological identification, which is based on spore color, size, surface ornamentation and wall structure, with reference to the descriptions provided by the International Culture Collection of 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 and interchanged among researchers. In general, the spore number

is square root transformed and statistically analyzed. The establishment of germplasm banks of Glomeromycota (AMF) was promoted [66] due to its relevance for scientific research; however, taxonomical and biodiversity studies of AMF are usually limited by the lack of experience in culturing and spore morphotyping by researchers. Until now 360 species were described [67]. Difficulties associated with measuring microbial soil diversity have been pointed out [68], as molecular techniques can overestimate AMF diversity. Moreover, as new undescribed AMF species are increasingly recorded , whereas the sampling effort can affect the detection of the AMF community structure and species richness [69]. Therefore, there are traditional and molecular methods to characterize fungal communities. For example, 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. Biochemical and molecular techniques based on DNA analysis or sequencing of ribosomal genes) are used to identify the AMF spores and also AMF in roots). The use of molecular techniques has contributed to morphological identification. The polymerase chain reaction (PCR) can target specific AMF DNA sequences, the majority being ribosomal RNA (rDNA) genes [70]. Molecular techniques have served for the characterization of AMF to enhance our understanding of their ecology [71], evolution and phylogeny [72] as many AMF genotypes present in the field cannot be cultured [73]. Different PCR-based methods have been employed for the molecular characterization of AMF, such as denaturing gradient gel electrophoresis (DGGE) [74], and temperature gradient gel electrophoresis (TGGE) [75].

2.9. Use of Trap Cultures for Arbuscular Mycorrhizae

To obtain species that have not formed spores at the moment of collection in the field, trap plants established in pots under greenhouse conditions named "trap cultures" using diluted soil have helped to find species that had not been observed in field samples [76,77]. However, studies generally found fewer and different AMF species in the trap cultures than in fields [78,8]. For trap cultures using soil, the detailed procedure was reported: a part of field soil (250 g or less) (containing the three types of AMF propagules: spores, mycelium and colonized roots) from each soil sample is mixed with a tindalized substrate composed of perlite-vermiculite (1:1 v/v) and placed in 2 L pots [52]. A gramineae and a legume such as *Sorghum vulgare* and *Medicago sativa,* respectively, are planted. 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 systematic sampling of the study site is conducted (spores are isolated, segregated into species type groups, and initiated pot cultures). 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 monospecific cultures. Species richness can then be calculated. When familiarity with the AMF species of the site is achieved, the identification of spores directly from the field in response to management practices can be attained. Dominance, diversity and biovolume indices may be calculated to describe the sporulating community. 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 their suitability for

characterization of AMF communities; however, some AMF species can be successfully isolated and propagated. In a long-term subculturing experiment using a single plant host species, *Bracchiaria comata*, only a dominant species (*Claroideoglomus etunicatum*) persisted.

A complete description of the AMF community of soil would include the identity of fungi (spores, extraradical and intraradical hyphae and vesicles) as well as information on the relative abundance of each component for each species. The molecular techniques identify hyphae of a few described AMF species in roots and soil. These methods are useful to trace the persistence of introduced isolates, or interactions among several isolates under controlled conditions. Quantification of the effects of agricultural management practices upon communities of AMF requires to quantify the total hyphal length in soil and total mycorrhization of roots (Figure 2). 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, a description of the community based on spore counts and identification probably provides inaccurate evidence of 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 enhances nutrient uptake of roots and produces glomalin, thus playing a significant role in soil aggregation.

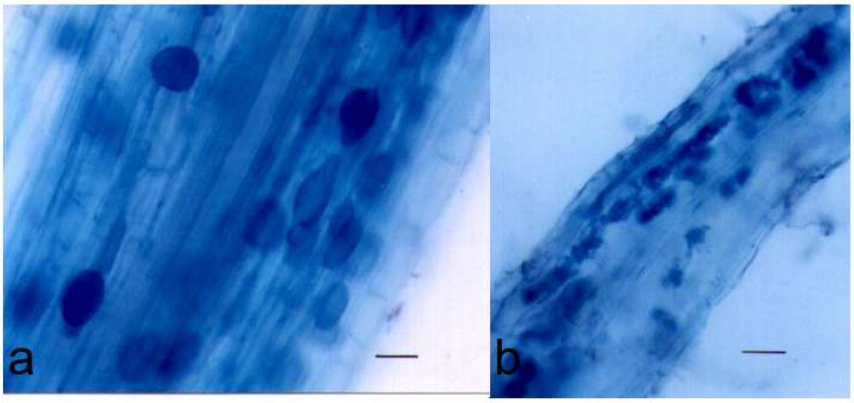

Figure 2. (**a**) AMF colonization in fine roots, scale bar: 100µm showing hyphae, vesicles and (**b**) Extraradical hyphae, scale bar: 50µm.

3. Functional Diversity of AMF

Variations in the function of fungal structures provide information about the functional diversity of AMF; however, studies of mycorrhizal functional diversity are scarce [79], despite the extensive impacts on nature and the effects of its use by human populations [67]. Thus, our ability to generalize about interactions between AMF and plants is limited. Earlier in 2005, Wolfe and Klironomos [80] assessed the functional diversity of soil microbial communities. Regarding AMF, it is known that there are significant differences among families [55]. Some authors have used the index of

diversity to describe the biodiversity of a site or to compare the diversity among different sites in the same biome; nevertheless, it is difficult to use it as an indicator of soil microbial biodiversity and ecosystem function. However, soil biodiversity regulates soil fertility. On the contrary, the most fundamental lack of knowledge concerns the functional roles of AMF assemblages in the field [81]. AMF functionally varies across a range of characters including the resistance to root parasites [82], improvement of drought tolerance [83] and mitigation of impacts of environmental stresses such as salinity [84]. The different ecological traits of *Glomus* are less investment in extraradical hyphae and more in intraradical root structures than *Gigaspora*, *Acaulospora*, and *Scutellospora*. Glomeraceae has different propagation strategies [85] and *Glomus* often dominates the AMF communities following N addition, when host plants are thought to reduce the investment of C in AMF; 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 appropriate when host plant C is limiting. This group 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 [86] found three species of *Glomus*, which could be useful indicators of eutrophication (N enrichment) in coastal sage scrub in southern California. Studies have revealed that Glomeromycota taxa may vary in their colonization strategies, regarding the use of different propagule types by the major AM families [87,88]. However, contrasting evidence exists on the ability of each Glomeromycota family to use each propagule type. According to Hart and Reader [87] *Gigasporaceae* is less sensitive to soil disturbance than *Glomeraceae* due to differences in their colonization strategies, as *Gigasporaceae* colonize primarily from spores whereas *Glomeraceae* colonize from hyphae. As hyphae are more sensitive to soil disturbance than spores, subsequent colonization of additional roots is affected. According to De Souza [55] the life history strategy of members of *Gigasporaceae* ("K" strategists) contrasts to *Glomus* (Figure 3). It was pointed out the need to carry out more studies in more realistic environments, such as microcosms or field plots, to ensure that a given microbial strain can persist. Moreover, Velázquez and Cabello [89] proposed the inoculation of agronomically important crops with different functional groups of fungi (mycobization) as a less costly biotechnological tool that does not have a negative impact on agrosystems such as organic orchards.

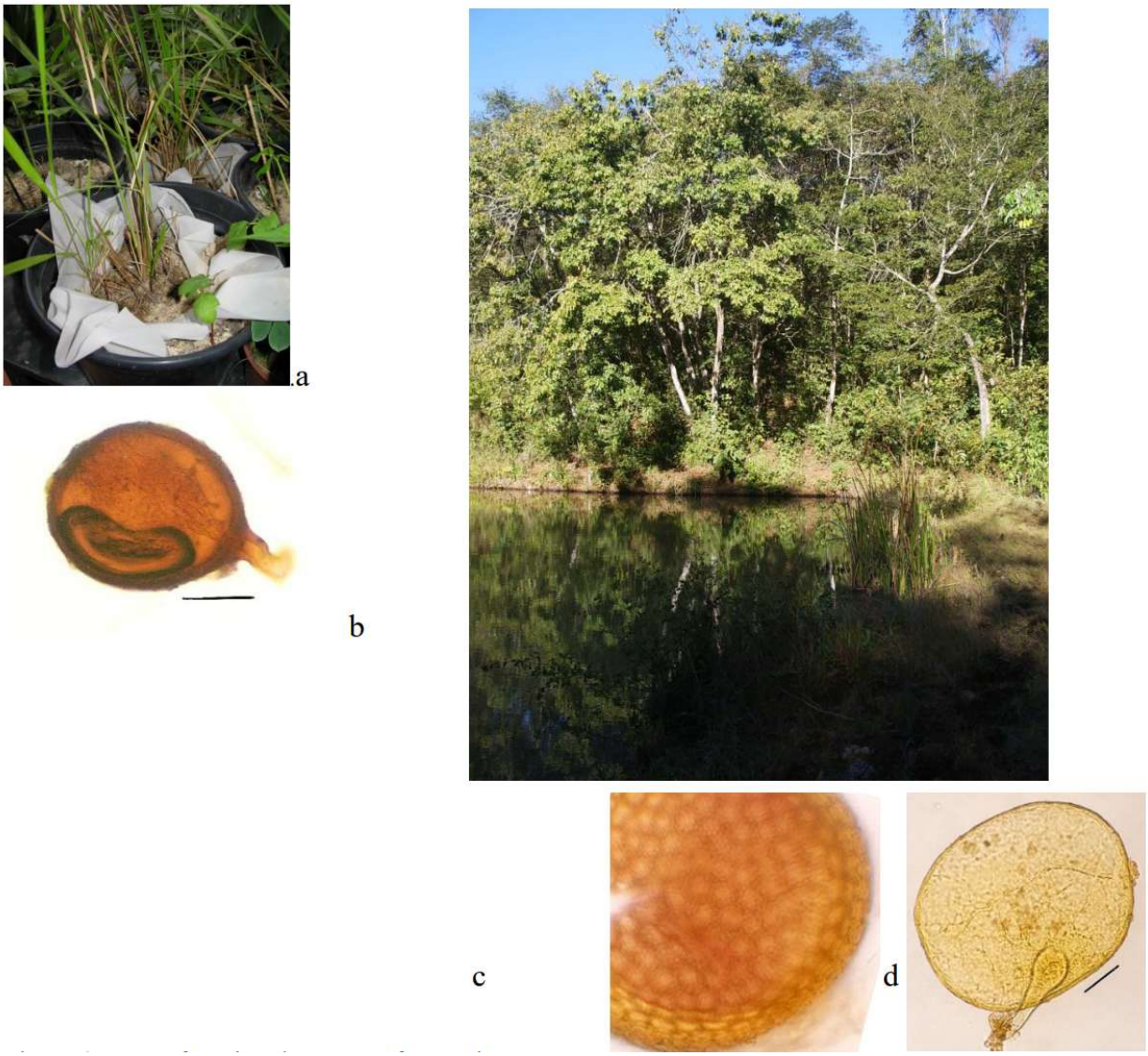

Figure 3. Some functional aspects of AMF in ecosystems. (**a**)Trap cultures in pots; AMF spores retrieved from natural soils: (**b**)Spore of *Glomus*; (**c**) *Acaulospora* and (**d**) *Scutellospora*. Scale bars: 10 μm

3.1 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 [90]. During AMF 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 change 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 AMF, being short-lived in the rhizosphere [91]. Although the role of the root exudates stimulating the formation of

mycorrhizal associations is very important, the primary colonization and number of entry points are highly dependent on the density of AMF propagules in soil [54]. A single root can be colonized simultaneously by 5 to 6, AMF species in the rhizospheric soil [92], thus, root colonization indicates the magnitude of benefits from AMF. Most of the host plant benefits obtained by AMF symbiosis, mainly phosphorus acquisition, depend on the early colonization of roots. The rapid colonization is related to AMF propagule density and composition in propagule banks. Higher AMF propagule density accelerates the process of mycorrhizal colonization [51]. Arbuscules play an important role in nutrient transfer between symbionts, being relatively short lived (less than 15 days). Other structures produced by some AMF include vesicles, auxiliary cells, and vesicles (thin-swelled, lipid-filled, structures in intercellular spaces for storage; however, vesicles can also serve as reproductive propagules. The increase in the number of vesicles is coincident with the last stages of culture where plants become senescent. Vesicles are resting structures; their number is increased in old or dead roots. *Gigaspora* and *Scutellospora* produce only arbuscules and inter- and intracellular hyphae, whereas *Glomus*, *Entrophospora* and *Acaulospora* also produce vesicles, vesicular-arbuscular mycorrhizal (VAM) fungi is a term used in the past, which are terminal, globose structures in intracellular areas of the root cortex. Biochemical and molecular techniques can be reliable tools for the identification and quantification of AMF in roots, which is frequently used nowadays. Nevertheless, they are time-consuming and costly, being not recommended for routine use). Despite the methods (destructive–non-destructive; vital–non-vital) to visualize AMF in roots, the staining of the roots and the counting of the stained fungal structures in the root by light microscopy is still the standard techniques. In Brazil, techniques for staining AMF in the roots of agricultural crop and fruit trees have been compiled [92]. Staining (e.g. trypan blue) reflects the presence of mycelium into roots; however, we cannot affirm that it is a living mycelium. Therefore, the succinate dehydrogenase analysis can be used to determine the active mycelium. For this evaluation 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 fungal mycelium by the reduction of tetrazolium salts at the expense of added succinate). Thus, the hyphal SDH activity, observed histochemically, is an index of the AMF metabolic activity. The active mycelium can follow the same pattern of the percentage of colonization [93], and thus, less SDH activity can be observed in colonized roots isolated from polluted substrates than in roots obtained from non-polluted ones. Moreover, there is no model of mycorrhizal plant species (such as *Arabidopsis*, which is non-mycorrhizal). Thus, a potential index for use in the 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 considering the phenological stage of these “model” species. Some crops, such as sweet potato, soybean, maize, sorghum, barley, sugarcane, tobacco, cotton, and cacao, frequently exhibit high colonization rates under natural conditions [89]. However, wheat, beans, coffee and tomato (Table 1) can have 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. *Sorghum sudanense* was 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*) [51]; therefore, their use should be indicated both for temperate or tropical regions, an inexpensive method to be used as an indicator of soil health could be planting *Plantago lanceolata*, and after that, the determination of the colonization level, and the sporulation after four months. Such methodology could be used to compare disturbed and undisturbed soils. Recently, Yanqing et al. [90] considered that AMF can be used as a biological indicator, and optimization models can be used to evaluate 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. Thus, several crops showed high colonization [94,95].

Table 2. Some trap plants colonized by AMF.

Source	Trap Plant	RC[†]	AMF Species	Glomeraceae Dominant	Total AM Species Number	NMP of AMF
[7]	*Sorghum vulgare*	-	*Glomus aggregatum, Glomus etunicatum, G. clarum* and *G. claroideum*	+	21	-
	Medicago sativa	-				
[51]	*Plantago lanceolata*	-	*Acaulospora* (6); *Gigaspora* (3); *Glomus* (10) and *Scutellospora* (2)	+	21	-
	Zea mays	-	*Gigaspora* (2); *Glomus* (5) and *Scutellospora* (2)	+	9	-
	Sorghum sudanense	-	*Acaulospora* (11 species); *Gigaspora* (5); *Glomus* (20) and *Scutellospora* (12)	+	48	-
[53]	*Plantago lanceolata*	-	*Glomus perpusillum*	-	-	-
[61,95]	Bean	76	67–97 spores 50 g^{-1} soil	-	NI	NI
	Sorghum	92	1028 spores 50 g^{-1} soil	-	NI	-
[96]	White clover	26–60	32–97 spores 50 g^{-1} soil	-	NI	119
[91]	*Arachis hypogaea*	24.5	*Entrophospora colombiana*	-	NI	-
	Sorghum bicolor	15.9	*Glomus geosporus*	+	NI	-
	Zea mays	19.7	*Acaulospora longula*	-	NI	-

[†]% maximal AM root colonization; RC= Root colonization (%); NI = Not informed; NMP of AMF = the most probable number of AMF propagules.

3.2 Composting of Wastes, Biochar and Vermicompost for Soil Health

Early investigation on the addition of organic amendments to counteract degradation of soil properties used agro-waste residues such as dry olive cake and sugar beet waste supplemented with rock phosphate as organic amendments after fermentation by *Aspergillus niger* showing positive effects of the amendments and beneficial microorganisms, such as AMF on the microbial soil status and the relevance of such biotechnological management in bioremediation. The effectiveness of AMF is usually influenced by environmental variables and soil conditions., compost, produced by *Trichoderma longibrachiatum* and vermicompost produced by *Amynthas gracilis* worms, can improve plant growth (232 %) and nutrient uptake of pistachio seedlings Enhancing soil conditions . The dual application of AMF and adequate doses of vermicompost (e.g. 5 ton ha^{-1}) better mitigated the effects of water deficit on quinoa growth.

4. Discussion

The aim of this work was to evaluate the methodology for studying soil quality and health, specifically, to understand how arbuscular mycorrhizas contribute to soil health. Chemical and physical properties were used as indicators of soil quality in several ecosystems, being static such as soil organic carbon, nutrient availability, and soil acidity. However, physical properties (soil texture, soil depth or topsoil depth, soil bulk density, available water holding capacity, saturated hydraulic conductivity, soil loss, soil strength, porosity, aggregate stability and size distribution are nowadays more employed as dynamic indicators (least limiting water range, trafficability, leaching and erosion potential). However, soil quality indicators would be specific to each soil type [19], thus biological parameters are considered of most importance, and among them, mycorrhizas have won more space together with bacteria.

Additionally, trap plants, under greenhouse conditions are increasingly employed to obtain species that have not formed spores in the field, including new ones reported. Thus, in the study of AMF communities at field sites, various methods can be employed, being spore number and species diversity the most employed nowadays.

5. Conclusions

Increasing studies indicate mycorrhizas for soil quality assessment; especially, on soil structure. Pioneer research with AMF and soil compaction as well as AMF propagules bank in soils showed variations of AMF in crops under different tillage systems, revealing AMF benefits to their plant hosts in most ecosystems. Research worldwide has focused on different aspects of AM symbiosis for maintaining healthy soils. Practical tools to study AMF related to soil health are increasingly promoted despite the growth of conventional agriculture. While there are significant gaps in the AMF world, research on soil health and the benefit of AMF is vital to increase, restore and manage soil fertility. Thus, the symbiotic plant association with AMF constitutes a promise for soil health, and thus for sustainable management. Additionally, the development of practical methods or indicators must be specifically adapted for each region according to its biological, social, and economic characteristics, to complete the wise management of ecosystem services. Molecular and biochemical tools can be

expensive, being less used in developing countries, which require cheaper methods to evaluate indicators of soil health. The choice of plant species would have great implications for 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 needs to be further investigated. Future research needs to be carried out to increase studies of soil health, especially regarding AMF functionality, soil characteristics and nutrient dynamics.

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