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Full Length Research Paper

The Diversity of Arbuscular Mycorrhizal Fungi Associated With Plants in Ethiopia and Its Role in Agro-Ecosystem

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In this review it has been tried to surface out the role of arbuscular mycorrhizal fungi to the agro ecosystem. Particularly, AMF's contribution to stabilize soil fertility, reduction of use of phosphate fertilizer, increase in plant tolerance against biotic & abiotic stresses, and enhancement of quality plant production was reviewed. Besides, this review has attempted to compile several efforts, conducted so far in the study of AMF diversity and abundance at different land use patterns in Central, Rift Valley, Northern and Southern Ethiopia. AMF diversity, plant-fungus assemblages, is generally influenced by the slope, soil physico-chemical nature. Especially soil PH, availability of extractable chemicals such as calcium & potassium, its cation exchange capacity, electrical conductivity and field capacity are determinant factors. However, the effects of factors such as plant community in the AMF community composition are less clear. In general, infectivity and diversity of AMF communities is often reduced in disturbed habitats such as agro ecosystems or post-mining sites. AMF are considered to have low specificities of association with host species, but this conclusion is mostly based on experiments in which individual isolates of fungal species are grown separately. When fungi are examined as a community, evidence suggests fungal growth rates are highly host-specific, hence it can be concluded that ecological specificity applied to some arbuscular mycorrhizal associations. However, the competitive balance between AM fungal species in terms of their ability to colonize roots may be affected by environmental conditions. This specificity of fungal response could contribute to the maintenance of diversity within the AMF community. Regarding the seasonal diversity of AMF, evidence has showed that fungal spore density differs seasonally, with some fungi sporulating in late spring and others sporulating at the end of summer. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. Though scholars in Ethiopia have contributed tremendously, yet there are still lots of tasks which are required to be carried out to help respond the outstanding questions allied with fungi-plant assemblages.

Keywords: Arbuscular mycorrhizal fungi, Diversity, Agroecosystem

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) is a group of obligate biotrophs, to the extent that they must develop a close

symbiotic association with the roots of a living host plant in order to grow and complete their life cycle (Parniske, 2008). The term “mycorrhiza” literally derives from the Greek *mykes* and *rhiza*, meaning fungus and root, respectively. AMF can symbiotically interact with almost all the plants that live on the Earth. They are found in the roots of about 80-90% of plant species (mainly grasses, agricultural crops and herbs) and exchange benefits with their partners, as is typical of all mutual symbiotic relationships (Wang and Qiu, 2006). They represent an interface between plants and soil, growing their mycelia both inside and outside the plant roots.

AMF provide the plant with water, soil mineral nutrients (mainly phosphorus and nitrogen) and pathogen protection. In exchange, photosynthetic compounds are transferred to the fungus (Bonfante and Genre, 2010). Taxonomically, all AMF have been affiliated to a monophyletic group of fungi, i.e. the Glomeromycota phylum (Schüßler *et al.*, 2001). They are considered to be living fossils since there is evidence that their presence on our planet dates back to the Ordovician Period, over 460 million years ago (Simon *et al.*, 1993). Investigations on AMF taxonomy began in the nineteenth century with the first description of two species belonging to the genus *Glomus*. Since that date, many Glomeromycotan species, genus and families have been discovered and characterized by means of traditional approaches based on the phenotypic characteristics (mainly spore morphology). Molecular DNA sequencing-based analyses have recently contributed to a great extent by shedding light on a previously unseen and profound diversity within this phylum (Schüßler and Walker, 2011)

Functionally, AMF form the so-called arbuscular mycorrhizae with plant roots. The most typical AMF structure, which also gives the name to this group of fungi, is the arbuscule (Figure 1). This structure, whose shape recalls that of a small shrub, forms inside the root cortical cells by branching in several very thin hyphae. In this way, the surface area, where the nutritional exchanges between the plant and fungus take place, is maximized. Fungal hyphae that grow between root cortical cells are able to produce other AMF structures, such as intercellular hyphae and vesicles (Figure 1). All these structures that grow inside the plant roots represent the intraradical phase of the fungus. Hyphae also grow outside the plant roots, and generate a network that extends over long distances and explores the soil beyond the nutrient depletion zone that normally characterizes the area surrounding the roots. At the end of the AMF life cycle, or in response to particular environmental conditions, spores (Figure 1) of variable size (up to 400 µm), depending on the species, are produced in the roots and/or in the soil. These, along with external explorative and running hyphae, represent the extraradical

phase of the fungus. The synergic action of the intra- and extra radical phases is responsible for the ecological significance of the AMF, a soil-root-living key group of organisms (Bonfante and Genre, 2010).

Arbuscular mycorrhizal fungi (AMF), or Glomeromycota are obligate symbiotic fungi that penetrate plant roots and form the arbuscule, that is, a specialized hyphal structure that develops inside cortex cells, and represents the main site of nutrient exchange between partners (Smith and Read, 2008). These fungi have played an important role in the evolution of land plants for more than four hundred million years (Remy *et al.*, 1994), and they today colonize the roots of most plants. In turn, the plants, despite their ability to live independently, may increase nutrient uptake, growth and reproductive success when associated with AMF (Simon *et al.*, 1993). Moreover, AMF ameliorate soil quality (Simon *et al.*, 1993) and improve the ability of host plants to withstand abiotic stress and disease (Singh *et al.*, 2011), thus increasing plant performances (Berruti *et al.*, 2016).

2. Services provided by AMF in agro-ecosystems

AM fungi, able to establish a symbiotic interaction with the root organs of 80% of plant families, not only improve the growth of plants through increased uptake of available soil phosphorus (P) and other non-labile mineral nutrients essential for plant growth, they have also ‘non-nutritional’ effects in stabilizing soil aggregates, in preventing erosion, and in alleviating plant stress caused by biotic and abiotic factors (Smith and Read, 2008). The beneficial effects of AM fungi on plant performance and soil health are essential for the sustainable management of agricultural ecosystems (Barrios, 2007).

Human society benefits from a multitude of resources and processes from natural and managed ecosystems, to which AMF make a crucial contribution. These resources and processes, which are called ecosystem services, include products like food and processes like nutrient transfer. Growing human needs and demands have led to an increase in resource demands imposed on ecosystems, greater global consumption of natural resources and a significant decline in ecosystem services. Many people have been under the illusion that these ecosystem services are free, invulnerable and infinitely available; taken for granted as public benefits, they lack a formal market and are traditionally absent from society’s balance sheet. Since 1997, economists and ecologists have joined forces to estimate the annual value of the services that ecosystems provide (Fisher and Turner, 2008).

Although most services lie outside the market and are difficult to calculate, minimum estimates equal or exceed global gross national product (Pimm 1997). In 1997, a team of researchers from the USA, Argentina and the Netherlands put an approximate price tag of US \$33 trillion a year on these fundamental ecosystem services. This is

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Table 1: Overview of AMF service provided to ecosystem (Source: Silvio *et al.*, 2010)

AMF Function	Ecosystem service(s) provided
Root Morphology modification and development of a complex, ramifying mycelial network in the soil	Increased plant/soil adherence and soil stability (binding action and improvement of soil structure)
Increasing mineral nutrient and water uptake by plant	Promote plant growth while reducing fertilizer requirement
Buffering effect against abiotic stresses	Increased plant resistance to drought, salinity, heavy metals pollution and mineral nutrient depletion
Secretion of "glomalin" into the soil	Increased soil stability and water retention
Protecting against root pathogens	Increased plant resistance against biotic stresses while reducing phytochemical input
Modification of plant metabolism and physiology	Bio regulation of plant development and increase in plant quality for human health

nearly twice the value of the world's gross national product. In this study, two major ecosystem services, 'soil formation' and 'nutrient cycling', were respectively estimated to represent US \$17.1 and 2.3 trillion (Costanza *et al.*, 1997).

The supply of agricultural products and ecosystem services are evidently essential to human existence and quality of life; however, recent agricultural practices that have greatly increased global food supply have had inadvertent, detrimental impacts on the environment and on ecosystem services. High-intensity agriculture has principally focused on productivity instead of integrating natural resource management into food production security; mechanization, monocultures, and increased use of synthetic inputs (chemical fertilizers, pesticides) have degraded water quality, reduced arable lands and forest resources, and soil fertility (Foley *et al.*, 2005).

In consequence, novel and expedient methods are needed to manage Earth's ecosystem services, the loss of which will have important consequences for sustainable food production in the face of an increasing world population. Agriculture is the largest interface between humans and environment, thus reconciling crop production and environmental integrity, in other words sustainable crop production, is a major challenge for agriculture and future farmers (Robertson and Swinton, 2005). This implies the need to develop crop management strategies that optimize soil fertility, biological diversity and crop robustness (Altieri, 1995) by creating forms of agro ecosystems that respect natural ecological processes and support productivity in the long term (Altieri, 1999).

In this context, the ecosystem services rendered by soil biota in maintaining soil quality, plant health and soil resilience are extremely pertinent (Smith and Read, 2008). In particular, soil microorganisms that form mutually beneficial relationships with plant roots have become a target of increasing interest in agricultural research and development because they offer a biological alternative to promote plant growth and reduce inputs in sustainable cropping systems (Hart and Trevors, 2005). The ubiquity of AM fungi at the interface between soil and plant roots

makes them a key functional group of soil biota which by their nutritional and non-nutritional activities profoundly influences ecosystem processes that contribute to the ecosystem services in agro ecology. AMF symbiosis can play as an ecosystem service provider (for an overview see Table 1) to guarantee plant productivity and quality in emerging systems of sustainable agriculture. The appropriate management of ecosystem services rendered by AM will impact on natural resource conservation and utilization with an obvious net gain for human society (Silvio *et al.*, 2010).

2.1 AMF role in soil stability

During development of AM, the fungal symbionts grow out from the mycorrhizal root to develop a complex, ramifying network into the surrounding soil which can reach up to 30 m of fungal hyphae per gram of soil (Wilson *et al.*, 2009). This network can make up to 50% of fungal mycelium in soil (Rillig *et al.*, 2002) there by representing a major part of the soil microbial biomass (Leake *et al.*, 2004). This mycelial network can have a binding action on the soil and improve soil structure. In addition, the secretion by AM fungi of hydrophobic, 'sticky' proteinaceous substances, referred to as glomalin (Rillig *et al.*, 2002), also contributes to soil stability and water retention (Bedini *et al.*, 2009). The combination of an extensive hyphal network and the secretion of glomalin is considered to be an important element in helping to stabilize soil aggregates (Rillig and Mummey, 2006), thereby leading to increased soil structural stability and quality (Caravaca *et al.*, 2006).

Agronomic practices such as monoculture cropping, ploughing, or fertilization have frequently been observed to have a negative impact on the amount as well as the diversity of AM fungi present in soils (Oehl *et al.*, 2005). A reduction in fungal biomass will result in a negative effect on soil stability and consequently increase the risk of soil erosion. This is not to be underestimated; in the UK, productivity loss due to soil erosion of agricultural soils has been estimated to 9.99 million €/year (Görlach *et al.*,

2004). Since soil is a non-renewable resource on a human time-scale, the impact of erosion is often cumulative and in most instances irreversible (Silvio *et al.*, 2010).

2.2 AMF role in reducing phosphate fertilizer input

Phosphate, which is an essential mineral nutrient for plant growth, is one of the three main mineral nutrients applied in agriculture. Rock phosphate sources are limited and on the basis of the presently known world phosphate reserves, most of the phosphate mines will be depleted in about 100 years (Herring and Fantel 1993; <http://minerals.usgs.gov/minerals/pubs/mcs/2008/mcs2008.pdf>).

Although the consumption of triple-phosphate has been reduced in developed countries between 2000 and 2006 by 36%, reaching an annual amount of 0.3 million tonnes, whereas in the mean time it increased by 36% in the developing countries reaching an annual amount of 2.1 million tonnes (www.fertilizer.org/ifa/ifadata/search). Excess application of phosphate fertilizers is an important cause of water eutrophication, and therefore improvement of phosphate uptake efficiency by plants is a priority. Inorganic phosphate (Pi) has very limited diffusion capacities in soils and its rapid absorption from the soil solution by plant roots generates Pi depletion zones at the root surface resulting in a decline of directly absorbed Pi by the plant surface (Roose and Fowler, 2004). The network of fungal mycelium connected to AM roots increases by several orders of magnitude the soil volume which can be explored by a plant so that a mycorrhizal root is more efficient in phosphate uptake than a non-mycorrhizal root (Smith and Read, 2008). Under given field conditions, it has been estimated that a reduction of 80% of the recommended phosphate fertilizer could be supplemented by inoculation with AM fungi (Jakobsen, 1995). It is evident that such reductions in phosphate application have important economical and environmental impacts (Silvio *et al.*, 2010).

2.3 The role of AMF in amplifying plants' tolerance against abiotic stresses

Abiotic stresses cause extensive losses to agricultural productivity. Mineral depletion, drought, salinity, heavy metals or heat are serious problems in many parts of the world, in particular in arid and semi arid areas (Evelin *et al.*, 2009). It is predicted that two thirds of cultivable land may disappear in Africa, a third in Asia and one fifth in South America by 2025 and that arable land area per inhabitant in the world will be reduced to 0.15 ha in 2050 (<http://www.un.org/esa/sustdev/documents/agenda21/french/action12.htm>). In the USA and Spain, one third of the country is undergoing desertification. The potential of AM fungi to enhance plant tolerance in abiotic stress conditions has long been recognized (Smith and Read, 2008) and their manipulation in sustainable agricultural systems will

be of tremendous importance for soil quality and crop productivity under severe edapho-climatic conditions (Lal, 2009).

Amongst more recent examples of the use of beneficial soil microbes to improve crop tolerance against abiotic stress conditions, studies of the synergistic effect of co-inoculated bacteria and AM fungi from dry environments on plant growth under drought stress (Marulanda and Barea, 2009) underlines the interest of manipulating autochthonous AM fungal isolates from dry soils for re-vegetation of degraded land sites to improve soil quality, and to fight desertification in Mediterranean ecosystems. For example an indigenous drought-tolerant strain of *Glomus intraradices* associated with a native bacterium reduced by 42% the water required for the production of *Retama sphaerocarpa* (Marulanda *et al.*, 2006). AM fungi alleviate stress salinity in olive tree plantations in Spain or in arid North Africa where palm grove yields are considerably affected by drought and soil salinity (Porrás-Soriano *et al.*, 2009).

Another area where AM fungal inoculation has become a prospective tool for enhancing plant tolerance to environmental stress conditions, is in the re-vegetation of naturally or industrially metal-contaminated soils. There are many examples in the literature to illustrate this role of the AM symbiosis, although the underlying mechanisms are not yet fully understood (Khade and Adholeyan, 2009). The occurrence of AM in Ni hyper accumulating plant species found naturally on metal-rich soils offers possibilities of using heavy metal hyper accumulating plants together with AM fungi for phytoremediation strategies (Gamalero *et al.*, 2009). Furthermore, many phosphate fertilisers are a major source of soil contamination by cadmium in agricultural systems (Nziguheba and Smolders, 2008) which again pleads for the reduction of crop reliance on phosphate fertilizers. AM fungi, through their mycelium network, not only improve Pi uptake by roots but they also have a buffering effect on the cadmium uptake, reducing the toxic effect of cadmium on plant growth (López-Millán *et al.*, 2009).

2.4 The role of AMF in protecting crops against biotic stresses

To limit the spread of pests causing great yield losses in cultivated crops, conventional agriculture has been using large quantities of pesticides as well as plant breeding programmes in order to obtain disease-resistant plants. However, pesticides are often only partially effective against soil-borne diseases. Moreover, they are detrimental to human health and to the environment and as a consequence an ever-increasing number of pesticides are being taken off the market. In addition, disease resistance obtained by plant breeding programmes is often due to single plant genes, which can be rapidly overcome by evolutionary biodiversity in pathogenic agents.

Complementary approaches have therefore to be developed to ensure durable tolerance of plants to pathogens. Numerous studies have demonstrated the beneficial effect of AM fungi in increasing plant tolerance to biotic stress caused by soil-borne pathogens interacting with many plant species. This has been consistently shown for a number of pathogenic fungi or Oomycetes such as *Fusarium*, *Rhizoctonia*, *Verticillium*, *Thielavopsis*, *Aphanomyces*, *Phytophthora* and *Pythium*, as well as nematodes from the genera *Heterodera*, *Meloidogyne*, *Pratylenchus* and *Radopholus* (Hao *et al.*, 2009). Most of the research has been carried out under very controlled conditions at early stages of plant growth but a few studies conducted in the field or in the greenhouse under real production conditions confirm these results (Utkhede, 2006).

Disease associated losses has shown a tremendous challenge in crop production arena. If we consider tomato, which is one of the most widely grown vegetables in the world, and which is susceptible to many insects, bacteria and nematodes causing significant reduction in fruit yield (34%) under current production practices (Engindeniz, 2006). Although this plant is not highly responsive to AM fungi in terms of plant growth (Smith *et al.*, 2009), it clearly benefits from mycorrhization when challenged by root pathogens such as *Fusarium oxysporum* f. sp. *radicislycopersici*, *Rhizoctonia solani*, *Phytophthora parasitica* or *Meloidogyne incognita*. In this case, root colonisation by AM fungi can largely reduce root infection and disease severity caused by the pathogens, resulting in increases in plant fresh weight (up to 198%) and fruit yield (14.3%) as compared with pathogen-infected non-mycorrhizal plants (Table 2).

This clearly justifies the comparison of mycorrhiza to a 'health insurance' for plants (Gianinazzi and Gianinazzi-Pearson, 1988). Variation, however, exists in the efficiency of bioprotection between AM fungal species or isolates (Martinez-Medina *et al.*, 2009). Nonetheless, even where there is no immediate positive effect on plant growth and yield (Table 2), a reduction in disease development can be beneficial for decreasing pathogen populations in the soil and this may have a positive impact on following crops. Overall, the greater tolerance of mycorrhizal plants against root pathogens provides bioprotection as an ecosystem service for sustainable agriculture since it can be active against a wide spectrum of pathogens and could complement innovative multiple allele quantitative resistance obtained by plant breeding (Silvio *et al.*, 2010).

2.5 The role of AMF to enhance plant quality for human health

Mineral content and secondary metabolites of crops used as food or medicinal remedies can be beneficial in preventing diseases such as cancer, cardiovascular and neurodegenerative diseases or microbial infection

(Cummings and Kovacic, 2009). For example, zinc deficiency alters the immune and gastrointestinal systems, blood cell development, thyroid hormone metabolism as well as pancreas, liver and brain activity, and can increase risks of diabetes, coronary artery disease and cancer (Cummings and Kovacic 2009). Approximately 30% of the world's soils are Zn deficient, particularly in tropical areas (Cavagnaro, 2008) and this leads to reduced yields and Zn content in crop products, resulting in inadequate dietary Zn intake for many human populations and a negative impact on human health. Several studies have reported that AM can increase Zn uptake by plants even under field conditions (Cavagnaro, 2008). For example, the Zn content in shoots and fruits of field-grown wild-type mycorrhizal tomato plants was found to be up to 50% higher than in a mutant with reduced mycorrhizal colonization (*rmc*) (Cavagnaro *et al.*, 2006).

It is also becoming evident that the AM symbiosis can stimulate the synthesis of plant secondary metabolites, which are important for increased plant tolerance to abiotic and biotic stresses or beneficial to human health through their antioxidant activity (Seeram 2008). These bioactive compounds include organosulfides, polyphenols (phenolic acids, anthocyanins, flavonoids), phytosterols, stilbenes, vitamins, lignans and terpenoids including carotenoids (Kirby and Keasling, 2009). Although it is well-known that AM fungi can stimulate synthesis of phenolic compounds (phenolic acids, flavonoids) and activate the carotenoid pathway in roots (Schliemann *et al.*, 2008), only a few analyses have targeted final crop products (leaves, roots or fruits) of mycorrhizal plants used in food or in medicinal remedies. Nonetheless, these studies indicate that activation of plant secondary metabolism in response to AM fungi can result in increases in essential oil concentration of plant tissues or in the content of individual molecules.

The reported data also show that even under field conditions AM fungi can enhance leaf, fruit or bulb accumulation of many molecules with medicinal interest. For example, the 95% increase in artemisinin concentration in leaves of mycorrhizal *Artemisia annua* (Chaudhary *et al.*, 2008) is of both medical and economical interest since artemisinin, which is highly priced, is considered as the best treatment for uncomplicated malaria when used as part of a combination therapy (Kirby and Keasling, 2009). It is however important to note that the beneficial impact of AM fungi on plant mineral and secondary metabolite contents depends not only on AM fungal species or isolates, but also on plant genotype and fertilization regime (Gianinazzi *et al.*, 2008) which again underlines the need to develop crop management strategies using appropriate plant/AM fungal combinations and culture practices for the production of mycorrhizal plants with nutritional quality (Silvio *et al.*, 2010).

3. AMF abundance and diversity

AMF diversity is generally influenced by the soil chemistry, and especially by the soil pH (Dumbrell et al., 2010), the abundance of AMF in the roots was related to some chemical (available extractable calcium and potassium) and physical (cation exchange capacity, electrical conductivity and field capacity) properties of the soil, thus suggesting an effect of AMF on improving the soil quality. The non-metric multidimensional scaling (NMDS) ordination of the AMF community composition showed that the diversity of AMF in the various sites was influenced not only by the soil quality, but also by the slope. Therefore slope, soil PH, and soil chemistry are vital to consider in the plant-fungus assemblages (Casazza et al., 2017).

However, the effects of factors such as plant community in the AMF community composition are less clear (Zobel and, O'È pik, 2014). In general, infectivity and diversity of AMF communities is often reduced in disturbed habitats such as agroecosystems or post-mining sites (PuÈschel et al., 2008).

Arbuscular mycorrhizal fungi are considered to have low specificities of association with host species, but this conclusion is mostly based on experiments in which individual isolates of fungal species are grown separately, (Bever et al., 2001). When fungi are examined as a community, evidence suggests fungal growth rates are highly host-specific, hence it can be concluded that ecological specificity applied to some arbuscular mycorrhizal associations (McGonigle and Fitter , 1990). However, the competitive balance between AM fungal species in terms of their ability to colonize roots may be affected by environmental conditions. This specificity of fungal response could contribute to the maintenance of diversity within the AMF community (Bever et al., 2001).

Evidence showed that fungal spore density differs seasonally, with some fungi sporulating in late spring and others sporulating at the end of summer. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. For example, *Gigaspora gigantea*, which sporulates most abundantly in the fall and appears over winter as spores, is likely to be physiologically active during the warm season. In an experiment in which AMF were trapped on different plant hosts, isolates of different fungal species sporulated differentially, with the relative dominance of fungal species being reversed, depending on the plant species with which they were associated (Bever et al., 1996).

As this pattern of host specificity of growth rates in this "non-specific" association has been observed in other systems, including tall grass prairie (Johnson et al., 1992), California grasslands (Nelson and Allen, 1993), chalk grasslands (Fitter and Sanders, 1992) and agricultural fields (Douds and Millner, 1999), this appears to be a general property of this interaction. The symbiotic

performance of AM fungi and the final mycorrhizal phenotype can be considered as resulting from the interaction of two main factors: infectivity and affectivity, both of which depend on fungal and plant determinants. Although plant mechanisms regulating mycorrhizal infection is complex, it is interesting that plant resistance to arbuscular mycorrhizal fungi can be induced by a simple mutation at one locus (Gollottee et al., 1993).

3.1. Diversity of AMF across different cropping systems of north Ethiopia

According to the unpublished study conducted in Northern part of Ethiopia, Jabi Tehnan woreda western Gojam by Moges Shenkutie, 2014 spore density of the different cropping systems varied significantly within and between land uses types ranging from 104 spores/100gm soil from Eucalyptus (*E.globulus*) mono (tree) cropping to 929 spores/100gm soil for mixed cropping system (cabbage+sunflower+maize). All plants formed AM symbiosis except cabbage (*Brassica oleraceae*). The AM fungal colonization pattern showed variations among the roots of the cropping types ranging from 22% (teff and eucalyptus) up to 73.4% from sunflower/maize/ (mixed crop). On the contrary, other studies showed that teff and Eucalyptus tree had mycorrhization rate of 58-67% (Cesra et al., 2009) 31-60% (Tekalegn Mamo and Killhalm, 1987), respectively. A total of 8 AMF genera and 43 morphospecies were identified from the different cropping systems of which the highest number of species was recorded from the genus *Acaulospora*(14 species), followed by the genera *Glomus* (9 species) and *Scutellospora* (7 species), *Funneliformis* (5 species), *Claroideoglomus* (3 species), *Gigaspora* (3), *Entrophosphora* and *Paraglomus* with one species each. Based upon Importance Value (IV), no dominant AMF species were recorded across all the land use types.

However, three common AMF species: *Funneliformis geosporum* (34%), *Acaulospora rehmii* (27%) and *Glomus clarum* (26%) were found comparatively with high importance value respectively. Although the genera *Acaulospora* and *Glomus* contained large number of species, they did not have dominant species distributed across the land use types. In this study, AMF species diversity was much lower in tree-based cropping system (Eucalyptus) or mixed croton+juniperus plantation than in the annual cropping systems (monocrops, mixed crops).The study showed that intensive land use (cropping) produced more spores than the relatively stable ecosystems of trees indicating that disturbance enhances sporulation. As per studies of Janos,1992, Picone, 2000 high spore densities were recorded from land use changes, it is justified as these changes induce AMF to sporulate due to grazing, disturbance and slow rate of decomposition. Moges's study has revealed a link between spore density, mycorrhization and species diversity in

relation to physico-chemical characteristics at $P < 0.05$. Based upon the Importance Value (IV), the different AMF genera were generally categorized into "Commonly distributed" and "Rarely distributed" species across the different land use types. However, according to the unpublished study of Moges Shenkutie (2014) there were no species found as dominant ($IV > 50\%$) that were distributed amongst all plants in all cropping systems. This is quite different from the report of Zerihun Belay et al., (2015), where the genera *Claroideoglossum* and *Funnelformis* were categorized into the dominant genera with IV 63 and 56, respectively. Furthermore, study outputs of Moges Shenkutie (2014) consolidates that all species from *Claroideoglossum* and a species from *Paraglossum* were categorized into the "common" group (with $IV 10\% < X < 50\%$) together with many species of *Acaulospora* (33%), *Glomus* (23%) and *Scutellospora* (16%) and that were distributed in many of the cropping systems (land uses). However, all species from *Gigaspora*, and *Entrophospora* were "Rare ($IV < 10\%$)" and distributed in one or in the other land use types. The other species, *Claroideoglossum caledonium*, *C. etunicatum*, *F. mossae*, *Glomus tortosum* and *Acaulospora morrowiae*, *Glomus ebureum*, and *G. lamellosum*, *G. clarum*, *Claroideoglossum luteum*, and *Acaulospora rehemii* were detected from most of the sites.

3.2. Diversity of AMF across different cropping systems in central & rift valley of Ethiopia

According to the root samples and rhizosphere soil of nine acacia species (*Acacia abyssinica*, *Faidherbia albida*, *A. nilotica*, *A. senegal*, *A. seyal*, *A. sieberiana*, *A. saligna*, *A. tortilis* and *A. robusta*) collected from Bishoftu, Zeway and Addis Ababa sites with different land use types, AMF were present in all roots and rhizosphere soil samples of acacia trees with low (12%) to moderate (67.3%) levels of colonization (Zerihun Belay et al., 2013). This pattern is similar to that observed in other tropical systems such as (0 to 75% colonization) in acacia and other woody legume species in dry deciduous forest areas of Northern Ethiopia (Birhane et al., 2010), (31 to 64% colonization) in acacia and prosopis tree species in Senegal (Ingleby et al., 1997), (35 to 65% colonization) in acacia tree species, in India (Lakshman et al., 2001) and (56 to 73% colonization) in *A. farnesiana* and *A. planifrons*, in India (Udaiyan et al., 1996).

Correlation analysis showed that arbuscular colonization was positively correlated with hyphal and vesicular colonization (Lingfei et al., 2005). Concerning soil parameters, Zerihun Belay et al., (2013) has revealed that, though not significant, there was an indication of positive correlation between percentages of hyphal colonization and organic carbon (0.536) and a negative correlation between hyphal colonization and available P (-0.454), a result that is similar to the work of Lingfei et al. (2005).

Also, Kahiluoto et al., 2001 suggested a negative correlation between available phosphorus and AM colonization.

Significant variation in the abundance of AMF spores was found in the rhizosphere soil of acacia tree species in the same or different plant community. The mean number of spores per 100 g of soil ranged from 307 to 1506 with an average of 994 (Zerihun Belay et al., 2013). Other studies in similar or different host plants of the tropical area corroborate this finding: 775 to 1240 spores 100 g⁻¹ soil in *A. albida* Del. in Senegal (Diop et al., 1994); 500 to 1500 spores 100 g⁻¹ soil in *A. farnesiana* and *A. planifrons* in moderately fertile alkaline soils in India (Udaiyan et al., 1996); 110 to 2600 spores 100 g⁻¹ soil in tropical forest and pasture (Picone, 2000) and 5 to 6400 spores 100 g⁻¹ soil in a valley savanna of the dry tropics (Tao et al., 2004). By contrast, low spore densities of 11 to 32 spores 100 g⁻¹ soil were detected in dry deciduous woodlands of Northern Ethiopia associated with different acacia species (Birhane et al., 2010).

The percentage of root length colonized by AMF was estimated. Spores, spore clusters and sporocarps extracted from soil samples were counted and morphologically identified to species or specific morphotype. Roots of all acacia species were colonized from low to moderate or relatively high levels by AMF with the occurrence of arbuscules, vesicles and hyphae. Arbuscules were however not detected in roots of *A. senegal*. The highest AM fungal colonization was found in *A. seyal* (67.3%) from open grazing field (OGF) at Zeway followed by *A. nilotica* (44%), whereas the lowest AMF colonization of 12% was recorded in *A. saligna* at Bishoftu. Rhizosphere soils harbored AMF fungal spores ranging from 3.7 spores g⁻¹ soil in *A. nilotica* to 15.0 spores g⁻¹ in *A. seyal* from open grazing field (OGF) at Zeway.

A total of 41 AMF species in 14 genera and 7 families of the Glomeromycota were identified. Nine species belonged to *Acaulospora*, 6 to *Funnelformis*, 4 each to *Gigaspora*, *Glomus*, and *Rhizophagus*, 3 each to *Claroideoglossum*, and *Scutellospora*, 2 each to *Racocetra* and *Diversispora*, and 1 each to *Entrophospora*, *Sclerocystis*, *Paraglossum* and *Pacispora*. Moreover, 2 unidentified morphotypes each of *Glomus*, and *Acaulospora* and 1 of *Archaeospora* were isolated. Based on relative abundance and isolation frequency of spores, *C. claroideum*, *C. etunicatum*, *C. luteum*, *F. geosporus* and *G. aggregatum* were the dominant species in the study. The study showed that the acacia species were characterized by relatively high AMF colonization and very high AMF diversity. AMF spore density and AM root colonization in acacia roots were influenced by soil factors such as available P and soil texture (Zerihun Belay et al., 2013).

As per the study conducted by Zerihun Belay et al., (2015) to estimate the effect of different land use types on arbuscular mycorrhizal (AM) fungal populations in soil and trap cultures from Showa robit, Ethiopia, the following out

puts were recorded. Seven land use types were selected. There were low-input arable systems, either having a mixture of crops (Arable1) or monocropped with sorghum (Arable2) or maize (Arable3). Arable4 was relatively a high-input system with monocropped sorghum. A fruit cropping area (FC) managed with composts and plant residues, a natural forest (NF) and an acacia plantation (AP) were also studied. AMF spore abundance, species richness, diversity indices and mycorrhizal inoculum potential (MIP) were studied. In field soil, significantly higher spore numbers were recorded from FC, Arable1 and Arable3 (5.8-6.1 spores g⁻¹ soil) than in Arable4, NF and AP (2.8-3.9 spores g⁻¹ soil). In trap cultures, AP, FC, and Arable2 had the highest spore numbers (9.8-11.1 g⁻¹ soil) and Arable 4 and NF the lowest (2.5-3.8 g⁻¹ soil). Slightly different MIP patterns also occurred with Arable1 (53.7%) and FC (52.6%), having significantly higher hyphal colonization, 53.7% and 52.6%, respectively, compared to the other land use types that fell within percentage colonization of 19.9-25.8 %. A total of 42 and 33 morphospecies of AMF were identified in field soil and trap culture soil, respectively. Trap culturing increased spore numbers but caused a loss of AMF species richness. Higher species richness was obtained in FC and Arable1 compared with the other systems. *Claroideoglossum* and *Funnelformis* were the dominant genera in all land use types in both trap culture and field soil. The results clearly imply that organic management and diversification of crops enhances AMF diversity of low-input agricultural systems.

Study findings of Tesfaye Wubet *et al.*, 2009 confirm previous results from the same area indicating distinct fungal communities associated with the diverse tree species and suggests the potential of these indigenous tree seedlings to trap a wide range of AM fungi appropriate for successful afforestation. According to the study carried out by Mengsteab Hailemariam *et al.*, 2013 on spore abundance, root colonization of *Albizia gummifera* (J.F. Gmel.) and *Croton macrostachyus* (Hochst Ex Del.) trees and their effect on colonization of maize has revealed that the number of spore count was significantly higher under the canopy of *A. gummifera* (791/100 g of dry soil) and *C. macrostachyus* (877/100 g of dry soil) trees than outside the canopy (547 and 588/100 g of dry soil, respectively).

The level of root colonization of *C. macrostachyus* (45 %) was higher than *A. gummifera* (41 %). Root colonization of maize crops grown under the canopy of *A. gummifera* and *C. macrostachyus* trees was significantly higher than outside the canopy ($P < 0.001$). Maize seedlings grown on non-sterilized soils collected under and outside the canopy of *A. gummifera* and *C. macrostachyus* trees recorded higher root colonization, plant height, shoot and root dry weight than grown on sterilized soils ($P < 0.001$). The percentage of AM colonized roots of *Zea mays* seedlings was significantly positively correlated with the number of spore counts for field soils. The study output consolidates the fact that the rhizospheres of indigenous agroforestry

perennial species are important source of inoculum for annuals. The integration of perennials and annuals in an agroforestry system enhances the maintenance of soil quality in the tropics.

3.3. Diversity of AMF in agroforestry systems of South Ethiopia

According to study conducted in agroforestry practicing areas of Sidama Zone, South Ethiopia, at moderate to low P and N concentrations the rate of AMF root colonization and spore density was high in comparison with the rhizosphere soils with the highest P and N concentration.

In the contrary, study out puts of Beyene Dobo *et al.*, 2016 revealed that there is increment in spore density and diversity with an increase in soil available P in culturally protected forest that may be because the concentration of P is not high enough to influence mycorrhizal development (Diriba Muleta *et al.*, 2007). The highest percentage of total AMF colonization was recorded for shade trees *Millettia ferruginea* (84%) and *Erythrina brucei* (80%) followed by intercropped perennial crops *Ensete ventricosum* (86%), *Catha edulis* (85%) and *Coffea arabica* (80%) and the lowest percentage AMF colonization was recorded for *Rhamnus prinoides* (53%) and *Colocasia esculenta* (52%).

Though found in almost all homegarden agroforestry practices and with broad coverage in Sidama agroforestry, some crops and vegetables such *Brassica integrifolia* and *Cucurbita pepo*, grown intercropped were found to be non-mycorrhizal as none of the AMF structures were recorded. The highest number of AM spore population was recorded in rhizosphere soils of *Croton macrostachyus* (1066±19.33) and *Catha edulis* (1054±53.12) and the lowest spore density was recorded for *Dioscorea alata* (100.00±2.89) spore per 100 g of dry soil.

The percentage fungal colonization in any individual plant species and spore population in the rhizosphere soils of that species did not correlate to each other (Beyene Dobo *et al.*, 2016a), Possibly because of the presence of a diverse population of AM fungal species or according to Biermann and Linderman (1983), it could be due to the fact that there are AMF species that rely more on extensive formation of hyphal networks instead of survival through spore formation as primary infective propagules. The relationship between spore numbers and mycorrhizal colonization has been found positive by several workers (Giovannetti and Nicolson, 1983) but negative by others (Louis and Lim, 1987). Some workers have also found no relationship between mycorrhizal colonization and spore density of AMF (Diaz and Honrubia, 1994). The factors like edaphic or climatic condition; host fungus compatibility, root properties and soil microorganisms might influence the abundance of spore population and mycorrhizal associations with a particular tree species (Beyene Dobo *et al.*, 2016a).

With regards to the association of land use pattern and

AMF abundance, it has been found that monocropping reduces spore density and AM colonization in comparison with the culturally protected forest and the agroforestry (Beyene Dobo et al., 2016b). Similar study conducted in South Ethiopia agroforestry practicing lands have indicated the presence of about 29 morphospecies belonging to nine genera (Acaulospora, Glomus, Claroideoglomus, Funneliformis, Pacispora, Septoglomus, Rhizophugus, Scutellospora and Gigaspora). Mean while, spores of four genera Rhizophugus, Glomus, Funneliformis, and Acaulospora had higher spore production, accounting for 36.22%, 21.20%, 19.39%, 17.54% and 11.74% of the total number of spores respectively.

Glomus and acaulospora species are found to be the most abundant among the glomeromycotan genera in tropical areas (Gerdemann and Trappe, 1974; Blaszkowski, 1989; Talukdar and Germida, 1993; Zerihun Belay et al., 2014). This makes them favorable fungi for mass multiplication as well as seedling inoculation for their better establishment (Beyene Dobo et al., 2016a). Glomus species is widely distributed regardless of the type and intensity of disturbance in the different ecosystems, whereas Acaulospora is dominant in least disturbed agro ecosystems (Snoeck et al., 2010). According to Muthukumar et al. 2003b, AM fungal sporulation is influenced by an array of factors which come from environment, host and fungus and spore density tend to decrease during early root growth but to increase during root inactivity or senescence. Study result of Beyene Bono et al., 2016b at Bokasso, Tellamo and Arroza forest areas in Sidama Zone, South Ethiopia has substantiated findings of Carrenho et al., 2007, in that soil texture of clay loam and sandy loam are types of soils that favor mycorrhizal development. Moreover, this Ethiopian Study has also revealed the significance of soil pH ranging from 6.18-6.28 to favour AMF development.

The study conducted in Southwestern Ethiopia by Diriba Muleta et al. (2008) to investigate the spatial distribution of AMF has revealed that the occurrence of significantly higher AMF spore densities at canopy bases and at 0–30 cm soil depth. Spore populations were found to belong to five genera: Acaulospora, Entrophospora, Glomus, Gigaspora and Scutellospora, with Glomus and Acaulospora dominating. Sampling points, sites and depths, shade tree species and shade tree/coffee plant age affected AMF spore density. Agroforestry practices including the use of leguminous shade trees effectively maintained AMF numbers in soils even at depth compared with unshaded coffee plants (monocultures).

Tadesse Chane and Fasil Assefa (2013), reported the number of spores produced by AMF in all rhizosphere soils of coffee forests as ranging from 578 to 1313 spores/100g of dry soil. The spores were grouped into four principal morphological types Glomus, Gigaspora, Scutellospora

and Acaulospora in descending order of their relative abundance.

In Ethiopia, Zerihun Belay et al. (2013), showed that land use types drastically affected AMF colonization and AMF diversity in a dryland agroforestry system in the central part of Ethiopia. Accordingly, land use changes from forest to agriculture contribute to declining of AMF abundance, root colonization and effectiveness in plant growth promotion.

AMF have also been shown to drive plant succession and may prevent invasion by alien species. The very few conditions where infective AMF are low in abundance and diversity is when the soil erodes, is disturbed and is devoid of vegetation cover. These are all common features of degraded lands. Meanwhile, degraded lands harbor low levels of infective AMF abundance and diversity. Therefore, the successful restoration of infective AMF can potentially improve the restoration success of degraded lands. Better AMF inoculation effects result when inocula are composed of native fungi instead of exotics, early seral instead of late seral fungi, and are consortia instead of few or single species (Asmelash F et al., 2016).

As per the study findings of Beyene Dobo et al., 2017 in tree-enset-coffee based agro-forestry practices there was significant ($P < 0.05$) effects on AMF spore abundance among different combinations. However, land uses with *Milletia ferruginea* and *Erythrina brucei* had higher spore numbers than land use types with *Cordia africana* as a shade tree. AMF species also seemed to show preference for specific tree-crop combination. Percentage root colonization of five months old Sorghum bicolor grown as a trap plant in field soils collected along the tree-crop combinations from the experimental sites showed significantly different ($P < 0.05$) root colonization. There was a positive correlation ($r = 0.458$, $P = 0.016$) between root colonization and spore abundance at the 0.05 level (2-tailed). A total of 28 AMF morphotypes from eight genera were isolated. Species diversity also responded differently to different tree-crop combinations, in tree-enset-coffee based agroforestry practices for all experimental sites. The response of AMF species to different tree-crop combinations therefore indicates the importance of selection of specific species of trees for better management of small holder at low external input agroforestry practices.

3.4. Diversity of AMF in afro-montane forests of Ethiopia

Michelsen (1993) has revealed findings of the study conducted on roots of 28 species of epiphytic vascular plants collected on tree trunks and branches at six afro-montane forest sites between 1700 and 3300 m above sea level in Bale Mountains National Park, Ethiopia. Seven of the 28 epiphyte species were colonized by vesicular-

arbuscular mycorrhizal fungi (VAM). Mycorrhizal colonization only occurred at two of the six sites examined, at 2900 m and 3300 m, but more than one type of VAM endophyte was present in each case. Three facultative epiphytic species were all highly colonized by VAM on the forest floor, whereas roots from epiphytic habitats were weakly colonized. No correlations were found between VAM colonization, fine root diameter and root hair length, but VAM colonization and root hair abundance were negatively correlated. The lack of VAM colonization of potential, epiphytic host species at the majority of the sites examined points to the dispersal of VAM propagules as the factor limiting mycorrhizal colonization of epiphytic habitats. It is suggested that root systems of hemiepiphytic tree species serve as corridors between forest floor and tree trunks through which VAM may spread via hyphal growth. Arbuscular mycorrhiza fungi (AMF) are vital in the regeneration of vegetation in disturbed ecosystems due to their numerous ecological advantages and therefore are good indicators of soil and ecosystem health at large. Emiru Birhane et al., 2017 has conducted study aiming at determining how the seasonal, vegetation cover density, edaphic and anthropogenic factors affect AMF root colonization (RC) and spore density (SD) in Desa'a dry Afromontane forest. AMF RC and SD in the rhizosphere of five dominant woody species, *Juniperus procera*, *Olea europaea*, *Maytenus arbutifolia*, *Carissa spinarum* and *Dodonaea angustifolia* growing in Desa'a forest were studied during the rainy and the dry seasons in three permanent study vegetation cover density plots (dense, medium, and poor). Spore density ranged from 50 to 4467 spores/100 g soil, and all species were colonized by AMF within a range of 4–95%. *Glomus* was the dominant genus in the rhizosphere of all species. Vegetation cover density strongly affected SD and RC. The SD was significantly higher ($p < 0.05$) in the poor vegetation cover density than in the other two and lowest in the dense cover; root colonization showed the reverse trend. Management practices significantly ($p < 0.05$) influenced AMF SD and RC, with the fenced plots being more favoured. Seasons significantly ($p < 0.05$) affected RC and SD. More RC and SD were observed in the wet period than the dry period. Correlating AMF SD and RC with soil physical and chemical properties showed no significant difference ($p > 0.05$) except for total nitrogen. Hence the study consolidates that Disturbance, vegetation cover density, season and total nitrogen are significant factors that control the dynamics and management interventions to maintain the forest health of dry Afromontane forests.

4. CONCLUSION AND RECOMMENDATION

4.1. CONCLUSION

The diversity of arbuscular mycorrhizal (AM) fungi and their

broad or narrow association with distinct plant species in natural environments are crucial information in the understanding of the ecological role of AM fungi on plant co-existence. This knowledge is also needed for appropriate mycorrhization of nursery-grown seedlings for forestation efforts and to help boost cop production. In a nut shell, knowing the diversity of AMF will contribute significantly to the environmental and agricultural applications and overall resilience of the ecosystem.

4.2. RECOMMENDATIONS

Based on the review studies done, the following recommendations are proposed:

- The diversity and occurrence of AMF in Ethiopia in association with some plants such as cereal crops, vegetables and livestock forage is less clear or very minimal information, so there is a need to trigger comprehensive studies in the indicated aspects.
- Most diversity studies conducted in Ethiopia are morpho-species based, thus it will be good to substantiate those findings with molecular techniques.
- As the recent Ethiopian horticultural production is pumped with excessive use of fungicides and other sorts of agrochemicals, it needs to trigger further investigation on the impact of these inorganic inputs in the diversity and abundance of AMF.
- Diversity studies in Ethiopia should be accompanied of estimating the socio-economic role of fungi-plant assemblage in organic agriculture; as compared to the use of excessive inorganic inputs.
- As a nation of potential agricultural production, Ethiopia needs to have a very comprehensive mapping of the spatial and temporal diversity and abundance of AMF across the various major vegetation coverage. To lessen the limitations of recent strives; they are very few and restricted more to the Northern, Southern, Central and Rift valley systems.

In a nut shell, it is well acknowledged that dedicated scientific compatriots have contributed tremendously to light on the understanding of AMF diversity and abundance in various vegetations in Ethiopia and pointed out the role of AMF contribution to the agro-ecosystem. Nonetheless, there are still lots of tasks which are required to be carried out to help respond the outstanding questions of the science world and new biotechnological intervention requiring agriculture lead economy of Ethiopia. Hence, this review would like to recommend the initiation of pertinent projects in the aforementioned five areas, coordinating with the national and global potential partners to help uncover the mysteries of fungi-plant diversity and the potential ecosystem service that the association could play in Ethiopia.

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