Effects of Land use Changes on Arbuscular Mycorrhizal Fungi Abundance and Diversity in Selected Agro-ecological Zones of Ethiopia: Implications for Sustainable Agro-ecosystems

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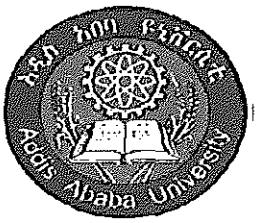

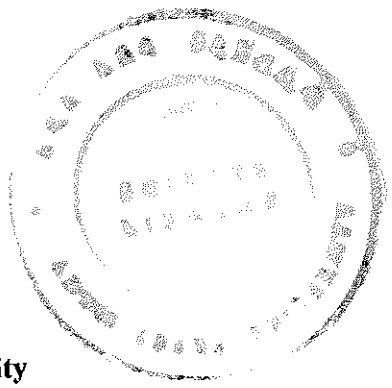

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Summary

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms that form the symbioses with majority of terrestrial plants including most of the agricultural crops. These fungi help plants in mineral and water uptake, and alleviation of abiotic and biotic stresses. They are particularly important in tropical ecosystems that are characterized by low soil fertility. The density and diversity of AMF are the major contributors to the maintenance of plant biodiversity, productivity, and ecosystem stability and function. AMF are also important in the reclamation and restoration of degraded ecosystems and in horticultural systems. However, AMF community structure and function are affected by changes induced by severe deforestation and land degradation that, in turn, affect the soil physical and chemical characteristics, fertility and stability. The land use and land cover change from forests and grasslands into settlements and agricultural systems for several decades in Ethiopia is supposed to drastically change plant diversity that directly influences the community structure of AMF. This necessitates the exploration of the AMF-land use change association within selected agroecosystems in the country. The main questions of this work, therefore, were; 1) Have different land use systems impacted the community composition and abundance of AMF species? and 2) If so, what is the community shift of AMF in terms of plant type and cropping systems in the changing land use and land cover of the ecosystem? 3) Is AMF species diversity and abundance influenced by the plant species composition or soil factors? For this purpose, abundance and diversity of AMF were studied in different land use systems in relation to vegetation types, soil physical and chemical properties, and different disturbances in humid lowland ecosystem at Showa Robit, Central Ethiopia, and dry acacia grassland ecosystem, Rift Valley, Ethiopia.

From the dry acacia grassland ecosystem, 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*) were collected from Bishoftu, Zeway with different land use types to assess AMF diversity, spore density and root colonization. The highest AM fungal colonization was found in *A. seyal* (67.3%) from open grazing field (OGF) at Zeway, whereas the lowest AMF colonization of 12% was recorded from *A. saligna* at Bishoftu. The AMF spore count from the rhizosphere of the different trees was within the range of 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 familes were identified. Apart from the common AMF genera of *Glomus, Claroideoglomus, Funneliformis,* other rare genera such as *Racocetra, Diversispora, Archaeospora, Entrophosphora* and *Sclerocystis* were also recorded. *Claroideoglomus claroideum, C. etunicatum, C. luteum, Funneliformis geosporum* and *Glomus aggregatum* were the dominant species. The study showed that the acacia species were characterized by relatively high AMF colonization and very high AMF diversity.

From the study of the humid lowland ecosystem at Showa Robit, the indigenous AM fungal populations in soil and in trap cultures were evaluated from different land use types. Accordingly, seven land use types were selected, that include low-input arable systems, either having a mixture of crops (Arable1) or monocropped with sorghum (Arable2) or maize (Arable3). Arable4 was a relatively high-input system with monocropped sorghum. The others were; fruit cropping area (FC) managed with composts and plant residues, a natural forest (NF) and an acacia plantation (AP). The parameters studied were; AMF spore abundance, species richness, diversity indices and Mycorrhizal Inoculum Potential (MIP). The result showed a significantly higher

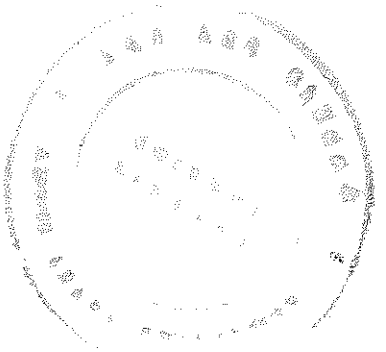
number of spores from FC, Arable1 and Arable3 (5.8-6.1 spores $^{-1}$g soil) than the land use types characterized by the lowest spore numbers (Arable 4, NF, AP) (2.8-3.9 spores $^{-1}$g soil) in field soil while AP, FC, and Arable2 had the highest numbers (9.8-11.1 $^{-1}$g soil) and Arable4 and NF were lowest number of spores (2.5-3.8 $^{-1}$g soil) in trap cultures. The data showed slightly different pattern of mycorrhizal infectivity potential with Arable1 (53.7%) and FC (52.6%), having significantly higher HC (%) compared to the other land use types that fell within percentage colonization of 19.9-25.8 %. A total of 38 and 28 morphospecies of AMF in 15 genera were identified in field soil and trap culture soil, respectively. The data showed that trap culturing increased spore number but caused a loss of AMF species richness. Higher species richness and MIP were obtained in FC and Arable1 compared to the other systems. The groups were categorized into Dominant, Common and Rare genera. *Claroideoglomus* and *Funneliformis* were found to be dominant that were distributed across all land use types in both trap culture and field soil. The results clearly showed that manuring and diversification of crops under low-input agricultural system enhances AMF diversity.

In the final component of the study, mycorrhizal fungi association of six fruit plant species [*Mangifera indica* (mango), *Musa acuminate* (banana), *Carica papaya* (papaya), *Citrus limon* (lemon), *Persea americana* (avocado), and *Psidium guajava* (guava)] was investigated. The result showed that fruit crops fell into higher spore density group of (7.2-8.8 spores g^{-1} of soil) and low spore density group (3.7-5.3 spores g^{-1} of soil). Accordingly, mango, avocado, banana, and lemon were from the high density spore group; whereas, papaya, and guava fell into the low spore density group at $P < 0.05$. The AM colonization also showed that mango and lemon fell into high mycorrhization (71.7%), and guava under low mycorrhization group (27.3%). A

total of 32 morphospecies into 12 genera were characterized from all the fruit crops. The highest AMF species richness was in mango (18 species) followed by banana (16 species) and guava (14 species). The species *Claroideoglomus claroideum* and *Glomus aggregatum* were the dominant species ("generalists") among the fruit trees.

Generally, the spore abundance and the species diversity of AMF identified from the selected agro-ecological zones of Ethiopia were relatively large compared to the previous works. The study also showed more diversity of AMF species was identified in dry acacia grassland ecosystem than in humid lowland ecosystem. However, future studies should be focused on the genera *Claroideoglomus, Glomus* and *Funneliformis* so as to develop AMF inocula for enhancing productivity of the different cropping system. Understanding the role of AMF over a broad range of land use systems is essential to establish seedling of woody plants for land rehabilitation and effective management for sustainable production through AMF technology

Key words: Abundance; Arbuscular mycorrhizal fungi (AMF); *Claroideoglomus,* diversity; *Glomus, Funneliformis;* land use change; Rift valley; Showa Robit



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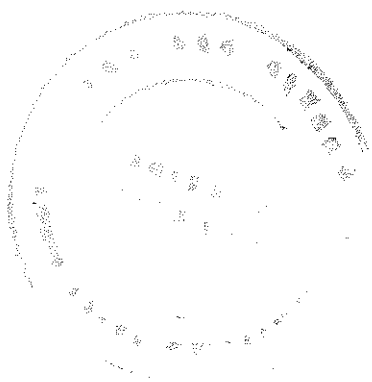

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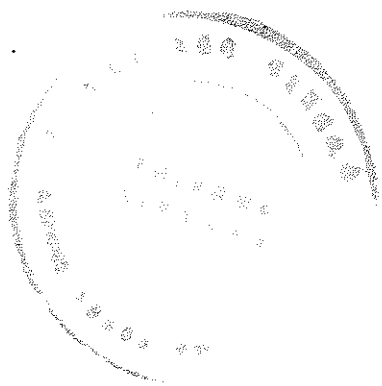

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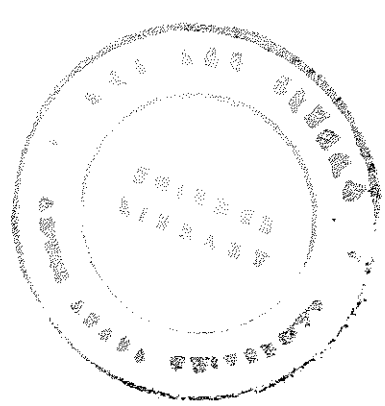

Chapter one
Literature review

1.1 Definition of mycorrhiza

Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi and roots of higher plants. The word "mycorrhiza" was introduced by Frank, a German plant pathologist means fungus root, from the Greek: *mykes* [mushroom] and *rhiza* [root] (Smith and Read, 2008). The term is used to describe the union of two different beings to form a single morphological organ, in which both partners can benefit from the association, i.e. the plant provides carbohydrates for the fungus, which in turn makes nutrients available for the plant.

Although the term mycorrhiza implies the association of fungi with roots, relationships called mycorrhizal associations, which are involved in the absorption of nutrients from soil, are found between hyphal fungi and the underground organs of the gametophytes of many bryophytes and pteridophytes, as well as the roots of seed plants and the sporophytes of most pteridophytes (Smith and Read, 2008).

Mycorrhizas, not roots, are the chief organs of nutrient uptake by land plants and recent work has confirmed that the earliest land plants, which had no true roots, were colonized by hyphal fungi that formed vesicles and arbuscules strikingly similar to modern arbuscular mycorrhizas. It is generally accepted that the colonization of the land was achieved by such symbiotic organisms, which were able to access nutrients unavailable to non-symbiotic individuals (Smith and Read, 2008).

1.1.1 Groups of mycorrhizas

The kinds of mycorrhiza are divided on the basis of their fungal associates into those involving aseptate endophytes in the Glomeromycota and those formed by septate fungi in the Phylum Ascomycetes and Phylum Basidiomycetes. The Glomeromycota has been recognized as a separate phylum of true fungi, instead of Zygomycetes because of the advances in molecular techniques (Schüßler *et al.,* 2001).

Based on structural features the mycorrhizae are divided into two groups; the ectomycorrhiza (ECM) and endomycorrhiza (EM) (Peterson and Massicotte, 2004). The ectomycorrhiza is characterized by a hyphal mantle ensheating the roots and an intercellular hyphal net; whereas, in the group endomycorrhiza, fungal hyphae invade the root cells and forms structures such as hyphal coils and arbuscules and/or vesicles. The endomycorrhizae are also subdivided into the arbuscular mycorrhiza (AM), ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, orchid mycorrhiza and the ectendo mycorrhiza on the basis of the nature and the structure of the intracellular hyphal development **(Table1:1; Figure1:1)** (Brundrett *et al.,* 1996; Smith and Read (2008).

Table 1.1 The plant and fungal partner characteristics of the important mycorrhizal types (Taxa). Adopted from Smith and Read, 2008

Kinds of mycorrhiza	Arbuscular mycorrhiza	Ectomycorrhiza	Ectendomycorrhiza	Arbutoid mycorrhiza	Monotropoid mycorrhiza	Ericoid mycorrhiza	Orchid mycorrhiza
Fungi septate	–	+	+	+	+	+	+
aseptate	+	–	–	–	–	–	–
Intracellular colonization	+		+	+	+	+	+
Fungal mantle	–	+	+ or –	+ or –	+	–	–
Hartig net	–	+	+	+	+	–	–
Achlorophylly	– (+)				+		+
Fungal taxa	Glomero	Basidio/Asco (Glomero)	Basidio/Asco	Basidio	Basidio	Asco	Basidio
Plant taxa	Bryo Pterido Gymno Angio	Gymno Angio	Gymno Angio	Ericales	Monotropoideae	Ericales Bryo	Orchidales

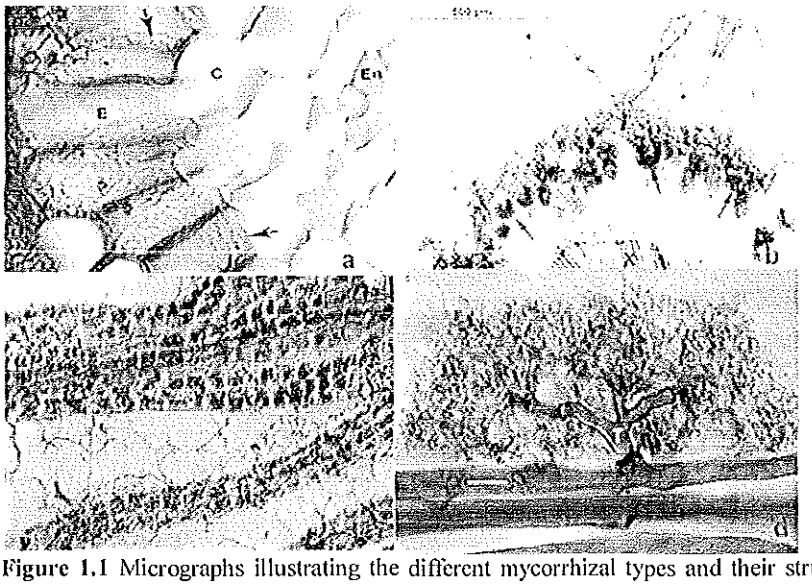

Figure 1.1 Micrographs illustrating the different mycorrhizal types and their structures. (a) *Populus tremuloides* ECM root cross-section showing Harting net hyphae (arrows) around elongated epidermal cells. (b) Orchid mycorrhizas with hyphae in trichomes and hyphal coils (arrows) in stem of *Pterostylis vittata* (c) Ericoid mycorrhizas with hyphal coils in hair roots of *Leucopogon verticillatus* (d) Arbuscule of a *Glomus* species in a root cortex cell. Modified from (Brundrett *et al.*, 1996)

Some aspects of the colonization process are common to all mycorrhizal fungi, whether they form ectomycorrhizas (ECMs), arbuscular (AMs), ericoid, or orchid mycorrhizas. The propagules (spores, hyphae, and rhizomorphs), in the rhizosphere which form the bridge between soil and plant roots are responsible for the so-called

woodwide web, network of connections for horizontal nutrient movement among different plants (Simard *et al.*, 1997).

As a result of signaling events, hyphae contact the root surface, and colonization takes place according to steps that are strictly choreographed by the mycorrhizal type (Smith and Read, 2008). ECM fungi produce a hyphal mantle that tightly covers the root tip, while epidermal (cortical) cells become separated by the development of labyrinth-like hyphae (the Hartig net), which increase the surface contact area with root cells (**Figure 1.1*a***).

In ericoid mycorrhizas, colonization is due to the development of fungus inside epidermal cells, forming a coil that gives rise to independent infection units. In orchid mycorrhizas, coils are produced mostly in the inner layers of the root (**Figure 1.1 b, c**). Colonization by AM fungi is the most complex, leading to the development of hyphopodia (or appressoria) at the root surface, inter- and intracellular hyphae, coils, and branched structures called arbuscules that develop inside cortical cells (**Figure 1.1d***)*. These structures, which give their name to the symbiosis, are considered the main site of nutrient exchange between the partners (Harrison, 2005).

1.1 Arbuscular mycorrhizal fungi

The arbuscular mycorrhiza (AM) is the most widespread terrestrial symbiosis and formed with more than 80% of the land plant species. This is probably a consequence of its ancestral character and the co-evolution of the two partners (plant and fungi) (Smith and Read, 2008). Fossil record of spores and hyphae indicated the existence of AM fungi more than 460 mya. It is suggested that the AM fungi may have assisted

plants to colonize the terrestrial ecosystem of land (Simon *et al.*, 1993; Redecker, 2002).

The close co-evolution of plants and AM fungi over time resulted in a sophisticated association in which the processes of recognition, penetration, and establishment of the fungi in roots are highly regulated. Recent discoveries have substantiated the existence of signal molecules and regulatory genes that mediate the communication between AM fungi and the plant root in the symbiosis (Parniske, 2008).

The driving force behind arbuscular mycorrhizal (AM) interactions is an exchange of nutrients between the fungal partner and the plants. The AM fungi are obligate symbionts and rely on the carbon provided by their plant hosts to complete their life cycle. According to Parniske (2008), AM fungi obtain carbohydrates (up to 20% of the photosynthesis products) from plants. In return, the fungus helps the plants in water and mineral uptake.

The majority of this nutrient exchange is thought to occur in root cortical cells containing the highly-branched fungal arbuscules from the Latin 'arbusculum', meaning bush or little tree) (**Figure 1.1d**) (Parniske 2008; Sawers *et al.*, 2008). Therefore, AM symbiosis contributes significantly to global nutrient (phosphate) and carbon cycling and influences primary productivity in terrestrial ecosystems particularly under conditions of limited nutrient availability.

AM fungi are unusual organisms because of their lifestyle and genetic make-up. The hyphal network of AM fungi is usually aseptate and coenocytic, with hundreds of

nuclei sharing the same cytoplasm. Individual spores contain hundreds of nuclei and the question of how the different polymorphic DNA-sequence variants that are present within a single cell are distributed between genomes or nuclei is the subject of an ongoing debate (Pawlowska and Taylor, 2004). Reproduction in AM fungal spores is thought to be solely asexual (Pawlowska and Taylor, 2004; Smith and Read, 2008). However, some reports showed that there is a possibility of exchange and recombination of genetic material between hyphae of closely related fungal strains know as anastomosis (Giovannetti *et al.*, 2004).

Although spores of AM fungi can germinate in the absence of host plants, they are obligate biotrophs, and depend on a living photoautotrophic partner to complete their life cycle and produce the next generation of spores (Hildebrandt *et al.*, 2006). Individual fungal strains colonize different plants under laboratory conditions (Smith and Read, 2008). Likewise, a single plant can be colonized by many different AM fungal species within the same root. Therefore, AM symbiosis is thought to show little host specificity at the level of colonization (Parniske, 2008).

1.2.1 Development of the AMF infection (symbiosis)

Spores of AM fungi under favorable environmental conditions germinate and undergo a sequence of structural morphogenesis (Barker *et al.*, 1998). These stages have been categorized mainly into the presymbiotic and the symbiotic stages ((Parniske, 2008). It is established fact that AM fungal spores are produced in the soil by the extraradical hyphae after symbiotic association with the host plant. These dormant spores (**Figure 1.2**) may remain alive in the soil for one or even two years depending upon species and genera (Giovannetti, 2000).

This stage is host independent as AM fungal spores not only carriers of genetic material but also contain energy reserves (stored lipids and carbohydrates) (Giovannetti, 2000; Bago and Bécard, 2002). These energy reserves are functional during spore germination to sustain the initial growth of the germ tube (Smith and Read, 2008). During the development of the germ tube no division of nuclei (spore contain 2000-3000 nuclei) takes place. Nuclei are distributed in the newly formed germination structures (Sieverding, 1991).

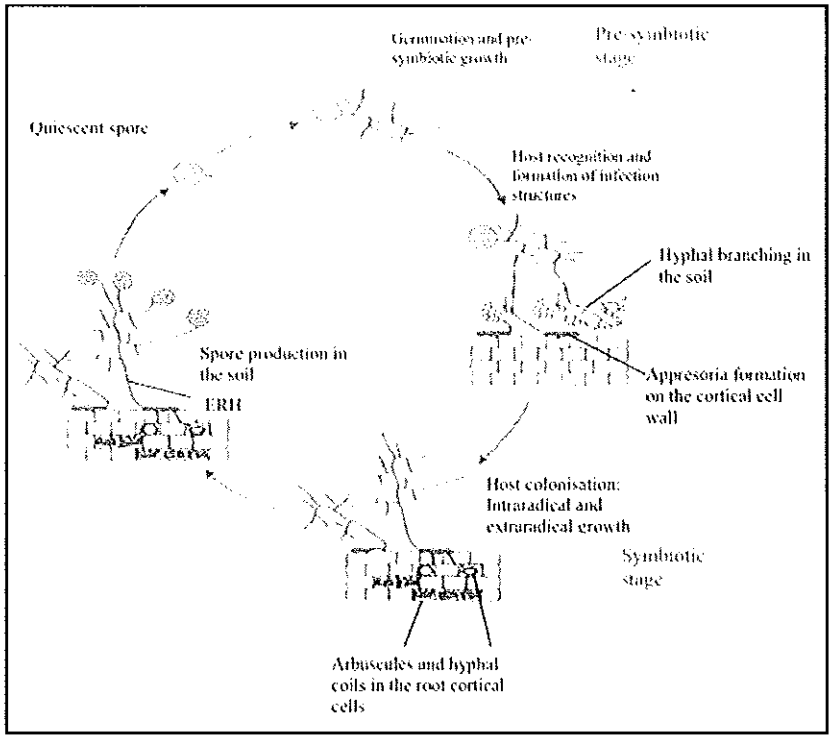

Figure 1.2 Life cycle of AMF showing pre-symbiotic and symbiotic stages of colonization (modified from Giovannetti, 2000).

In the absence of a plant root, hyphal growth will cease but this happens before spore reserves are depleted so that the fungus has an opportunity to germinate again and additional chances to find a host root. In general, germination takes place within a period ranging from a few days to several weeks depending up on the energy reserves

in the cytoplasm of the spore (Bago and Bécard, 2002). However, some spores retain sufficient carbon to allow repeated germination and further possibilities to encounter an appropriate host. The AMF with large spores such as *Gigaspora gigantea* can germinate up to 10 times (Koske, 1981).

1.2.1.1 The presymbiotic phase

In the presymbiotic stage, germinated spores grow toward the host root by producing hyphal branches. This occurs before the formation of structures such as appressoria that occur on the host root epidermal cell walls (Giovanetti, 2000). For both symbionts, the period before physical contact (appressoria formation) involves recognition and attraction of appropriate partners and other events promoting an alliance. In the vicinity of a host root, there is enhanced hyphal growth and extensive hyphal branching, for the fungus senses a host-derived signal ('branching factor'), leading to intensified hyphal ramifcation to have contact with a host root (Giovanetti *et al.*, 1993; Buee *et al.*, 2000).

The stimulatory effect of plant root exudates on AM fungal hyphae has been recognized for a long time, but the molecular identity of the 'branching factors' has only recently been identified by Akiyama *et al.* (2005). This branching factor is 5-deoxy-strigol, belonging to the strigolactones. Strigolactones are produced and exuded by plants, as signaling compounds in the rhizosphere. Strigolactones have been isolated from different plants and were found to stimulate seed germination of parasitic weeds such as Striga and Orobanche (Bouwmeester *at al.*, 2003).

Before direct physical interaction between the symbionts the fungus is induced to produce diffusible signaling molecules, which are perceived by the plant root (Kosuta *et al.,* 2003). AM fungi would produce a signal analogous to Nod factor and as result of the signal perception, activation of specific genes and morphogenetic programs occur both in the plant (Kosuta *et al.,* 2003), and in the fungus (Breuninger and Requena, 2004).

1.2.1.2 The endosymbiotic phase

Once in contact with the plant root, the fungus forms an appressorium on the root epidermis through which it enters the root (**Figure1.3**). The development of appressoria considered as successful presymbiotic recognition events when fungal and plant partners are committed to an interaction. Appressoria differ from hyphae in structure that it is flattened, elliptical hyphal tips that adhere by unknown means to the surface of host rhizodermal cells (Garriock *et al.,* 1989).

The signals that trigger appressoria formation in AM fungi are not yet known. Attempts to induce appressoria formation on synthetic surfaces were not successful (Giovannetti *et al.,* 1993), but *Gigaspora margarita* was induced to form appressoria on isolated epidermal cell wall fragments. It would not form appressoria on fragments of vascular or cortical cell walls, which suggests that the signaling molecule is specific to the epidermal cell wall. In addition, AMF also do not form appressoria on the surface of non-mycorrhizal plants, suggesting that these fungi recognize specific cues on the roots, and non-mycorrhizal plants may not synthesize the signal molecule necessary for appressorium differentiation.

present in the apoplast. New spores are typically synthesized outside of the plant root at the leading tip of individual fungal hyphae (Parniske, 2008).

1.2.2 Arbuscular mycorrhizal fungi classification

The modern taxonomic approach for AMF is based on a combination of morphology and small subunit (SSU) ribosomal DNA gene sequences (Schüßler and Walker, 2010). However, the history and complexity of the taxonomy and systematics of these organisms is addressed by recognizing four periods (Stürmer, 2012).

The initial discovery period (1845–1974) of the classification was mainly depending up on description of sporocarp-forming species. The following alpha taxonomy period (1975–1989) established a solid morphological basis for species identification and classification. The taxonomy of AM fungi has been dependent on morphological and anatomical characteristics of their spores. All descriptions during this time were based on morphological features of spores. Spore subcellular structures, which are diverse and largely accounted for most differences between species. The cladistics period from 1990 to 2000 classified AMF based on phenotypic characters. Other techniques such as serology, isozyme and fatty acid variation and DNA based methods have been used in a clearer phylogenetic analysis than was possible using morphological and microscopic identification (Hepper *et al.*, 1988; Morton and Redecker, 2001; Schüßler *et al.*, 2001).

The recent approach involves the phylogenetic synthesis period (2001 to present) with a new classification based on genetic characters using sequences of the multicopy rRNA genes (Stürmer, 2012). The phylogenetic analysis of SSU rDNA sequences has

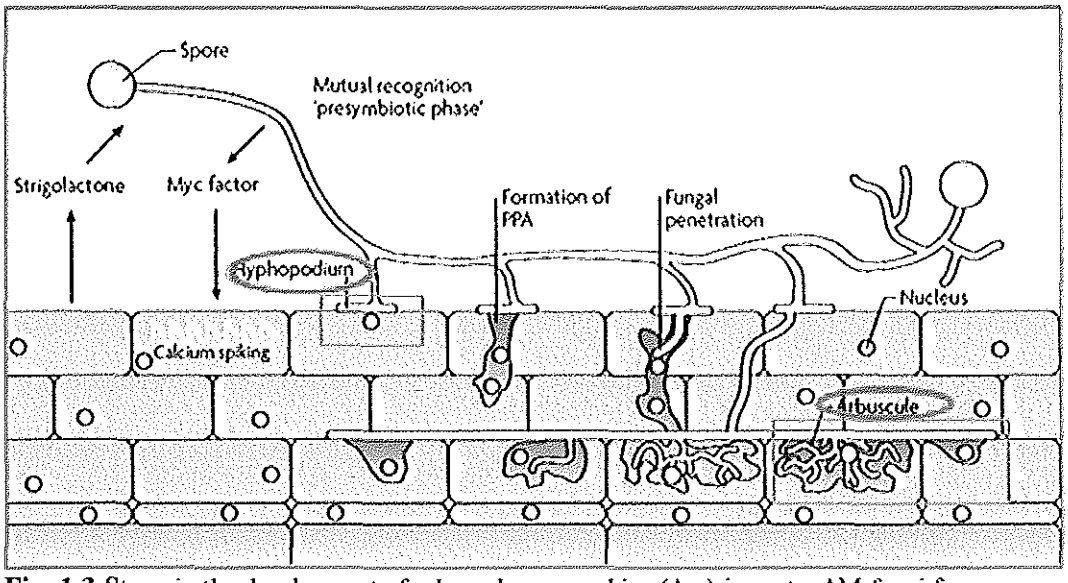

Fig. 1.3 Steps in the development of arbuscular mycorrhiza (Am) in roots. AM fungi form special types of appressoria called hyphopodia, which by definition develop from mature hyphae and not from germination tubes (Modified from Parniske, 2008).

Subsequently, a fungal hypha that extends from the hyphopodium enters the prepenetration apparatus (PPA), which guides the fungus through root cells towards the cortex. Here, the fungus leaves the plant cell and enters the apoplast, where it branches and grows laterally along the root axis. These hyphae induce the development of PPA-like structures in inner cortical cells, subsequently enter these cells and branch to form arbuscules (**Fig. 1. 3**). Arbuscules are the result of coordinated subcellular development of the host plant cell and the AM fungus.

The exact structure of arbuscules varies depending on the fungal and host genotype. However, arbuscules have a shorter lifetime than the host cell (perhaps as short as days) and consequently, a single host cell is thought to be competent for several rounds of successive fungal invasions. Vesicles, which are proposed to function as storage organs of the fungus, are sometimes, but not always, formed in AM and are

present in the apoplast. New spores are typically synthesized outside of the plant root at the leading tip of individual fungal hyphae (Parniske, 2008).

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The initial discovery period (1845–1974) of the classification was mainly depending up on description of sporocarp-forming species. The following alpha taxonomy period (1975–1989) established a solid morphological basis for species identification and classification. The taxonomy of AM fungi has been dependent on morphological and anatomical characteristics of their spores. All descriptions during this time were based on morphological features of spores. Spore subcellular structures, which are diverse and largely accounted for most differences between species. The cladistics period from 1990 to 2000 classified AMF based on phenotypic characters. Other techniques such as serology, isozyme and fatty acid variation and DNA based methods have been used in a clearer phylogenetic analysis than was possible using morphological and microscopic identification (Hepper *et al.*, 1988; Morton and Redecker, 2001; Schüßler *et al.*, 2001).

The recent approach involves the phylogenetic synthesis period (2001 to present) with a new classification based on genetic characters using sequences of the multicopy rRNA genes (Stürmer, 2012). The phylogenetic analysis of SSU rDNA sequences has

placed the AMF in a monophyletic group, the phylum Glomeromycota (Schüßler *et al.*, 2001). According to Schüßler *et al.* (2001) a phylogenetic tree of higher fungal taxa established using the small subunit (SSU) rRNA genes. Accordingly, Glomeromycota form a separate monophyletic clade and form a sister clade to the Basidio- and Ascomycota **(Fig.1.4).**

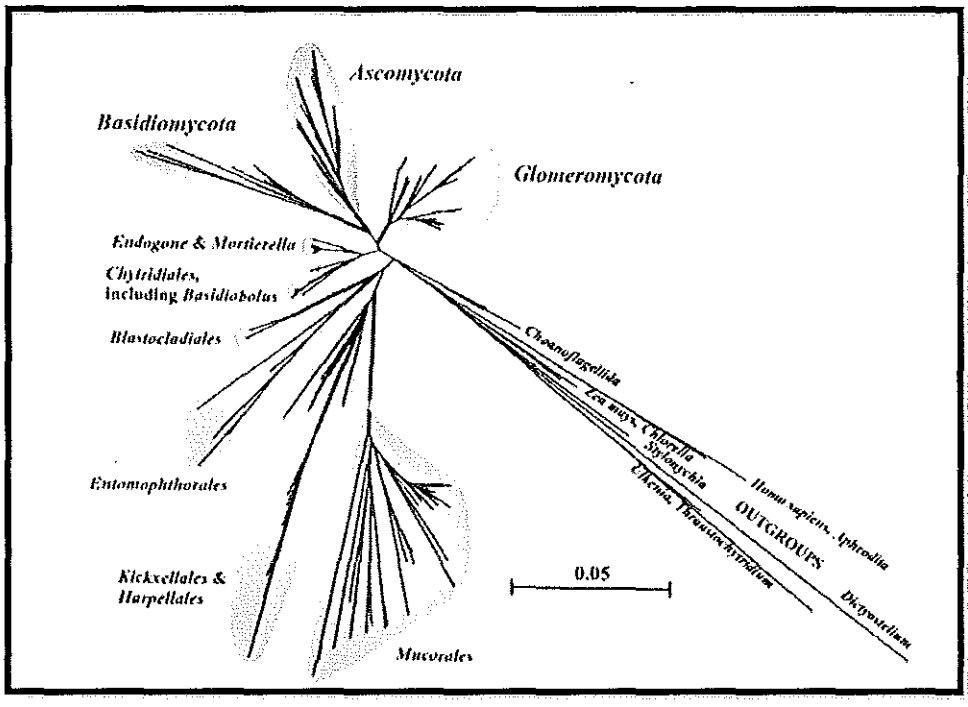

Fig.1. 4 Phylogeny of fungi based on SSU rRNA sequences. Thick lines delineate clades supported by bootstrap values above 90%. The Zygomycota and the Chytridiomycota do not form monophyletic clades and therefore are shown as the respective taxa representing the clade (From Schüßler *et al.* 2001).

Recently Redecker *et al.* (2013) revised the classification of the Glomeromycota into four orders **(Table 1.2)**; *Clomerales, Diversisporales, Archaeosporales,* and *Paraglomerales.* Within this, 11 families, 25 genera and more than 214 species have been identified **(Table1. 2)** (www.amf-phylogeny.com).

Table 1. 2. List of orders, families and genera in the phylum Golmeromycotan (Modified Redecker *et al.*, 2013)

Orders (4)	families (11)	genera (25)
Glomerales	*Glomeraceae*	*Glomus* *Funneliformis* *Rhizophagus* *Sclerocystis* *Septoglomus*
	Claroideoglomeraceae	*Claroideoglomus*
Diversisporales	*Gigasporaceae*	*Cetraspora* *Dentiscutata* *Gigaspora* *Intraornatospora* *Paradentiscutata* *Racocetra* *Scutellospora*
	Acaulosporaceae	*Acaulospora*
	Pacisporaceae	*Pacispora*
	Diversisporaceae	*Corymbiglomus* *Diversispora* *Otospora* *Redeckera* *Tricispora*
	Sacculosporaceae	*Sacculospora*
Paraglomerales	*Paraglomeraceae*	*Paraglomus*
Archaeosporales	*Geosiphonaceae* *Ambisporaceae* *Archaeosporaceae*	*Geosiphon* *Ambispora* *Archaeospora*

1.2.3 The role of AM fungi in terrestrial ecosystems

It is established that plant–microbe interactions in the rhizosphere are the primary determinants of plant health and soil fertility. The plant-AMF association is one of these microbial symbioses in terrestrial ecosystems. It influences nutrient and water uptake, plant community development and above-ground productivity. AMF also protects plant against pathogens and toxic stresses (Jeffries *et al.*, 2003).

1. 2.3.1 Nutrient up take

The most relevant contribution of AMF is the mobilization of phosphate which is critical for plant growth and makes up about 0.2% of dry weight. It is poorly available

in the soil because of the very low solubility and mobility (Schachtman *et al.,* 1998). There are two distinct phosphate uptake pathways that have been described in plants. The first one is a direct pathway of orthophosphate (Pi) uptake by root epidermal cells including root hairs, and the second pathway is through the absorption of P by external fungal mycelium and translocated into structures inside the root and thence across the symbiotic interface to plant cortical (Smith *et al.,* 2010) **(Fig.1.5 and 1.6).**

Physiological and molecular study has provided new insights into the integration of these two uptake pathways and how they influence plant nutrient acquisition (Bucher, 2007; Smith and Read 2008). It has shown that the outcome of establishment of AM symbioses in low-P soil is a marked increase in plant growth and P uptake, compared with NM control plants of the same species. The explanation of these effects is that direct uptake of P is supplemented by uptake via the AM pathway.

The extent to which an AM plant grows better than a non mycorrhizal (NM) counterpart depends in part on the size of its root system, including numbers and extent of root hairs (Smith *et al.,* 2010). However, growth differences between AM and NM plants tend to disappear as available soil P is increased, because of lower P depletion in rhizospheres (Smith and Read, 2008). The operation of the AM P uptake pathway is accompanied by expression of genes for individual transporters for orthophosphate (Pi) in root cortical cells that are strongly up regulated in AM plants, regardless of the plant responsiveness (Bucher 2007).

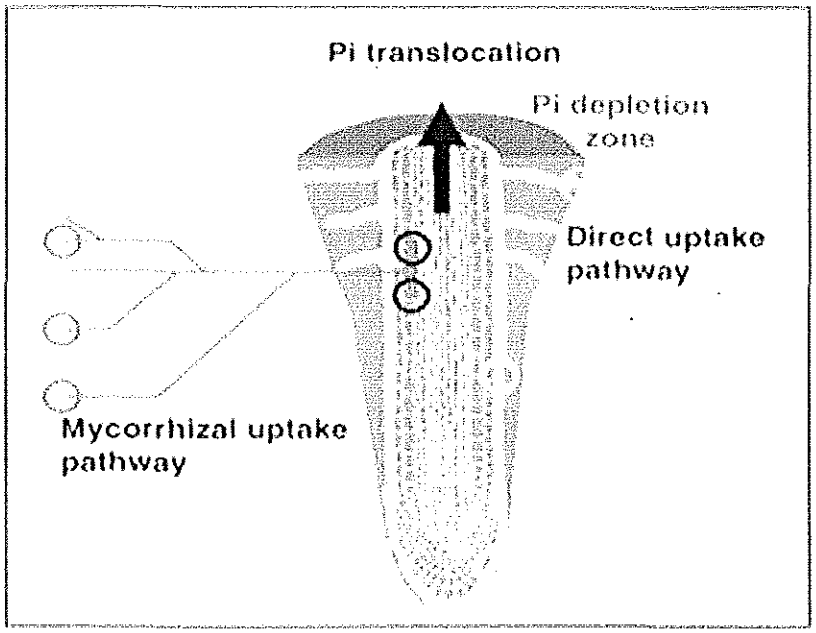

Fig.1. 5 Diagrammatic representation of the direct and AM pathways regarding P uptake as orthophosphate from the soil (Bucher, 2007).

Similarly to phosphate, nitrogen is a major limiting nutrient of plant growth, especially in the production of cereal crops. Nitrogen is available in the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Hyphae of AM fungi, as well as roots, are able to absorb both ammonium and nitrate. Details of the mechanisms involved in absorption, translocation and transfer of N to AM plants are beginning to be revealed. Ammonium is frequently present at very low concentrations in soil and its assimilation depends on the activity of glutamine synthetase (GS) in the external mycelium (Miflin and Lea, 1976). Following absorption and initial assimilation of either nitrate or ammonium, new accumulation of amino acids and particularly arginine in the extraradical mycelium is occurred. Most of the N is rapidly incorporated into arginine, which is the dominant amino acid in the extraradical mycelium (Jin *et al.,* 2005) and shown to be transferred between hyphal and plant root compartments. The arginine delivered to the intraradical mycelium is broken down and, whereas the ammonium released is transferred to the plant, the other breakdown

products are apparently recycled in the fungal tissue (Smith and Read, 2008) (**Figure 1.6**).

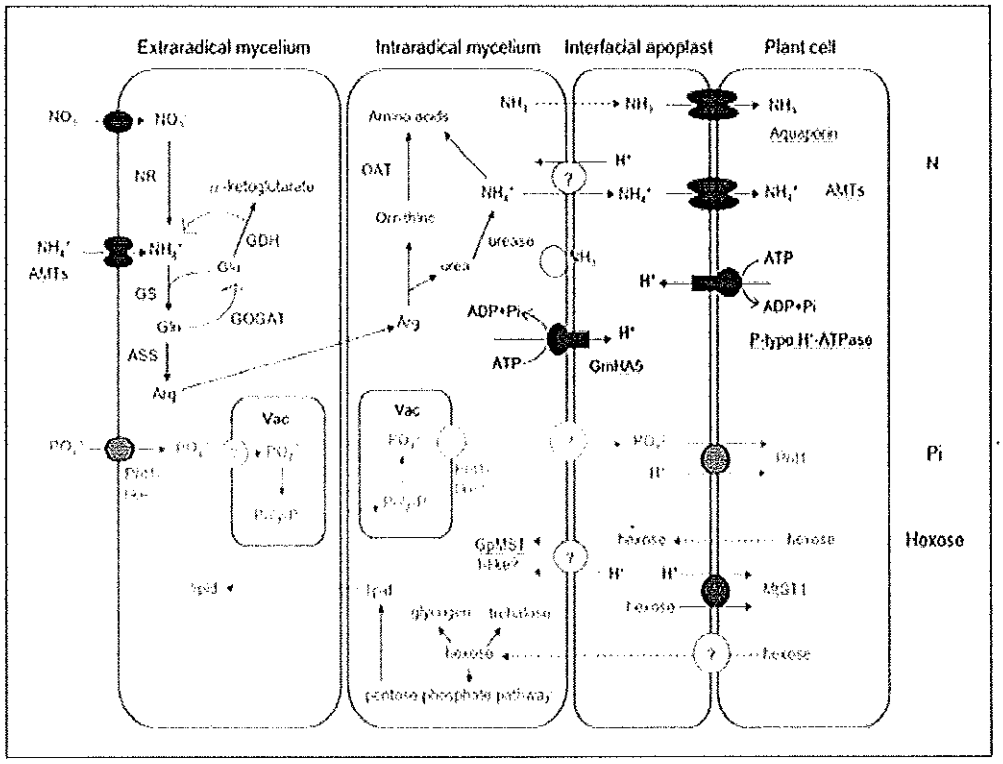

Fig. 1. 6 Transport of nitrogen, phosphate and carbohydrate between arbuscular mycorrhizal Fungi and plant (Sawers *et al.*, 2008).

1.2.3.2 Water relations and AMF

It is established that AM colonization does affect the water relations of plants (Allen and Boosalis, 1983; Augé, 2001; Ruiz-Lozano, 2003). According to Allen and Boosalis (1983), plant colonized by AM fungi can tolerate and recover more rapidly from soil water deficits than plants without AM fungi. Early studies also showed that extramatrical hyphae can also bind soil to roots and maintain better root-soil contact during drought and facilitate water uptake. Improved drought tolerance and better recovery by mycorrhizal plants is often related to improved P uptake (Allen, 1982).

It has been suggested that mycorrhizal hyphae may directly enhance root water uptake, providing adequate water to preserve physiological activity in plants, particularly under severe drought conditions. The relationships between AM colonization and plant water relations were comprehensively reviewed by Augé (2001), who concluded that AM effects on water relations included direct effects, as well as effects that were strictly related to changes in plant nutrition and size. In general, a variety of mechanism may help to improve drought stress in mycorrhizal plants. Mycorrhizal plants increase root length density or alter the root system morphology, enabling colonized plants to explore more soil volume and extract more water than non colonized plants during the drought (Khade and Rodrigues, 2009).

Mycorrhizal fungi have been shown to improve osmo-regulation in tissue of colonized plants by increasing leaf tissue solute concentration. The AM fungi may induce increase in the level of soluble proteins which contribute to plant tolerance to stresses, such as drought. Sugar accumulation in mycorrhizal maize plants had been reported under drought conditions. Higher levels of sugar may improve the ability of the plants to withstand drought stress and recover after the condition is restored (Augé, 2001; Khade and Rodrigues, 2009).

1.2.3.3 Impact on soil structure

In addition to their well-recognized roles in plant nutrition, mycorrhizas can influence the key ecosystem process of soil aggregation. Soil aggregation is governed by several biotic and abiotic components including land use management (Borie *et al.*, 2008). Aggregation is essential to maintain soil physical properties and facilitate biogeochemical cycling (Rillig and Mummey, 2006). Hyphae of AMF are considered

to be primary soil aggregators and there is a positively correlation between AMF hyphae and aggregate stability in natural systems (Rillig and Mummey, 2006).

The mechanisms by which the mycorrhizal fungal mycelium may influence soil aggregation can be divided into biochemical, biological and biophysical processes and their interrelationship with one another. In the biochemical mechanism: fungal mycelium products have long been implicated as an important mechanism in soil aggregation (Borie *et al.*, 2008) Recent evidence suggests that a glomalin-related soil protein (GRSP), produced by AMF hyphae has a cementing capacity to maintain soil particles together (Rillig and Mummey, 2006; Borie *et al.*, 2008).

Similarly, mycorrhizal fungi also influence soil aggregation through biophysical mechanisms such as enmeshment, alignment, and altered water relations (Rillig and Mummey, 2006). Other microbiota, together with mycorrhiza within the soil food web mechanisms also contribute to soil aggregation.

1.2.3.4 Ecological significance of AMF

1.2.3.4a Tolerance to biotic stress

Arbuscular mycorrhizal (AM) fungi can confer protection to host plants against some root pathogens. Elsen *et al.* (2008) reported whether the AMF *Glomus intraradices* was able to incite systemic resistance in banana plants towards *Radopholus similis* and *Pratylenchus coffeae*, two plant parasitic nematodes. They showed that the AMF reduced both pathogenic species by more than 50%, and suggested that AMF have the ability to induce systemic resistance against plant parasitic nematodes in a root system.

and antioxidants), physiological changes (photo-synthetic efficiency, relative permeability, water status, abscissic acid accumulation, nodulation and nitrogen fixation) (Porcel *et al.*, 2012).

Soil acidity is a major constraint for crop production worldwide as nearly half of the potential arable lands are acidic (von Uexküll and Mutert, 1995). Plant productivity in acid soils is limited by toxic levels of aluminum (Al), manganese (Mn), and iron (Fe) as well as deficiencies of plant available nutrients, especially phosphorus (P). Plants have developed several morphological, biochemicals, and physiological adaptations against acidity stress (Muthukumar *et al.*, 2014).

Among these, symbiosis with AM fungi is one of the strategies of plants evolved to survive and thrive in acid soils. The AM symbiosis increases plant growth in acid soils through enhanced uptake of nutrients. AMF symbiosis also alleviate Al and Mn phytotoxicities through a number of mechanisms such as binding of the toxic ions by the fungal hyphae, vesicles or auxiliary cells, exudation of organic acids, phosphatases, and production of glomalin (Muthukumar *et al.*, 2014).

These characteristics of the AMF enable plants to grow on severely disturbed sites, including those contaminated with heavy metals (HMs) (Karimi *et al.*, 2011). To this effect, isolation of the indigenous and presumably stress-adapted AMF can be a potential biotechnological tool for inoculation of plants for successful restoration of metal contaminated ecosystems.

Alleviation of these heavy metals by arbuscular mycorrhiza may occur by both direct and indirect mechanisms (Javaid, 2011). Strategies used by AM-fungi in direct method is phytostabilization that includes complexation of metals in the soil, compounds secreted by the fungus, adsorption to fungal cell walls, and chelation of metals inside the fungus. Indirect effects may include the mycorrhizal contribution to balanced plant mineral nutrition, especially P nutrition, leading to increased plant growth and enhanced metal tolerance (Javaid, 2011).

1.2.3.5 AMF and ecosystem functioning

Several workers have explored the contribution of AMF in maintenance of plant biodiversity, productivity and ecosystem functioning. Van der Heijden *et al.* (1998) reported that the diversity of AMF is a major factor contributing to the maintenance of plant biodiversity and ecosystem functioning. They showed that below-ground diversity of AMF is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning by using two independent, but complementary, ecological experiments. They concluded that the plant species composition and overall structure of microcosms fluctuated greatly at low AMF diversity in the European calcareous grassland.

There is also a correlation between plant biodiversity, and nutrient capture, and productivity increase significantly with increasing AMF-species richness. This indirectly indicates that microbial interactions can drive ecosystem functions such as plant biodiversity, productivity and variability (Vogelsang *et al.* 2006). These authors also investigated the effects of AMF species richness and composition on plant

management practice can be selected to increase the density of indigenous propagules or, alternatively, inoculate with effective isolates.

1.2.4 Management of effective AMF (Natural ecosystem and agriculture)

The arbuscular mycorrhizal (AM) symbiosis is the most commonly underground symbiosis in plants. Knowledge about the presence and diversity of AMF in a specific area is an essential first step for practical application of AMF in agroecosystem. Interest in the propagation of AM fungi for agriculture and reclamation of degraded ecosystem is increasing due to their role in the promotion of plant health, improvement in soil nutrition, soil aggregation and stability (Dalpé and Monreal, 2004).

1.2.4.1 Identification of AMF

Practically indirect (spore isolation from soil) and direct (DNA isolation from roots and spores) methods have been used for assessing AMF diversity within an ecosystem. Identification of morpho-species of AMF is based on spore characters, such as spore wall structure (i.e., number of layers, size, color, refractivity, flexibility, histologic reactivity, ornamentation, etc.) and developmental sequence of the spore (Douds and Millner, 1999). Direct isolation and identification of spores from the soil is inadequate and sequential trap cultures must be used to reveal the initially non-sporulating fungi in order to record the full range of species present in the sample (Suchitra *et al.*, 2012).

In contrast, direct molecular analyses reveal the diversity of fungi occupying the root and presumably contributing to the mycorrhizal effect on plant growth. Molecules other than DNA, e.g. fatty acids (Graham *et al.* 1995) or isozymes (Dodd *et al.* 1996) can also be used to authenticate species diversity in AMF.

However, AMF tissue is embedded deeply within the roots and therefore DNA extraction can be more of a problem with this method. AMF-specific primers are required because numerous pathogenic and saprophytic fungi can be co-detected. To design one primer for all glomalean fungi excluding plants and other fungi has proven to be difficult. Moreover, ribosomal DNA (rDNA) is highly polymorphic in single spores of AMF. This is in contrast to many other fungi, in which variable rDNA sequences (e.g. the internal transcribed spacers, ITS) are often identical within a species (Redecker *et al.,* 2003). Hence, the morpho-typing coupled with ribosomal based identification of AMF would give more information than the individual approach in studying arbuscular mycorhhizal fungal ecology and diversity.

It has been established that species of AM fungi differ in their effectiveness (ability to improve plant growth) due to differences in the rate and extent of infection (Abbott and Robson 1981) and due to differences in the transfer of P to the host, which may be related to characteristics of the plant/fungus interface (Dodd *et al.,* 1990).

Thus, before effectively utilize these fungi in agriculture, horticulture of forestry; it is imperative to understand their ecology including propagule number, species composition, infectivity and effectiveness (Sieverding, 1991). Therefore, 'a

management practice can be selected to increase the density of indigenous propagules or, alternatively, inoculate with effective isolates.

The selection of the most effective AM fungi is the first step toward development of an AM inoculation system in any management of AMF in natural or agroecosystems. With this aim, a number of methodological approaches and parameters have been proposed (Camprubí and Calvet, 1996; Wu *et al.,* 2002). These parameters includes the effectiveness in promoting plant growth, and in protecting against plant pathogens and/or abiotic stress such as salinity or drought. Once fungal selection has been made, the next step is the production of large quantities of inoculum, which must be based on well-defined fungal cultures (Azcbn-Aguilar and Barea, 1997). The INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi, invam.caf.wvu.edu) and the BEG (La Banque Europeene de Glomales, www.kent.ac.uk/bio/beg) represent useful germplasm stores of voucher specimens which allow accurate taxonomic positioning and preservation.

Different raw materials are used for inoculum production. These include seeds, seedlings, cuttings or micropropagated plants. Considering the genetic variability for both the plant and the fungal partners, biotests which assess the functional compatibility between the symbionts must be performed. These will enable the selection of the suitable fungus for the cultivar (genotype) or plant species against the prevalent edopho-climatic conditions. Inocula possessing more than one AM fungal species can also be tried (Lovato *et al.,* 1995; Azcbn-Aguilar, and Barea,1997).

1.2.4.2 Inoculum production and inoculation of AMF

The main problem in production of efficient and reliable AM fungal inoculum lies in their symbiotic behaviour, the fungi are obligatory biotrophs requiring a host plant for growth. Mycorrhizal fungi are propagated through pot-culture. The starting fungal inocula are spores and colonized root segments which, are incorporated to a growing substrate for seedling production (Brundrett *et al.*, 1996). The fungi spread in the substrate and colonize root seedlings. Both colonized substrates and roots can then serve as mycorrhizal inoculum. Culture systems without soil such as aeroponic cultures could be used for the production of cleaner spores and facilitate uniform nutrition of colonized plants (Sylvia and Hubbell, 1986). The successful propagation of some AM fungal strains on root-organ culture allowed the cultivation of monoxenic strains that can be used either directly as inoculum or as starting inoculum for large-scale production (Dalpé and Monreal, 2004).

1.2.4.2a Pot-culture propagation

The pot-culture propagation requires control and optimization of both host growth and fungal development. The microscopic sizes of AMF, together with the complex identification processes also contribute to the difficulty of inoculum propagation (Dalpé and Monreal, 2004). The inoculum propagation process needs the following stages: isolation of AMF pure culture strain, choice of a host plant and optimum growing conditions. Pure culture strains can be obtained from a single spore that germinate and colonize roots of a host plant or from colonized root segments isolated directly from field plants.

Monospecific cultures will then be produced through successive pot-culture generation, using isolated spores or fine root segments as starting inoculums. The next step is the choice of a host plant. The important criteria required for the host plant is its high mycorrhizal potential (i.e., its capacity to be colonized by the AMF strain and to promote its growth and sporulation), tolerance to growth under growth chamber and greenhouse conditions, and an extensive root system with solid but non-lignified roots (Struble and Skipper, 1988).

Leek (*Allium porrum* L.), Sudan grass (*Sorghum bicolor* (L.) Moench), corn (*Zea mays* L.), and bahia grass (*Paspalum notatum* Flugge) are the most frequently used plant host for inoculum propagation (Struble and Skipper, 1988). A preferable substrate is coarse texture sandy soil mixed with vermiculite or perlite or Turface (Dehne *et al.*, 1986). Optimum P levels and a selection of micro-element ratio is needed for inoculum development. Other edaphic factors such as pH, soil temperature, light intensity, relative humidity, and environment aeration must also be controlled to optimize AMF propagation (Dalpé and Monreal, 2004).

1.2.4.2b *In vitro* propagation on tissue culture

Root tissue cultures consist of excised roots that proliferate under axenic conditions on a synthetic nutrient media supplemented with vitamins, minerals, and carbohydrates. Several AMF species and strains have been propagated *in vitro* with various synthetic growth media and growth conditions (Fortin *et al.*, 20 02). The mono-specific strains available can be used directly as starting material for large-scale inoculum production, a sole Petri dish culture being enough to generate several thousand of spores and meters of hyphae within 4 months. *In vitro* bulk production of

AMF inoculum is promising, offering clean, viable, contamination-free fungi (Dalpé and Monreal, 2004).

The most frequently used AMF species for commercial inoculum is typically *Glomus intraradices*. This species is well adapted to both *in vivo* and *in vitro* propagation, can colonize a large variety of host plants, survive to long-term storage, and is geographically distributed all over the world (Dalpé and Monreal, 2004). These characteristics make the *G. intraradices* species an excellent candidate for commercial inoculum. Several other AMF belonging mainly to *Glomus* species, and *Gigaspora*, *Scutellospora*, and *Acaulospora* genera, are formulated for commercial inoculum production.

1.2.4.3 Co-inoculation of AMF with other rhizobacteria

The rhizosphere/ mycorrhizosphere microorganisms are able to interact with AMF to develop biological activities of great relevance to plant growth and health. The hitherto microorganisms have been used as co-inoculants with AMF, to enhance production of horticultural crops : (i) *Rhizobium* spp.; (ii) Plant growth promoting rhizobacteria (PGPR) certain strains of *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Enterobacter* and *Serratia* and (iii) Biocontrol agents such as fungi (*Trichodenna* spp. and *Gliocadium* spp.) (Barea *et al.*, 1992; Azcbn-Aguilar and Barea, 1997).

Several researches have been carried out on the co-inoculation of *Rhizobium* - myocrrhizal fungi on leguminous plants in agricultural, horticultural and restoration of degraded ecosystems (Barea *et al.*, 1992; Requena *et al.*, 2001; Jaizme-Vega *et al.*,

2006). Barea *et al.* (2002) reviewed the interactions between AMF and *Rhizobium* species that improved nodulation due to enhanced P-uptake by the plant. In addition, Requena *et al., (*1997) showed that the AMF supply trace elements and plant hormones to enhance nodulation and N_2–fixation.

Requena *et al.* (2001) undertook two long-term experiments on the effect of co-inoculation of indigenous AMF and nitrogen-fixing rhizobia on *Anthyllis cytisoides*, a drought-tolerant legume in a decertified Mediterranean ecosystem. The results showed significant improvements in the performance of *Anthyllis* plants inoculated with native AMF and rhizobial ecosymbionts. Survival rates were higher in AMF-inoculated than in uninoculated plants (80 and 65%, respectively). They reported that AM not only enhanced the establishment of key plant species but also increased soil fertility and quality. The dual symbiosis increased the soil nitrogen (N) content, organic matter, and hydrostable soil aggregates and enhanced N transfer from N-fixing to non-fixing species associated within the natural succession.

Jaizme-Vega *et al.* (2006) investigated the effect of the combined inoculation of AMF and PGPR on papaya (*Carica papaya* L.) infected with the root-knot nematode *Meloidogyne incognita*. They showed that nematode reproduction was significantly reduced in the presence of either any of the AMF isolates or any of the AMF-PGPR combinations. Compared with non-mycorrhizal plants, percentages of galled root decreased 3.6 times in mycorrhizal ones, treated or not with PGPR. They concluded that co- application of AMF and PGPR must be considered for papaya. de Lima *et al.* (2011) evaluated triple inoculation of *Bradyrhizobium, Glomus* and *Paenibacillus* on cowpea (*Vigna unguiclata* L.) and showed that microbial combinations were

production stage had a significant effect on plant growth after plants were transplanted into an abandoned agriculture field

Recently, Zhang *et al.* (2012) evaluated the effects of AMF on the restoration process of degraded grasslands. The results indicate that the inoculation with the AM fungi species (*Glomus mosseae, Glomus etunicatum,* and *Glomus intraradices*) increased the total cover of ephemeral plants from 7% to 14% in 2005, 15% to 38% in 2006, and 39% to 62% in 2009 than control treatment. Moreover, community productivity (shoot dry weight, g/m^2) in mycorrhizal treatments significantly increased from 6 to 29 in 2005, 11 to 36 in 2006, and 27 to 81 in 2009 compared with the control treatment. They concluded that AMF can speed up the regeneration process of grassland and this may be used as an effective biological approach in the restoration of degraded desert ephemeral plant communities.

1.2.4.5b Role of AM fungi in phytoremediation of heavy metal (HM) contaminated soils

AMF are considered to be ecologically important as they play important roles in the restoration of contaminated ecosystems by improving plant nutrition and fertility of degraded land (Chen *et al.,* 2001). Babu and Reddy (2011) evaluated AMF species associated with plants growing in fly ash pond and their potential role in ecological restoration. The result indicated that inoculation of plants with spores of AM fungal consortia along with colonized root pieces increased the growth (84.9%), chlorophyll (54%), and total P content (44.3%) of *Eucalyptus tereticornis* seedlings grown on fly ash compared to non-inoculated seedlings. The growth improvement was the consequence of increased P nutrition and decreased Al, Fe, Zn, and Cu accumulations.

These observations showed that the inoculation of tree seedlings with stress adapted AM fungi aid in the reclamation of fly ash ponds.

1.2.4.5c Role of AMF in polycyclic aromatic hydrocarbons (PAH) polluted soils

Polycyclic aromatic Hydrocarbons (PAH) are hydrophobic organic molecules consisting of two or more fused benzene rings. Bhalerao (2013) reviewed a role of AMF in two aspects of bioremediation of polycyclic aromatic hydrocarbons (PAH). The major mechanism of AM under such conditions is an establishment of plant covers on polluted soil and modification of PAH degradation rates or pathways. An establishment of plant covers on polluted soil is due to an improvement of plant nutrient acquisition, water relations, pollutant tolerance and sequestration.

Effects of AMF on PAH degradation in the rhizosphere may be direct or indirect. Since PAH are not absorbed by plants (Binet *et al.*, 2000) and are metabolized intracellularly all degrading activity would take place in soil or inside soil organisms other than AM. The fact that AMF have poor saprophytic capacities, the only probable direct effect of AMF on PAH degradation would be through enhanced production of extracellular peroxidases. The indirect effects may be due to changes in the microbial community.

1.3 AM fungal research in Ethiopian land use systems

Different studies indicated that conversion of natural ecosystems to agriculture affects the above- ground plant and animal biodiversity (Wardle and Lavelle, 1997; Bossio *et al.*, 2005) which, in turn affects below- ground macro and microbial diversity and their functions. Changes in total microbial community in response to different management

regimes, show management of vegetation and soils can have a considerable impact on microbial community structure and function in soils (Bossio *et al.*, 2005). AMF are influenced not only by the growing plants but also by different physical and chemical changes induced by severe deforestation and land degradation.

In general, the influence of land use systems on AM fungal diversity and abundances is not well studied in Ethiopia, not the least they were limited to researches on diversity and symbiosis of AMF fungi. These studies were on the mycorrhizal status of some indigenous trees and an endangered medicinal tree species (Wubet *et al.*, 2003ab), and accaia trees (Michelson, 1993; Yohannes and Assefa, 2007) and coffee and shade trees in southwestern Ethiopia (Muleta *et al.*, 2007). Most of the diversity studies were based on spore abundance and root colonization of AMF under different tree management systems. Berehane *et al.* (2012) also assessed the arbuscular mycorrhizal (AM) status of *Boswellia papyrifera* (frankincense-tree) that dominates dry deciduous woodlands in relation to season, management and soil depth in Ethiopia. The result showed that all trees are mycorrhizal and suggested that this phenomenon has profound consequences for rehabilitation efforts of the deciduous woodlands.

Chanie and Assefa (2013) examined AMF associated with shade trees and *Coffee arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. They identified four genera and nine different species of AMF.

1. 4 References

Abbott, L. K. and Robson, A. D. (1981). Infectivity and effectiveness of vesicular arbuscular mycorrhizal fungi: effect of inoculum type. *Aust. J. Agric. Res.* 32:631-639.

Akiyama, K., Matsuzaki, K. and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–827.

Allen, M. F. (1982). Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.B.K.) Lag Ex Steud. *New Phytol.* 91:191-196.

Allen, M. F. and Boosalis, .G. (1983). Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytol.* 93: 67-76.

Augé, R. M. (2001). Water relations, drought and vesicular- arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3 – 42. doi:10.1007/s005720100097.

Azcbn-Aguilar, C. and Barea, J.M. (1997). Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Sci. Hortic.* 68: I-24.

Babu, A. G. and Reddy, M. S. (2011). Diversity of arbuscular mycorrhizal fungi associated with plants growing in fly ash pond and their potential role in ecological restoration. *Curr. Microbiol.* 63:273–280.

Bago, B. and Bécard, G. (2002). Bases of biotrophy of arbuscular mycorrhizal fungi. In: *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts.* (Gianinazzi, S., Scüepp, H., Barea, J. M. and K. Haselwandter, K. eds.) Birkhäuser Verlag, Basel. pp. 33-48.

Barea, J. M., Azcón, R. and Azcón-Aguilar, C. (1992). Vesicular-arbuscular mycorrhizal fungi in nitrogen-fixing systems. In: *Methods in Microbiology,*

(Norris J. R., Read, D. J. and Varma, A. K. Eds) Academic Press, London, pp 391–416 .

Barea, J-M., Azc´on, R. and Azc´on-Aguilar, C. (2002). Mycorrhizosphere interactions to improve ,lant fitness and soil quality. *A. Van. Leeuw.* 81: 343–351.

Barker, S., Stummer, B., Gao, L., Dispain, I., O'Connor, P. and Smith S. (1998). A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterization. *Plant J.* 15: 791–797.

Bhalerao, S.A. (2013). Arbuscular mycorrhizal fungi: a potential biotechnological tool for tphytoremedation of heavy metal contaminated soils. *Int. J. Soil sci. Nat.* 4(1): 1-15.

Binet, P., Portal, J.M. and Leyval, C. (2000). Dissipation of 3-6-ring Polycyclic Aromatic Hydrocarbons in the Rhizosphere of Ryegrass. *Soil Biol. Biochem.* 32:2011-2017.

Birhane, E., Kuyper, T. W., Sterck, F. J. and Bongers, F. (2010). Arbuscular mycorrhizal associations in *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *For. Ecol. Manage.* 260:2160-2169.

Bodker, L., Kjoller, R. and Rosendahl, S. (1998). Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativ m*) caused by *Aphanomyces euteiches*. *Mycorrhiza.* 8: 169–174.

Borie, F., Rubio, R. and Morales, A. (2008). Arbuscular mycorrhizal fungi and soil aggregation. *J. Soil Sc. Plant Nutr.* 8: 9-18.

Bossio, D. A., Girvan, M. S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K. M., Ball, A. S., Pretty, J. N., Osborn, A. M. (2005). Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microb. Ecol.* 49:50-62.

Bouwmeester, H. J., Matusova, R., Zhongkui, S. and Beale, M. H. (2003). Secondary metabolite signalling in host–parasitic plant interactions. *Curr. Opin. Plant Biol.* 6: 358–364.

Breuninger, M. and Requena, N. (2004). Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. *Fungal Genet. Biol.* 41: 794–804.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture.* Australian Center for International Agricultural Research, Canberra. PP. 374.

Bucher, M. (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol.* 173: 11–26.

Buee, M., Rossignol, M., Jauneau, A., Ranjeva, R. and Becard G. (2000). The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *Mol. Plant–Microbe. Interact.* 13: 693–698.

Cameron, D. D. (2010). Arbuscular mycorrhizal fungi as (agro) ecosystem engineers. *Plant Soil* 333:1 – 5.

Camprubí, A. and Calvet, C. (1996). Isolation and Screening of Mycorrhizal Fungi from Citrus Nurseries and Orchards and Inoculation Studies. *Hortscience* 31(3):366–369.

Chanie, T. and Assefa, F. (2013). Arbuscular mycorrhizal fungi associated with shade trees and *Coffea arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afrika focus* 26:11-131.

Chen, B. D., Christie, P., Li, X. L. (2001). A modified glass bead compartment cultivation system for studies on nutrient uptake by arbuscular mycorrhiza. *Chemosphere* 42:185–192.

Dalpé, Y., and Monreal, M. (2004). Arbuscular mycorrhiza inoculum to support sustainable cropping systems. Online. *Crop Manage.* doi:10.1094/CM-2004-0301-09-RV.

de Lima, A. S. T., Xavier, T. F., de Lima, C. E. P., Oliveira, J. P., Mergulhão, A. C. E. S. Figueiredo, M. V. B. (2011). Triple inoculation with *Bradyrhizobium, Glomus* and *Paenibacillus* on cowpea (*Vigna Unguiculata* [L] walp.) development. *Bra. J. Microbiol.* 42: 919-926.

Dehne, H. W., and Backhaus, G. F. (1986). The use of vesicular-arbuscular mycorrhizal fungi and plant production. I. Inoculum production. *J. Plant Dis. Prot.* 93:415-424.

Dodd, J. C., Arias, I., Koomen, I., Hayman, D. S. (1990). The management of populations of vesicular–arbuscular mycorrhizal fungi in acid infertile soils of savanna ecosystem. II The effects of pre-crops on the spore populations of native and introduced VAM-fungi. *Plant Soil* 122:241 – 247.

Dodd, J.C., Rosendahl, S., Giovannetti, M., Broome, A., Lanfranco, L. and Walker, C. (1996). Inter- and intraspecific variation within the morphologically-similar arbuscular mycorrhizal fungi *Glomus mosseae* and *Glomus coronatum*. *New Phytol.* 133:113–122.

Douds, D. D. and Millner, P. (1999). Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agricult. Ecosyst. Environ.* 74:77 – 93.

Elsen, A., Gervacio, D., Swennen, R. and Waele, D. D. (2008). AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. *Mycorrhiza* 18:251 – 256

Evelin, H., Kapoor, R. and Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress. *Ann Bot* 104:1263 – 1280. doi:10.1093/aob/mcp251.

Fortin, J. A., Bécard, G., Declerck, S., Dalpé, Y., St-Arnaud, M., Coughlan, A. P., and Piché, Y. (2002). Arbuscular mycorrhiza on root-organ cultures. *Can. J. Bot.* 80:1-20.

Garriock, M., Peterson, R. and Ackerley, C. (1989). Early stages in colonization of *Allium porrum* (leek) roots by the vesicular-arbucular mycorrhizal fungus, *Glomus versiforme*. *New Phytol.* 112: 85–92.

Genre, A., Ortu, G., Bertoldo, C., Martino, E. and Bonfante, P. (2009). Biotic and abiotic stimulation of root epidermal cells reveals common and specific responses to arbuscular mycorrhizal fungi. *Plant Physiol.* 149:1424–1434.

Giovannetti, M. (2000). In Arbuscular Mycorrhizas: Physiology and Function. (Kapulnik, Y and J.D.L. Douds, J.D.D. eds). Kluwer Academic publishers, Netherlands. pp. 47-68.

Giovannetti, M., Sbrana, C., Avio, L. and Strani, P. (2004). Patterns of below-ground plant inter-connections established by means of arbuscular mycorrhizal networks. *New Phytol.* 164: 175–181.

Giovannetti, M., Sbrana, C., Avio, L., Citernesi, A. S. and Logi, C. (1993). Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages. *New Phytol.* 125:587–93.

Graham, J.H., Hodge, N.C. and Morton, J.B. (1995). Fatty acid methyl ester profiles for characterization ofglomalean fungi and their endomycorrhizae. *Appl. Environm. Microbiol.* 61: 58–64.

Gutjahr, C., Casieri, L. and Paszkowski, U. (2009). *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. *New Phytol.* 182: 829–837.

Harrison M. (2005). Signaling in the Arbuscular Mycorrhizal Symbiosis. *Annu. Rev. Microbiol.* 59:19–42.

Hepper, C. M., Sen, R., Azcon-Aguilar, C. and Grace, C. (1988). Variation in certain isozymes amongst different geographical isolates of the vesicular-arbuscular mycorrhizal fungi Glomus *clarum, Glomus monosporum* and *Glomus mosseae. Soil Biol. Biochem.* 20: 51-59.

Hildebrandt, U., Ouziad, F., Marner, F. J. and Bothe, H. (2006). The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. *FEMS Microbiol. Lett.* 254:258–267

Jaizme-Vega, M.C., Rodriguez-Romero, A.S. and Barroso Nunez, L. A. (2006). Effect of the combined inoculation of arbuscular mycorrhizal fungi and plant-growth promoting rhizobacteria on papaya (*Carica papaya* L.) infected with the root-knot nematode *Meloidogyne incognita. Fruits* 61: 1–7.

Javaid, A. (2011).Importance of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. In: *Biomanagement of Metal-contaminated soils.* Volume 20 (Kha.., M., S., Zaidi, A., Goel, R., and Musarrat, J., Eds), Springer Science+Business Media B.V. pp 125-141.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea, J-M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fertil. Soils* 37:1–16.

Jin, H., Pfeffer, P., Douds, D., Piotrowski, E., Lammers, P. and Shachar, H. Y. (2005). The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytol.* 168: 687–696.

Karimi, A., Khodaverdiloo, H., Sepehri, M., and Sadaghiani, M., R. (2011). Arbuscular mycorrhizal fungi and heavy metal contaminated soils. *Afr. J. Microbiol. Res.* 5(13):1571-1576.

Khade, S. W. and Rodrigues, B. F. (2009). Applications of arbuscular mycorrhizal fungi in agroecosystems. *Trop. Subtrop. Agro-Ecosyst.* 10: 337 – 354.

Koske, R. E. (1981). Multiple germination by spores of *Gigaspora gigantea*. *Trans. Br. Mycol. Soc.* 76:328–30.

Kosuta, S., Chabaud, M., Lougnon, G., Gough, C., Denarie, J., Barker, D. G. and Bécard, G. (2003). A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. *Plant Physiol.* 131:952–62.

Lovato, P. E., Schilepp, H., Trouvelot, A. and Gianinazzi, S. (1995). Application of arbuscular mycorrhizal fungi (AMF) in orchard and ornamental plants. In: *Mycorrhiza Structure, Function, Molecular Biology and Biotechnology*, (Varma, A. and Hock, B. Eds). Springer, Heidelberg, pp. 521-559.

Michelsen, A. (1993). Growth improvement of Ethiopian acacias by addition of vesicular-arbuscular mycorrhizal fungi or root of native plants to non sterile nursery soil. *For. Ecol. Manage.* 59:193-206.

Miflin, B. J. and Lea, P.J. (1976). The path of ammonia assimilation on the plant kingdom. *Trends Biochem. Sci.* 1:103–106.

Morton, J. B. and Redecker, D. (2001). Two new families of *Glomales, Archaeosporaceae* and *Paraglomaceae*, with two new genera *Archaeospora* and *Paraglomus,* based on concordant molecular and morphological characters. *Mycologia* 93:181 – 195.

Muleta, D., Assefa, F., Nemomissa, S. and Granhall, U. (2007). Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. *For. Ecol. Manage.* 241:145-154.

Muthukumar, T., Priyadharsini,P., Uma, E., Jaison, S., Pandey, R. R. (2014). Role of arbuscular mycorrhizal fungi in alleviation of acidity stress on plant growth. In: *Use of Microbes for the Alleviation of Soil Stresses,* Volume 1 (Miransari, M. ed), pp 43-71. Springer Science+Business Media New York.

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6:763 – 775.

Pawlowska, T. E. and Taylor, J.W. (2004). Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature.* 427: 733-737.

Peterson, R. L. and Massicotte, H. B. (2004). Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. *Can. J. Bot.* 82:1074–1088.

Porcel, R., Aroca, R. and Ruiz-Lozano, J. M. (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. *Agron. Sustain. Dev.* 32:181 – 200.

Pozo, M. J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J. M. and Azco´ n-Aguilar, C. (2002). Localized versus systemic effect of arbuscular

mycorrhizal fungi on defense responses to Phytophthora infection in tomato plants. *J. Exp. Bot.* 53:525–534.

Quoreshi, A. M. (2008). The use of mycorrhizal biotechnology in restoration of disturbed ecosystem. In: Mycorrhizae: Sustainable agriculture and forestry. Springer. Oxford, pp 303-320.

Redecker, D. (2002). Molecular identification and phylogeny of arbuscular mycorrhizal fungi. *Plant and Soil* 244: 67–73.

Redecker, D., Hijri, I. and Wiemken, A. (2003). Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems. *Folia Geobot* 38:113 – 124.

Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S. L., Morton, J. M. and Walker, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* DOI 10.1007/s00572-013-0486-y.

Requena, N., I. Jimenez, M. Toro, and J. M. Barea. (1997). Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and Rhizobium spp. in the rhizosphere of Anthyllis cytisoides, a model legume for revegetation in mediterranean semi-arid ecosystems. *New Phytol.* 136:667–677.

Requena, N., Pérez-Solís, E., Azcón-Aguilar, C., Jeffries, P. and Barea, J.M. (2001). Management of indigenous plant-microbe symbiosis aids restoration of desertified ecosystems. *Appl. Environ. Microbiol.* 67: 495–498.

Richter, B. S., and Stutz, J. C. (2002). Mycorrhizal inoculation of big sacaton: implications for grassland restoration of abandoned agricultural fields. *Restor. Ecol.* 10:607–616.

Rillig, M. C. and Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytol.* 171: 41-53.

Ruiz-Lozano, J. M. (2003). Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza* 13: 309–317.

Sawers, R. J. H., Yang, S-Y., Gutjahr, C., and Paszkowski, U. (2008). The molecular components of nutrient exchange in arbuscular mycorrhizal interactions. In: *Mycorrhizae: sustainable agriculture and forestry.* Siddiqui ,Z. A., Akhtar, M. S., and Kazuyoshi Futai, K. (Eds). Springer Science + Business Media B.V. pp. 37-59.

Schachtman, D. P., Reid, R. J. and Ayling, S. M. (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiol* 116: 447–453.

Schüßler, A., Schwarzott, D. and Walker, C. (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 105:1413 – 1421.

Schüßler, A. and Walker, C. (2010). The Glomeromycota. A species list with new families and new genera. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Electronic version freely available at www.amf-phylogeny.com.

Secilia, J. and Bagyaraj, D.J. (1987). Bacteria and actinomycetes associated with pot cultures of vesicular arbuscular mycorrhizas. *Can. J. Microbiol.* 33:1069–1073.

Sieverding, E. (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems. GTZ, Eschborn and Hartmut Bremer Verlag, Friedland, 365 pp.

Simard, S.W., Perry, D.A., Jones, M. D., Myrold, D. D., Durall, D. M. and et al. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388:579–82.

Simon, L., Bousquet, J., Lévesque, R.. C. and Lalonde, M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363:67 – 69.

Smith, M. R., Charvat, I. and Jacobson, R. L. (1998). Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. *Can. J. Bot.* 76:1947–1954.

Smith, S. E., Facelli, E., Pope, S. and Smith, F.A. (2010). Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil.* 326:3 – 20.A

Smith, S. E. and Read, D. J. (2008). Mycorrhial symbiosis, 3rd Ed. Elsevier Ltd.

Struble, J. E., and Skipper, H. D. (1988). Vesicular-arbuscular mycorrhizal fungal spore production as influenced by plant species. *Plant Soil* 109:1194-1196.

Stürmer, S. L. (2012). A history of the taxonomy and systematics of arbuscular mycorrhizal fungi belonging to the phylum Glomeromycota. *Mycorrhiza* 22: 247 – 258.

Suchitra, R., Kumutha, K and Balachandar, D. (2012). Morpho-typing and molecular diversity of arbuscular mycorrhizal fungi in Sub-tropical soils of coimbatore region, Tamil Nadu, India. *Indian J. Microbiol.* 52(2):145–152.

Sylvia, D. M. and Hubbell, D. H. (1986). Growth and sporulation of vesiclar arbuscular mycorrhizal fungi in aeroponic and membrane systems *Symbiosis* 1: 259–67.

Toljander, J.F., Lindahl, B.D., Paul, L.R., Elfstrand, M. and Finlay, R.D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* 61:295–304.

Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. and Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* 396: 72–75.

Vogelsang, K.M., Reynolds, H.L. and Bever, J.D. (2006). Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytol.* 172: 554–562.

von Uexküll, H.R. and Mutert, E. (1995). Global extent, development and economic impact of acid soils. In: *Plant–Soil Interactions at Low pH: Principles and Management*, (Date, R. A., Grundon, N. J., Raymet, G. E. and Probert, M. E. eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, p. 5-19

Wardle, D. A., Lavelle, P. (1997). Linkages between soil biota, plant litter quality and decomposition. In: *Driven by nature: Plant litter quality and decomposition*, (Cadisch, G., Giller, K. E., eds.). CAB International, Wallingford (UK). pp.107-125.

Wehner, J., Antunes, P. M., Powell, J. R., Mazukatow, J. and Rillig, M. C. (2009). Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? *Pedobiologia*, doi:10.1016/j.pedobi.2009.10.002.

Whipps, J.M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Can. J. Bot.* 82: 1198–1227.

Wu, T., Hao, W., Lin, X. and Shi, Y. (2002). Screening of arbuscular mycorrhizal fungi for the revegetation of eroded red soils in subtropical China. *Plant and Soil* 239: 225–235.

Wubet, T., Kottke, I., Teketay, D. and Oberwinkler, F. (2003a). Mycorrhizal status of indigenous trees in dry Afromontane forests of Ethiopia. *Forest Ecol. manag.* 17: 1-3.

Wubet, T., Weigß, M., Kottke, I., Teketay, D. and Oberwinkler, F. (2003b). Molecular diversity of arbuscular mycorrhizal fungi in *Prunus africana*, an endangered medicinal tree species in dry Afromontane forests of Ethiopia. *New Phytol.* 161: 517-528.

Yohannes, Y., Assefa, F. (2007). Phenotypic characteristics of rootnodule bacteria and arbuscular mycorrhizal fungi infecting *Acacia polyacantha* growing in Ghibe wooded grasslands. *Ethio. J. Natu. Sci.* 9(1):123-139.

Zhang, T., Sun, Y., Shi, Z. and Feng, G. (2012). Arbuscular mycorrhizal fungi can accelerate the restoration of degraded spring grassland in Central Asia. *Rangeland Ecol. Manage.* 65:426–432.

Chapter two:

Full Length Research Paper

Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia

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Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia

ABSTRACT

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*) were collected from Bishoftu, Zeway and Addis Ababa sites with different land use types to assess their arbuscular mycorrhizal fungal (AMF) diversity, spore density and root colonization. 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 *Funneliformis* 4 each to *Gigaspora, Glomus*, and *Rhizophagus*, 3 each to *Claroideoglomus*, and *Scutellospora*, 2 each to *Racocetra* and *Diversispora*, and 1 each to *Entrophospora, Sclerocystis, Paraglomus* 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.

Key words: *Acaulospora;* AM colonization; Arbuscules; *Funneliformis;* Glomeromycota; Rhizosphere soils

INTRODUCTION

The acacia trees are important legumes in the tropics represented by more than 1200 species (Anon, 1983). They are multi-purpose and fast growing woody plants used as fuel wood, fodder, for improving soil fertility, and as shade for tree crops (Brewbaker, 1986). *Acacia* species, with few exceptions, nodulate and fix nitrogen with root nodule bacteria in the range of 20-300 kg $ha^{-1}yr^{-1}$ (Dommergues, 1987), and symbiotically associated with arbuscular mycorrhizal fungi (AMF) which is a widespread phenomenon occurring in more than 80% of terrestrial plants (Smith and Read, 2008).

AMF enhance nutrient, particularly phosphorus (P), and water uptake by acacia species and improves nitrogen fixation which enables them to establish in marginalized lands in the tropics (Requena *et al.*, 2001). These associations contribute to their tolerance to drought, and induce resistance against soil pathogens (Smith and Read, 2008). These associations, in general, enable many of the acacia species perform well in degraded soils with high acidity, high salinity, high Aluminum saturation, and low soil fertility (Craig *et al.*, 1991). Consequently, acacia trees/shrubs are integrated in the traditional agroforestry systems and for the rehabilitation of fast disappearing and marginalized agro-ecosystems in the tropics and subtropics (Ngulube *et al.*, 1993).

In Ethiopia, the genus *Acacia* is the third dominant group of woody leguminous plants, represented by more than 49 indigenous species, and is widely distributed in altitudes ranging from 0-3400 m a.s.l (Hunde and Thulin, 1989). The legume is the most important component of the acacia woodland, which is major vegetation from

the arid and semi- arid parts of Ethiopia that are utilized in many different ways for the rural economy, and to rehabilitate and stabilize degraded ecosystems especially in the Rift Valley of Ethiopia (Eshete and Stahl, 1999).

Although several studies have been carried out in relation to diversity and density of AMF on coffee and shade trees in montaine forests (Wubet *et al.*, 2003; Muleta *et al.*, 2007), and in the dry deciduous woodlands of Northern Ethiopia (Birhane *et al.*, 2010), studies on the AMF-acacia relationship was limited to the co-inoculation of AMF and rhizobia. The AMF species found in earlier studies of acacia trees belong mainly to the genera *Glomus* and *Gigaspora* (Michelson, 1993; Yohannes and Assefa, 2009).

In Ethiopia, there is an increase in the land use change of different ecosystems for crop production in the country. The Rift Valley area is one of the regions that suffer most from rapid deforestation and decrease in the biodiversity of the woodlands for intensive agriculture and settlement for the ever increasing small-hold farming community (Garedew *et al.*, 2009). It is also established that agricultural development can change the whole spectrum of AMF associations that are specifically associated with fitness of specific plants, plant community structure, and ecological variability (Van der Heijden *et al.*, 1998).

Sanders *et al.* (1996) reported that plant species respond differently to different AMF species and that density and diversity of naturally occurring AMF were reduced, particularly in disturbed arid and semi arid habitats (Mason and Wilson, 1994). Oehl *et al.* (2003) also reported that increased land use intensity was correlated with a

decrease in AMF species richness and with a preferential selection of species in agro-ecosystems of Central Europe. Another study in Mexico also showed that land use change from temperate forest to avocado plantation had minimal effect on AMF communities, but conversion of forests to maize fields reduced AMF diversity (González-Cortés *et al.*, 2012).

Several studies on AMF and land use systems have been conducted in tropical ecosystems. In a study with *Acacia senegal* in the sahelian regions of Senegal, Ndoye *et al.* (2012) found that plant species and land-use systems have positive effects on AMF spore density and diversity as well as on soil microbial functions. In tropical dry ecosystems of Mexico, Gavito *et al.* (2008) found higher AMF morphospecies richness in primary forests than in secondary forests and pastures. In a study in Brazil, da Silva Sousa *et al.*, (2013) showed that the presence of trees such as *Gliricída* and *Maniçoba* increased sporulation, mycorrhizal colonization and the production of infective propagules of AMF in three land use systems.

According to Wubet *et al.* (2003), there is a need for research on the relationship between land use changes and mycorrhizal diversity and density because the establishment of seedlings for ecosystem rehabilitation depends upon the mycorrhizas in the terrestrial ecosystems.

The objective of this investigation was to study the arbuscular mycorrhizal fungal colonization, spore density and diversity of nine acacia species that grow in different land use systems in montaine highland woodland and lowland savanna woodland vegetation from parts of the Rift Valley of Ethiopia.

MATERIALS AND METHODS

Sampling sites

Acacia trees were sampled from three sites, i.e. Addis Ababa (2400 m a.s.l.) and Bishoftu and Zeway in the Rift Valley system (1600-1960 m a.s.l.). The Addis Ababa site is a high montaine woodland system, while the Rift Valley sites are naturally characterized by dry woody grassland dominated by different acacia species (Hunde and Thulin, 1989). The sampling areas represented six different land use types and vegetation covers. The Addis Ababa site was a (1) protected park (PP). Land use types occurring at Zeway were (2) sorghum cropping in agro-forestry system (SCAFS), (3) protected forest relics managed by Hawassa University (PFR-HU), (4) protected forest relics with natural vegetation (PFRNV) and (5) open grazing field (OGF). The land use type at Bishoftu was a (6) community preserved forest relics for reforestation programmes (CPFR) (**Fig. 2.1, Table 2. 1**).

Nine acacia tree species (*Acacia abyssinica, A. nilotica, A. robusta, A. saligna, A. senegal, A. seyal, A. sieberiana, A. tortilis* and *Faidherbia albida*) were studied. Of these *A. saligna* is of Australian origin while all the other ones are native to Africa. Sampling was conducted during dry seasons from Nov. 2010 to Dec. 2011. The average annual temperature and precipitation of Addis Ababa, Bishoftu and Zeway were 17, 20 and 22^{0}C, and 44, 35 and 74mm, respectively.

Table 2. 1 List of acacia species studied from different land use types of the sampling sites

Name of the acacia species	Plant density /100m^2	Agro- ecology	Altitude (m)	Sampling sites	Land use type
A. abyssinica	2	Woodland and forest margins	2400	Addis Ababa	Protected park
F. albida	10	Wooded grassland	1661	Zeway	SCAFS
A. nilotica	15	Wooded grass land	1660	Zeway	PFR-HU
A. senegal	15	Wooded grass land	1650	Zeway	PFRNV
A. tortilis	15				
A. seyal	10				
A. tortilis	10	Wooded grass land	1651	Zeway	OGF
A.seyal	10				
A. sieberiana	12	Wooded grass land	1954	Bishoftu	CPFR
A. saligna	15				
A. seyal	15				
A. robusta	10				

m: meter; SCAFS: sorghum cropping in agro-forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relics for reforestation programmes

The samples were collected in alcohol sterilized plastic containers and stored at room temperature until further analysis. Root samples were washed with tap water and stored in 50% of alcohol at 4^{0}C for determination of AMF root colonization. Soil chemical and physical parameters such as pH, organic carbon (OC), total nitrogen (TN), available phosphorus (P) and soil texture were determined using standard methods at the Addis Ababa City Administration Environmental Protection Authority (Table **2. 2**). Voucher specimens of the acacia trees were brought and deposited at the National Herbarium, Addis Ababa University for identification.

Assessment of AMF root colonization

The stored root samples were washed carefully with tap water and cut into segments about 1 cm long. About 0.5 g of root segments were cleared in 10 % (w/v) KOH at 90^{0}C in a water bath for 2-3h depending on the structure of the root and its

pigmentation (Brundrett *et al.*, 1996). Dark roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3 minutes at room temperature. Thereafter, the roots were treated with 10% HCl (v/v) for 15-20 minutes at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90 ^{0}C for 30 minutes in a water bath (Brundrett *et al.*, 1996). Fungal colonization was quantified using the magnified intersection method of McGonigle *et al.* (1990) under a compound-light microscope (OLYMPUS-BX51) at a magnification x200. Accordingly, 150 intersections were observed for each sample. The presence of arbuscular mycorrhizal hyphae. vesicles, and arbuscules were recorded.

Table 2.2 Physical and chemical parameters of soil samples under the acacia trees

Name of the acacia species	pH	T.N %	Avail. P (ppm)	O.C %	C/N	Clay %	Silt %	Sand %	Soil class	Land use type
A. abyssinica	6.8	0.19	24.54	2.95	16	46	20	34	Clay	PP
F. albida	7.2	0.15	5.82	2.02	14	38	26	36	Clay loam	SCAFS
A. nilotica	6.4	0.37	20.68	4.77	13	48	22	30	Clay	PFR-HU
A. senegal	6.6	0.34	4.72	3.72	11	16	30	54	Sandy loam	
A. tortilis	6.4	0.32	4.44	3.65	11	18	38	44	Loam	PFRNV
A. seyal	6.7	0.32	5.88	3.93	12	14	34	52	Loam	
A. tortilis	6.5	0.33	5.42	4.02	12	18	34	48	Loam	OGF
A. seyal	6.6	0.33	5.32	4.02	12	19	33	48	Loam	
A. sieberiana	6.4	0.08	13.06	1.58	20	22	24	54	Sandy clay loam	
A. saligna	6.5	0.12	12.88	1.75	15	44	24	32	Clay	CPFR
A. seyal	6.5	0.15	4.47	1.92	13	24	30	46	Loam	
A. robusta	6.5	0.21	13.86	3.15	15	26	24	50	Sandy clay loam	

T.N: total nitrogen; Avail.P: available phosphorus; O.C: organic carbon; C/N: carbon nitrogen ratio; PP: protected park; SCAFS: sorghum cropping in agro forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relics for reforestation programmes.

Spore extraction and identification

Soil samples were air-dried before extraction, counting and identification of AM fungal spores. The AMF spores were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia and Agrifood Research Finland (MTT), Laukaa, Finland. AMF spores were extracted by

the wet sieving and decanting method (Gerdemann and Nicolson, 1963), followed by centrifugation in water and in 50% sucrose solution (Walker *et al.*, 1982). Sieves of size of 500, 250 and 50 µm were used for the wet sieving procedure. Spores, spore clusters and sporocarps obtained from 250 and 50µm sieves were counted and observed using a dissecting microscope. Thereafter, spores were mounted on slides in polyvinyl-lactic acid-glycerol (PVLG, Omar *et al.*, 1979) or in PVLG mixed with Melzer's reagent (1:1 v/v).

Spores were examined under a compound microscope and identified to the species level or attributed to a specific morphotype. Identification and classification were based on a current species descriptions and identification manual (Schenck and Perez, 1990), online references of species description INVAM *http://invam.caf.wvu.edu*, http://www.zor.zut.edu.pl/Glomermycota/ University of Agriculture in Szczecin, Poland, Schüßler and Walker (2010) and the Schüßler AMF phylogeny website http://www.lrz.de/~schuessler/amphylo/

Statistical analysis

Spore density (SD) is expressed as the number of AMF spores per gm of soil. Species richness (SR) is measured as a number of AMF species per sample. Isolation frequency (IF) is (the number of samples in which a given species was isolated/ the total number of samples) x100%. Relative abundance of spores (RA) is (the number of spores in a given species / total number of spores) x100%. The dominant AMF species were determined according to relative abundance (RA>5%) and isolation frequency (IF >50%) (Li *et al.*, 2007). Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 18.0).

Transformed data were subjected to one-way ANOVA to test the differences in AM colonization and spore density among plant species. Multiple mean comparisons were performed using Tukey's HSD post hoc test at the 0.05 level of probability with one-way ANOVA. The relationship between AM colonization and spore density as well as spore density, and species richness and soil parameters were determined by Pearson's correlation analysis.

RESULTS

AMF root colonization

Acacia roots showed mycorrhization with typical structures (arbuscules, hyphae and vesicles) except that arbuscules were not detected in *A. senegal.* AMF root colonization varied from 12% to 67.3% (**Table 2. 3**). The highest colonization (67.3%) was found in *A. seyal* from OGF (open grazing field) followed by 44% colonization in *A. nilotica* from PFR-HU (protected forest relics managed by Hawassa University), whereas, *A. saligna* from CPFR (community preserved forest relics) showed the lowest AM fungi colonization (12%).

Arbuscule and vesicle colonization were the highest in the roots of *A. seyal* from OGF, 11.8% and 17.3 %, respectively. In contrast, low percentages of arbuscules (0%) and vesicles (1.6%) were recorded from roots of *A. senegal* (PFRNV) and from *A. abyssinica* (PP), respectively. The percentage of AMF root colonization of the same species from different land use types did not show significant difference except, that the percentage of vesicles recorded from *A.seyal* (17.3%) at OGF, was significantly higher than that of the same plant species (8%) from PFRNV (**Table2.3**). The data also showed slight, but not significant negative correlation between the root

colonization levels and the available P concentration in soil (r = - 0.40). However, arbuscular colonization was strongly correlated with vesicular and hyphal colonization (r^2= 0.76 and r^2 = 0.67, respectively; p< 0.05).

Tble 2. 3 AMF roots colonization rate and spore density in the rhizosphere of acacia trees

Significant differences between the samples are indicated by different letters in column and were determined by using Tukey HSD at the 0.05 level after one –way ANOVA. SD: spore density; AC, VC and HC are percentage

Name of the acacia species	AM colonization				Land use type
	AC %	VC %	HC%	SD (g^{-1} of soil)	
A. abyssinica	1.4 ± 0.8 a	1.6 ± 0.9a	15.3 ± 3.8a	7.5ab	PP
F. albida	1.7 ±0.3a	1.7 ± 0.3a	24.5 ± 0.8a	9.0 abc	SCAFS
A. nilotica	2.8 ± 1a	8.9 ± 0.2ab	44 ± 1.1ab	3.7a	PFR-HU
A. Senegal	0	3.1 ± 1.7a	20.2 ± 7.3a	11.9bc	PFRNV
A. tortilis	6.6 ± 1.9ab	6.9 ± 1.5a	37.6 ± 5.4ab	12.6bc	
A. seyal	2.8 ± 1ab	8 ± 1.1a	38 ± 1.7ab	8.7abc	
A. tortilis	2.3 ± 0.2a	2.3 ± 1.3a	37.5 ± 2ab	11bc	OGF
A. seyal	11.8 ± 3.9b	17.3 ± 1.3b	67.3 ± 4.4b	15c	
A. sieberiana	4.5 ± 2.5ab	4.5 ± 2.5a	32.3 ± 18ab	11.5bc	CPFR
A. saligna	2.5 ± 1.4a	5 ± 2.8a	12 ± 6.9a	9abc	
A. seyal	3.3 ± 1.9ab	10.3 ± 1.2ab	28.5 ± 11ab	12.7bc	
A. robusta	2.5 ± 1.4a	5.0 ± 2.8a	23.7 ± 6.4a	7.3ab	

of root length with arbuscule, vesicle and hyphal colonization, respectively; PP: protected park; SCAFS: sorghum cropping in agro forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes.

AMF spore density and species diversity

Rhizosphere soils from all acacia species in different land use systems harbored high numbers of AMF spores ranging from 3.7 spores g^{-1} soil to 15 spores g^{-1} soil with an average of 9.9 spores g^{-1} soil (**Table 2.3**). The highest average spore density of 15 spores g^{-1} soil was observed under *A. seyal* (OGF), and the lowest of 3.7 spores g^{-1} of soil under *A. nilotica.*

Significant difference (p<0.05) in spore density was observed between *A. seyal* (15.0 g^{-1}), *A. abyssinica* (7.5 g^{-1}), *A. robusta* (7.3 g^{-1}) and *A. nilotica* (3.7 g^{-1}). Similarly,

spore numbers obtained under *A. senegal* (11.9 spores g^{-1} of soil), *A. tortilis* (12.6 g^{-1}) from PFRNV, *A. sieberiana* (11.5g^{-1}) and *A. seyal* (12.7 g^{-1}) from CPFR were significantly different from spore numbers obtained under *A. nilotica* (3.7 spores g^{-1} of soil). Though not statistically significant there was an indication of slightly higher spore density in the rhizosphere soil of *A. seyal* from OGF (15.0 spores g^{-1} of soil) than in the rhizosphere soil of the same acacia species from forest relics in Bishoftu and Zeway (12.7 spores g^{-1} and 8.7 spores g^{-1} of soil, respectively).

Correlation analysis showed a significant negative correlation between AMF spore density and available P in soil ($r = 0.-728$, $p < 0.01$) (**Fig. 2. 2**). AMF spore density was positively correlated with the percentage of soil texture such as silt and sand ($r= 0.649$ & $r=0.604$, $p<0.05$, respectively), but negatively correlated with the percentage of clay ($r= -0.710$, $p<0.01$).

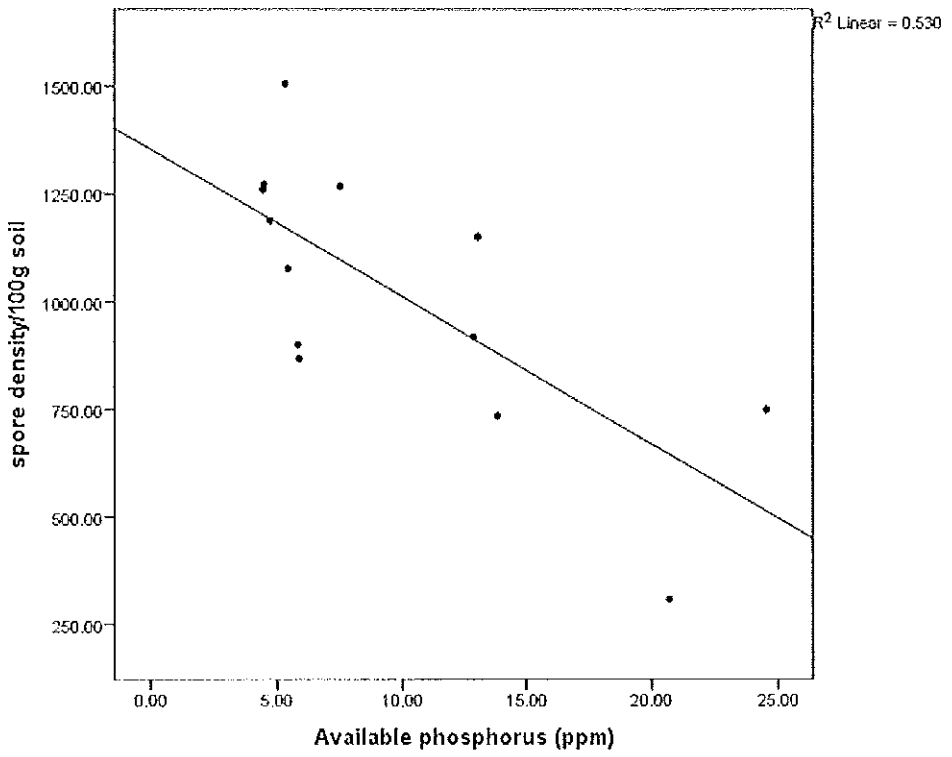

Fig. 2. 2 Correlation between available phosphorus and spore density

A total of 41 AMF species belonging to 14 genera and 7 families were identified from all rhizosphere soil samples of acacia species (**Table 2. 4 and Fig. 2. 3**). The data showed that the genus *Acaulospora* was the most diverse group represented by nine species, followed by the Genera *Funneliformis* (6 species), and by the genera *Gigaspora, Glomus*, and *Rhizophagus* represented each by four species. In addition, the genera *Claroideoglomus*, and *Scutellospora* on one hand, and the genera *Racocetra* and *Diversispora,* on the other were represented by three and two species, respectively. The genera *Entrophosphora, Sclerocystis, Paraglomus* and *Pacispora* also contained one species each. Additionally, 2 unidentified morphotypes each of *Glomus,* and *Acaulospora* and 1 of *Archaeospora* were also recorded.

The highest species diversity of 19 species from 7 genera was detected in the rhizosphere soil of *A. saligna* followed by 18 species in 6 genera from *A. abyssinica.* The relative species diversity distribution among the remaining trees was 11 species each from *A. tortilis* and *A. seyal,* with 6 and 5 genera, respectively, and of 10 species in 5 genera from *A. robusta* (**Table 2. 5**).

Among the land use types CPFR, OGF, and PFRNV harbored more diverse groups compared to the other land use types (**Table 2.5**). Accordingly, 31 AMF species in 9 genera were detected in CPFR at Bishoftu followed by 25 species in 11 genera from OGF, and 22 species in 9 genera from PFRNV. Based on relative abundance and isolation frequency of spores, the dominant five species identified were from the genera *Claroideoglomus* (*C. claroideum, C. etunicatum, C. luteum*), from g enera *Funneliformis (, F. geosporus)* and from genera *Glomus* (*G. aggregatum)* (**Table 2.4**).

Generally, 32.6%, 19.5% and 19.5% of AMF species were identified from the families of *Glomeraceae, Acaulosporaceae* and *Gigasporaceae,* respectively. However, most AMF species from the *Acaulosporacae* were characterized by low relative abundance and frequency of isolation compared to the other families.

Table 2. 4 The distribution of mycorrhizal fungi, their frequency of occurrence and relative abundance in rhizosphere of acacia species

AMF species	*A. abyssinica*	*F. albida*	*A. nilotica*	*A. senegal*	*A. tortilis* (PFRNV)	*A.seyal* (PFRNV)	*A.tortilis* (OGF)	*A.seyal (OGF)*	*A.sieberiana*	*A.saligna*	*A.seyal(CPFR*	*A.robusta*	IF (%)	RA (%)
Acaulospora													158	6.85
A. capsicula Błaszk.				x									8.3	0.25
A. cavarnata Błaszk.										x			8.3	0.25
A. denticulata Sieverd. & S. Toro		x								x	x	x	33.3	1.79
A. faveata Trappe & Janos	x												8.3	0.25
A. laevis Gerd. & Trappe										x			8.3	0.25
A. rehmii Sieverd. & S. Toro	x		x		x						x		33.3	1.53
A. sorbiculata Trappe							x					x	16.6	1.02
A. spinosa Walker & Trappe	x								x				16.6	0.76
A. tuberculata Janos & Trappe					x								8.3	0.25
Acaulospora sp1										x			8.3	0.25
Acaulospora sp2									x				8.3	0.25
Archaeospora													8.3	0.25
Archaeospora sp								x					8.3	0.25
Claroideoglomus													258	27.1
C. claroideum (Schenck & Sm.) Walker & Schuessler	x	x	x	x		x	x	x	x	x	x	x	91.6	7.16
C. etunicatum (Becker & Gerd.) Walker & Schuessler	x	x	x	x	x		x	x		x	x		75	5.37
C. luteum (Kenn *et al)* Walker & Schuessler	x	x	x	x	x	x	x	x	x		x	x	91.6	14.57
Diversispora													24.9	1.27
D. celata Walker, Gamper & Schuessler	x												8.3	0.51
D. epigaea (Daniels & Trappe) Walker & Schuessler					x			x					16.6	0.76
Entrophosphora													8.3	0.25
E. nevadensis Błaszk., Madej & Tadych; Palenzuela, . Ferrol, Azcón-Aguilar & Oehl							x						8.3	0.25
Funneliformis													316	19.9
F. badius (Oehl, Redecker & Sieverd.) Walker & Schuessler						x		x					16.6	1.53
F. caledonius (Nicolson & Gerd.) Walker & Schuessler	x			x			x		x	x	x		50	1.53
F. constrictus (Trappe) Walker & Schuessler	x	x	x	x					x	x		x	58.3	4.34
F. coronatus (Giovann.) Walker & Schuessler		x										x	16.6	0.76
F. geosporus Nicolson & Gerd.) Walker & Schuessler	x		x	x	x	x	x	x	x	x	x	x	91.6	6.9
F. mossae (Oehl, Redecker & Sieverd.)Walker & Schuessler	x	x	x	x		x	x	x	x	x			83.3	4.85
Glomus													275	24.5

G. aggregatum Schenck & Sm.	x	x	x	x	x	x	x	x		x	x	x	91.6	13
G. hoi Berch & Trappe										x			8.3	0.25
G. microaggregatum Koske, Gemma & Olexia									x	x	x		25	1.02
G. microcarpum Tul. & Tul.	x		x	x	x	x		x					50	4.6
Glomus sp1(sporocarpic , thick wall & smooth, 80-110µm)							x		x	x	x	x	41.6	2.81
Glomus sp2 (red brown geosporum like)	x	x		x	x	x		x	x				58.3	2.81
Rhizophagus													66.5	2.54
R. clarus (Nicolson & Schenck) Walker & Schuessler									x				8.3	0.25
R. diaphanus (Morton & Walker) Walker & Schuessler			x							x			16.6	0.76
R. fasciculatus (Thaxt.) Walker & Schuessler					x						x	x	25	1.02
R. intraradices (Schenck & Sm.) Walker & Schuessler	x							x					16.6	0.51
Sclerocystis													25	0.76
S. sinuosa Gerd. & Bakshi	x							x	x				*25*	*0.76*
Gigaspora													58.2	2.28
G. albida Schenck & Sm				x			x	x					25	1.02
G. decipiens Hall & Abbott							x						8.3	0.25
G. gigantea (Nicolson & Gerd.) Gerd. & Trappe						x				x			16.6	0.76
G. margarita Becker & Hall		x											8.3	0.25
Racocetra													41.6	2.55
R. fulgida (Koske & Walker) Oehl, Souza & Sieverd.		x											8.3	0.25
R. gregaria (Schenck & Nicolson) Oehl, Souza & Sieverd.		x		x			x	x					33.3	2.3
Scutellospora													108	6.59
S. calospora Nicolson & Gerd.) Walker & Sanders				x			x			x			25	1.79
S. cerradensis Spain & Miranda			x	x	x			x	x				41.6	2.3
S. pellucida (Nicolson & Schenck) Walker & Sanders		x		x		x	x		x				41.6	2.5
Pacispora													41.6	3.06
P. scintillans (Rose & Trappe) Walker, Vestberg & Schuessler	x		x					x		x	x		41.6	3.06
Paraglomus													41.6	1.79
P. occultum (Walker) Morton & Redecker	x	x				x	x			x			41.6	1.79

IF: isolation frequency; RA: relative abundance; PFRNV: protected forest relies with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies

Table 2. 5 Summary of total AMF genera and species identified in the plant species and land use types

Plant species	Total AMF identified from the plants		Land use type	Total AMF identified in the land use type	
	Genera	Species		Genera	Species
A. abyssinica	8	18	PP	8	8
F. albida	8	14	SCAFS	8	8
A. nilotica	6	12	PFR-HU	6	6
A. senegal	7	16	PFRNV	9	22
A. tortilis	6	11			
A. seyal	5	11			
A. tortilis	9	16	OGF	11	25
A. seyal	9	17			
A. sieberiana	7	15	CPFR	9	31
A.saligna	7	19			
A.seyal	6	12			
A. robusta	5	10			

PP: protected park; SCAFS: sorghum cropping in agro-forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes

DISCUSSION

In this study, AMF spore density, diversity and root colonization were studied on selected species of acacia growing in different land use types in two agroecosystems in Ethiopia (**Table 2.1**). AMF were present in all rhizosphere soil and acacia root samples with low (12%) to moderate (67.3%) levels of colonization.

This pattern is similar to that observed in other tropical systems, such as (0-75% colonization) in acacia and other woody legume species in dry deciduous forest areas of Northern Ethiopia (Birhane *et al.*, 2010), (31-64% colonization) in acacia and prosopis tree species in Senegal (Ingleby *et al.*,1997), (35-65 % colonization) in acacia tree species, in India (Lakshman *et al.*, 2001) and (56-73% colonization) in *A. farnesiana* and *A. planifrons,* in India (Udaiyan *et al.*, 1996). This study showed intra- and interspecific variations in AM colonization among acacia plants (**Table 2. 3**).

Correlation analysis showed that arbuscular colonization was positively correlated with hyphal and vesicular colonization (Lingfei *et al.*, 2005). Concerning soil parameters, though not significant at $p<0.05$, there was an indication of positive correlation between percentages of hyphal colonization and organic carbon ($r=0.54$) and a negative correlation between hyphal colonization and available P ($r=-0.45$), 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 (**Table 2.3**). The mean number of spores per g of soil ranged from 3.7 to 15 with an average of 9.9. Other studies in similar or different host plants of the tropical area corroborate our finding: 7.8 to 12.4 spores g^{-1} soil in *A. albida* Del. in Senegal (Diop *et al.*, 1994); 5-15 spores g^{-1} soil in *A. farnesiana* and *A. planifrons* in moderately fertile alkaline soils in India (Udaiyan *et al.*, 1996); 1.1- 26 spores g^{-1} soil in tropical forest and pasture (Picone, 2000) and 5–64 spores g^{-1} soil in a valley savanna of the dry tropics (Tao *et al.*, 2004).

By contrast, low spore densities of 0.1-0.3 spores g^{-1} soil were detected in dry deciduous woodlands of Northern Ethiopia associated with different acacia species such as *A. abyssinica, A. asak, A. etbaica, A. oerfota, A. polyacantha* and *A. tortilis* (Birhane *et al.*, 2010). Low AMF spore numbers were also recorded in a survey of acacia tree species (0.5-0.7 spores g^{-1} soil) in India (Lakshman *et al.*, 2001) and in acacia and prosopis tree species (0.8-0.5 spores g^{-1} soil) in Senegal (Ingleby *et al.*, 1997). The variation in AMF spore density between samples could be due to factors

such as climatic and edaphic properties and host preference of AM fungi (Bever *et al.* 1996; Muthukumar and Udaiyan, 2002; Mathimaran *et al.*, 2007).

The highest number of AMF spores was recorded in the rhizosphere soil of *A. seyal* from the open grazing field at Zeway, which was also higher than the number of spores of the same species at Zeway (13 spores/gm soil) and at Bishoftu protected forest relics (9 spores/gm soil). In addition, the spore count obtained in the rhizosphere soil of *A. seyal* from OGF was 2-5 times higher than the spore counts in *A. nilotica* from PFR-HU, *A. abyssinica* from PP and *A. robusta* from CPFR, land use types (**Table 2. 3**). According to Janos (1992) and Picone (2000), disturbed ecosystems induce AMF to sporulate because of grazing, disturbance and slow rate of decomposition. On the contrary, Birhane *et al.* (2010) reported that management in the form of exclosure (that excludes grazing) had a positive effect on spore abundance. They showed that spore abundance in exclosures ranged between 2.9 and 7.1 spores g −1 soil while in open areas it ranged between 0.8 and 4.5 spores g −1 soil.

The percentage of root colonization and the number of AMF spores observed in the sampled acacia trees did not correlate significantly ($r = 0.48$, $p > 0.05$). Becerra *et al.* (2009) showed that the relationship between AMF spore density and percentage of root colonization is influenced by many biotic and abiotic environmental factors such as AM fungal species, plant host and soil nutrients (Stutz and Morton, 1996). The present study showed a significant negative correlation between spore density and available P ($r = -0.73$, $p < 0.01$, **Fig. 2. 2**), which is similar to some reports from India and Northern Europe (Udaiyan *et al.*, 1996; Kahiluoto *et al.*, 2001). The decrease in

spore density with an increase in soil available P observed in the study can be attributed to the fact that, available soil phosphorus suppresses AM root colonization as well as AMF density (Kahiluoto *et al.,* 2001).

In contrast to this finding, Muleta *et al.* (2007) observed a positive relationship between spore number and available P in soil samples from natural coffee forest in Ethiopia. The authors suggested that the available P level in the study was not high enough to influence mycorrhizal development. As far as soil texture is concerned, spore density showed a significant positive correlation with sandy soil and negative correlation with clay soil ($r= 0.60$ and $r= -0.71$; $p< 0.05$ and $p< 0.01$, respectively). This is similar to the findings of Carrenho *et al.* (2007) that showed mycorrhizal association could be better developed in sandy soils than in clay soils. Sylvia and Williams (1992) argued that sandy soils are usually more porous, warmer, drier, and less fertile than those of a finer texture and these conditions have direct and indirect effects on AMF.

In this study both high AMF spore density and species diversity was observed in the rhizosphere of acacia trees. Based on spore morphology, 41 AMF species and 5 morphotypes were identified (**Table 2. 4**). Similarly, 44 and 60 AM fungal species were detected from semiarid grasslands of Namibia (Uhlmann *et al.*, 2004) and sub-Saharan Savannas of Benin, West Africa (Tchabi *et al.*, 2008), respectively. Likewise 43 species of AMF were isolated from Western Brazilian Amazon (Stürmer and Siqueira, 2011). The high AMF species richness observed in our acacia study is in contrast to the only 17 AMF species isolated from tropical humid high land of Kenya (Jefwa *et al.*, 2009). Mathimaran *et al.* (2007) found 18 species in Kenyan ferralsol

soil and Emmanuel *et al.* (2010) recorded 17 AMF species from soil fertility management systems in Nigeria. According to Helgason *et al.* (1998) woodlands have higher AM fungal species richness and diversity compared to agricultural ecosystems.

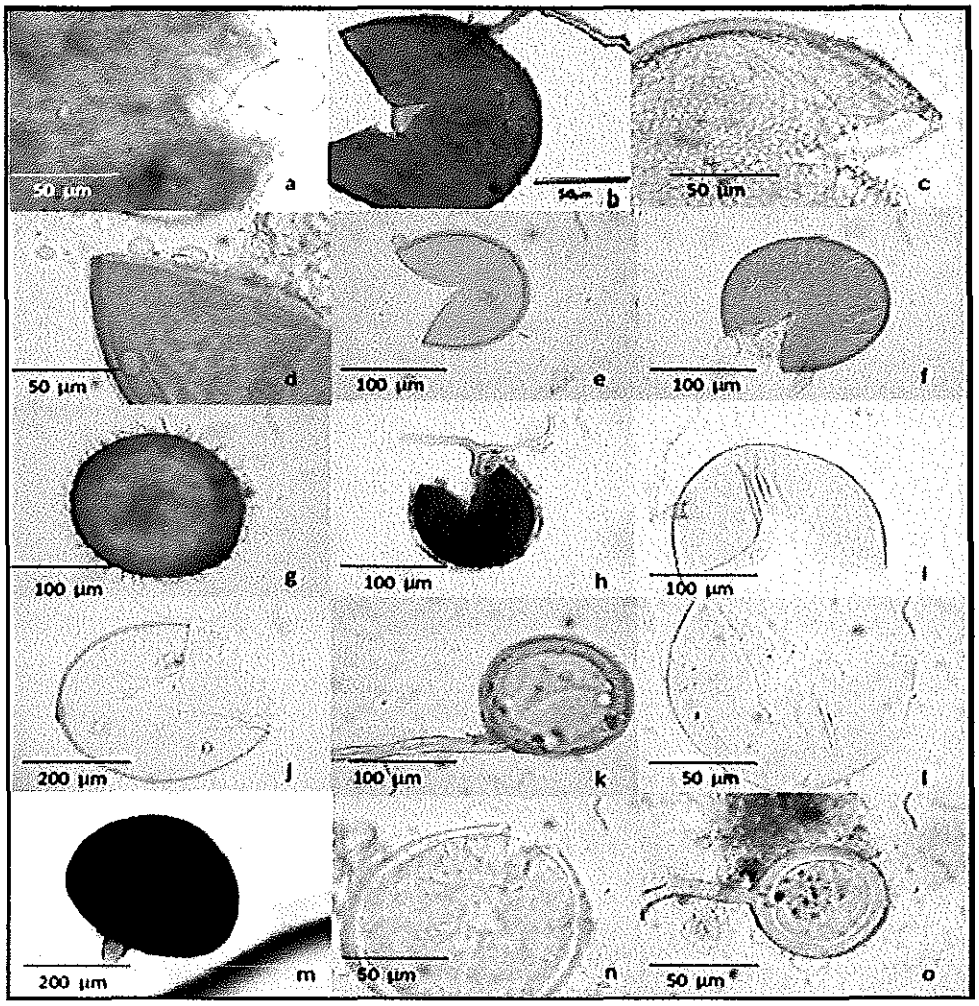

Figure 2. 3 Glomeromycotan species identified from rhizosphere soil samples of acacia species in Ethiopia. All photos are from slides made in PVLG. A) *Acaulospora dentidulata,* b) *Claroideoglomus etunicatum* c) *A. scrobiculata,* d) *A. spinosa,* e) *Claroideoglomus claroideum,* f) *Diversispora epigaea,* g) *Entrophospora nevadensis,* h) *Funneliformis geosporus,* i) *F. mosseae,* j) *Gigaspora gigantea,* k) *Glomus hoi,* l) *Pacispora scintillans,* m) *Racocetra gregaria,* n) *Rhizophagus diaphanus,* o) *R. fasciculatus.*

Generally, *Acaulospora* and *Funneliformis* were the dominant genera accounting for 9 and 6 species, respectively. This result is similar to that observed in tropical systems in the hot-dry valley of the Jinsha River, Southwest China (Zhao and Zhao, 2007), in Panama (Mangan *et al.*, 2004), and in Brazil (Stürmer *et al.*, 2006). The dominance of these two genera may be related to their sporogenous characteristics, i.e. the production of relatively small spores within a short period of time compared with the large spores of the genera *Gigaspora* and *Scutellospora* (Hepper, 1984; Bever *et al.*, 1996).

Among the 41 species identified, the most frequently encountered were *Claroideoglomus luteum, Glomus aggregatum, C. claroideum, C. etunicatum* and, *Funneliformis geosporus* (**Table 2.4**). Other studies have also shown that these species are repetitively detected in temperate and tropical ecosystems (Stutz *et al.*, 2000; Oehl *et al.*, 2003; Zhao and Zhao, 2007). On the other hand, AMF species such as *G. hoi, A. capsicula, A. cavarnata, A. faveata, A. tuberculata, Diversispora celata, Entrophospora nevadensis, Rhizophagus clarus, G. decipiens, G. margarita* and *Racocetra fulgida* occurred only in any one of the acacia species suggesting that they are specific in their distribution.

There was also a significant positive correlation between relative abundance and isolation frequency of AMF species ($r=0.88$, $p< 0.01$) indicating that species producing more spores usually had a wider distribution, while species with small geographic ranges usually produced fewer spores (Zhao and Zhao, 2007). Within the limits of the sampling areas, the study showed relatively high mycorrhizal colonization but very high AMF species diversity in the rhizosphere soil of different

acacia species growing in Ethiopia. The study also indicated that AMF spore density and the extent of AMF colonization in acacia roots was influenced by soil factors such as available P and soil texture. The results obtained have wider implications for improving restoration success of soil fertility in degraded soils. Our small-scale field survey confirms that attention should be given to woody legumes with high mycorrhization property and are of high agroforestry importance in the country.

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REFERENCES

Anon (1983). *Acacia mangium* and other Acacias of the humid tropics. National Academy Press, Washington, D.C.

Becerra, A. G., Cabello, M, Zak, M. R. and Bartoloni, N. (2009). Arbuscular mycorrhizae of dominant plant species in Yungas forests, Argentina. *Mycologia* 101: 612–621.

Bever, J.D., Morton, J. B., Antonovics, J. and Schultz, P. A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in Mown grassland. *J. Ecol.* 84: 71-82.

Birhane, E., Kuyper, T. W., Sterck, F. J. and Bongers, F. (2010). Arbuscular mycorrhizal associations in *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *For. Ecol. Manage.* 260: 2160–2169.

Brewbaker, J. L. (1986). Nitrogen fixing trees for foder and browse in Africa. In: *Alley farming in the humid and sub humid tropics* (Kang, B. T. and Reynods, L. eds) Proceeding of a working held at Ibalan Nigeria, IDRC, Ittawa, pp 58-70.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. 374+x p.

Carrenho, R., Trufem, S. F. B., Bononi, V. L. R. and Silva, E. S. (2007). The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta. Bot. Bras.* 21: 723-730.

Craig, G. F., Atkins, C. A. and Bello, D. T. (1991). Effects of salinity on growth of four strains of Rhizobium and their infectivity on two species of acacia. *Plant Soil* 133:253-262.

da Silva Sousa, C., Cezar Menezes, R. S., de Sá Barreto Sampaio, E. V., de Sousa Lima, F., Oehl, F. and Costa Maia, L. (2013). Arbuscular mycorrhizal fungi within agroforestry and traditional land use systems in semi-arid Northeast Brazil. *Acta Scient. Agron.* 35:307-314.

Diop, T.A., Gueye, M., Dreyfus, B. L., Plenchette, C. and Strullu, D. G. (1994). Indigenous arbuscular mycorrhizal fungi associated with *Acacia albida* Del. in different areas of Senegal. *Appl. Environ. Microbiol.* 60:3433-3436.

Dommergues, Y. R. (1987). The role of biological nitrogen fixation in agroforestry. In: *Agroforestry, a decade of development* (Steppler, H. A. and Nair, P. K. R. eds). ICRAF, Nairobi, Kenya, pp 245-271.

Emmanuel, B., Fagbola, O., Abaidoo, R., Osonubi, O. and Oyetunji, O. (2010). Abundance and distribution of arbuscular mycorrhizal fungi species in long-term soil fertility management systems in Northern Nigeria. *J. Plant Nutr.* 33: 1264-1275.

Eshete, G. and Stahl, G. (1999). Tree rings as indicators of growth periodicity of acacias in the Rift Valley of Ethiopia. *For. Ecol. Manage.* 116: 107-117.

Garedew, E., Sandewall, M., Söderberg, U. and Campbell, B. M. (2009). Land-Use and Land-Cover Dynamics in the Central Rift Valley of Ethiopia. *Environ. Manage.* 44:683–694.

Gavito, M. E., Pérez-Castillo, D., González-Monterrubio, C. F., Vieyra-Hernández, T. and Martínez-Trujillo, M. (2008). High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land use types in a tropical dry ecosystem. *Mycorrhiza* 19:47-60.

Kahiluoto, H., Ketoja, E., Vestberg, M. and Saarela, I. (2001). Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant and Soil* 231: 65–79.

Lakshman, H. C., Rajanna, L., Inchal, R. F., Mulla, F. I. and Srinivasulu, Y. (2001). Survey of VA- mycorrhizae in agroforestry and its implications on forest trees. *Trop. Ecol.* 42(2): 283-286.

Li, L. F., Li, T. and Zhao, Z. W. (2007). Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. *Mycorrhiza* 17: 655-665.

Lingfei, L., Anna, Y. and Zhiwei, Z. (2005). Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiol. Ecol.* 54: 367–373.

Mangan, S. A., Eom, A-H., Adler, G. H., Yavitt, J. B. and Herre, E. A. (2004). Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. *Oecologia* 141:687–700.

Mason, P. A. and Wilson, J. (1994). Harnessing symbiotic associations: vesicular-arbuscular mycorrhizas. In: *Tropical trees: the potential for domestication and the rebuilding of forest resources.* (Leakey, R. R. B. and Newton, A. C. eds.) London, HMSO, pp 165-175.

Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E. and Jansa, J. (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agricult. Ecosyst. Environ.* 119: 22-32.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol.* 115: 495–501.

Michelsen, A. (1993). Growth improvement of Ethiopian acacias by addition of vesicular-arbuscular mycorrhizal fungi or root of native plants to non sterile nursery soil. *For. Ecol. Manage.* 59:193-206.

Mulcta, D., Assefa, F., Nemomissa, S. and Granhall, U. (2007). Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. *For. Ecol. Manage.* 241: 145–154.

Muthukumar, T. and Udaiyan, K. (2002). Seasonality of vesicular– arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta. Oecologica* 23: 337–347.

Ngulube, M. R., Chapola, G. B. J. and Mwabumba, L. (1993). The potential of Australian dry zone acacias for agroforestry in Malawi. *For. Ecol. Manage.* 56: 83-97.

Ndoye, F., Kane, A., Mangaptché E. L. N., Bakhoum, N., Sanon, A., Diouf, D., Ourèye Sy, M., Baudoin, E., Noba, K. and Prin, Y. (2012). Changes in land use system and environmental factors affect arbuscular mycorrhizal fungal density and diversity, and enzyme activities in rhizospheric soils of *Acacia senegal* (L.) *Willd. ISRN Ecology* doi:10.5402/2012/563191.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T. and Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Appl. Environ. Microbiol.* 69: 2816–2824.

Chapter three

Diversity and abundance of arbuscular mycorrhizal fungi across different land use types in a humid low land area of Ethiopia

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DIVERSITY AND ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI ACROSS DIFFERENT LAND USE TYPES IN A HUMID LOW LAND AREA OF ETHIOPIA

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SUMMARY

The aim of this work was to study the effect of different land use types on arbuscular mycorrhizal (AM) fungal populations in soil and trap cultures from Showa Robit, Ethiopia. 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 a relatively 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 taken to study AMF spore abundance, species richness, diversity indices and mycorrhizal inoculum potential (MIP). 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 Arable4 and NF showed the lowest (2.5-3.8 g^{-1} soil). Slightly different MIP patterns also occurred with Arable1 and FC, 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. *Claroideoglomus* and *Funneliformis* 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.

Keywords: *Claroideoglomus; Funneliformis; Glomus;* mixed cropping; monocropping; mycorrhizal inoculum potential

Verbruggen *et al.* (2010) showed a decrease in mycorrhizal fungal communities in relation to land use intensity in that the average number of AMF taxa identified was highest in grasslands (8.8), intermediate in organically managed fields (6.4) and significantly lower in conventionally managed fields (3.9) in the agricultural soils of the Netherlands. It is also shown that AMF diversity and species richness in organically managed fields and natural vegetation fields was higher than that of conventionally managed fields (Oehl *et al.*, 2004; Tchabi *et al.*, 2008).

In Ethiopia, the land cover has been changed from natural forest to farmland, open grazing and fast growing plantation forests for several decades. According to FAO (2007), the country lost an average of 141,000 ha, or 1.1% per year, of its forest covers between 1990 and 2005, due to deforestation. Another study in the Central Ethiopian Rift Valley indicated that woodland cover declined from 40% to 9% at one site, while another site lost 54% of its woodland cover due to rapid deforestation (Garedew *et al.*, 2009). In all these years, deforestation has resulted in massive soil degradation with a decline in soil organic matter and available nitrogen in the highlands of Ethiopia (Lemenih *et al.*, 2005; Girmay *et al.*, 2008). A recent study also showed drastic changes in several of the physical and chemical properties of soils from different parts of the country due to rapid land use changes (Getachew *et al.*, 2012).

The drastic change in deforestation and land use in Ethiopia decimated the large biodiversity and plant community structure of the country. This is also presumed to affect the underground microbial composition including the AMF, because several studies have showed that plant community structure affects diversity and community

composition and species richness of AMF (Burrows and Pfleger, 2002; Vandenkoornhuyse *et al.*, 2002; Johnson *et al.*, 2003: Scheublin *et al.*, 2004; Sýkorová *et al.*, 2007).

It has also been reported that changing the vegetation cover from tree-based intercrops to mono-cropping systems can reduce AMF fungal richness (Chifflot *et al.*, 2009). Lower AMF species richness has been found in arable fields, compared to different natural ecosystems and perennial communities such as tropical forests (Snoeck *et al.*, 2010). It may well be that the intensive land use change in Ethiopia for several decades has brought a reduction and/or shift in abundance and diversity of AMF under monocropping and intercropping systems.

The hypothesis of this study was that AMF abundance and diversity may have been affected by land management practices such as monocropping cultivation and use of fertilizer in the agricultural systems of the country. It is also equally important that understanding the role of AMF over a broad range of land use systems is essential for land rehabilitation and effective management for sustainable production through AMF technology in the future (Estaún *et al.*, 1997; Oehl *et al.*, 2003).

The objectives of this study were; (1) to compare AM fungal diversity and community composition among different land-use systems within a single agro-ecosystem; (2) to determine mycorrhizal inoculum potential in soil of these systems (3) and to determine whether AM fungal species richness, mycorrhizal inoculum potential (MIP) and spore abundance are influenced by land use changes.

MATERIAL AND METHODS

Study site description

The study site is located in Showa Robit (10^0 06' 650" - 09^0 57'957" N, 039^0 54'37"-$039^0$56'579" E), (**Fig. 3. 1**) in north Showa Zone of Amhara Regional State, 225 km north of Addis Ababa, Ethiopia. The agro-ecology of the study site is low-land or Erteb Kola (sub-moist warm) with altitude ranging between 1120 and 1350 m a.s.l. The climate data of the study area recorded for the last ten years shows average annual maximum and minimum temperature and precipitation of 32.1 and 16.1 ^{0}C, and 968 mm, respectively (NMA, 2002-2010).

The land use of the area is mainly characterized by agroforestry practices such as agrisilvicultural (crops including and shrub/tree crops-trees) and agropastoral systems (trees+ crops+ pasture/animals) (Nair, 1993). The vegetation cover of the area is wood-land dominated by trees such as *Acacia, Erythrina, Cordia* and, *Ficus* species. The main grain crops of the area are sorghum, teff, finger millet and maize, whereas horticultural and commercial crops such as mango, banana, sugar cane, coffee, orange, tobacco, onion, tomato and cabbage are also grown in a mixed and/or rotation cropping system.

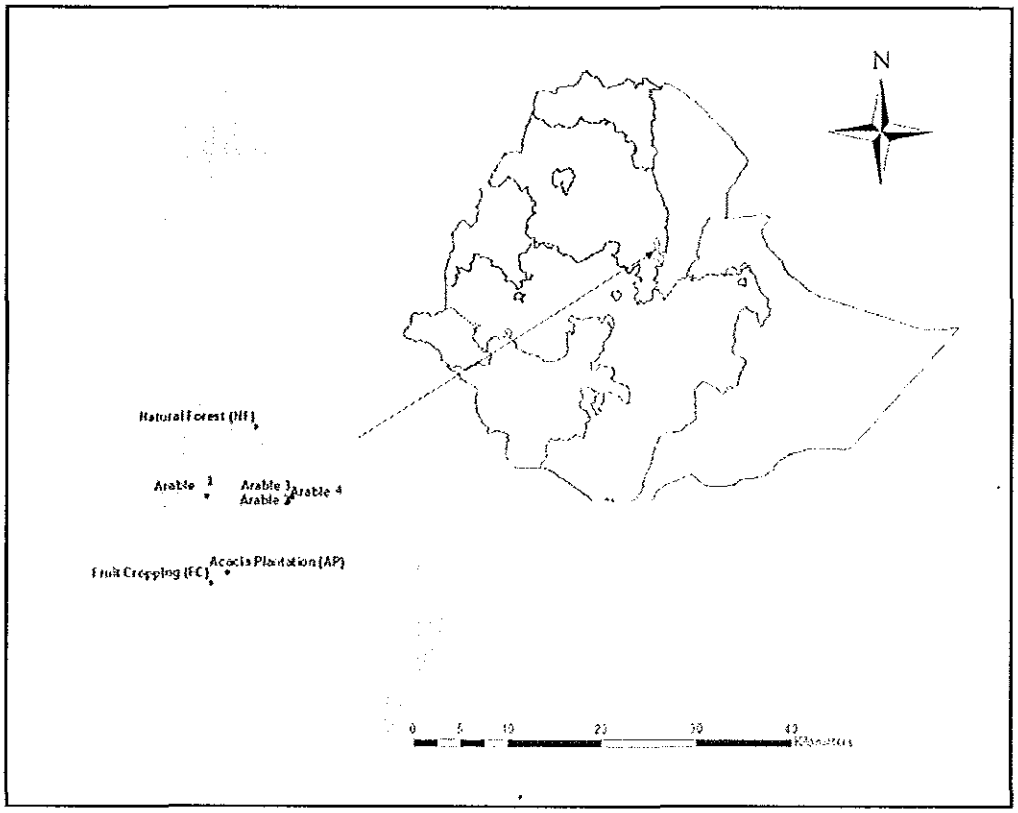

Fig. 3. 1. Map of the study site and sampling location.

Characteristics of the sampling site

The sampling area included seven different land use types and vegetation covers. There were four arable lands (Arable1-Arable4), one fruit cropping area (FC), one natural forest (NF) and one acacia plantation (AP). Arable1 was a low-input, mixed subsistence cropping (teff, sunflower and sesame). Arable2 and Arable3 were low-input sorghum and maize monocrop fields, respectively. Arable4 was relatively a high-input, conventional field cropped with sorghum. In the fruit cropping area (FC), mainly fruits, vegetables and garden cash crops were grown in an intercropping system with banana, papaya, mango, lemon, avocado, tomato and coffee. Two forest relic sites were also included. The natural forest site was dominated by acacia, fig

trees and red stinkwood. The other forest relic site was an acacia plantation dominated by two acacia species (*Acacia seyal* and *A. nilotica*). A description and agricultural management practices of the study sites are given in **Table 3.1**.

Sampling was conducted during the dry season from November to December 2011. Three replicate sampling locations (approximately 100 m^2) were established for each land use type and dominant plant species. Three replicates of each dominant plant species were randomly selected. From each sampling site, 500 g of rhizosphere soil samples were taken from a depth of 0-30 cm and subsequently pooled into one composite sample per location. A total of 54 samples, 3×1 each from the three arable monocrop fields, 3×3 from the arable mixed crop field, 3×7 from the fruit crop field, 3×3 from the natural forest and 2×3 from the acacia plantation were collected. The samples were collected in alcohol sterilized plastic containers, air dried and stored at room temperature for further analysis. Subsamples were used as inoculum for the trap culture system and for the spore extraction.

Table 3. 1: Charactersitcs of study sites; management practices, standing crops or dominant plant species, fertilization and plant protection system. (Sources:Debere Brehan Agricultural Research Centere and Kewit Wereda Agriculture Office).

	Management practices				
Land use type	Cropping system, land history	Fertilization (kg ha^{-1})	Plant protection	Standing crops or dominant plant species during sampling	Common name
Arable1	Crop rotation; three crops in mixture	Low-input urea (50) DAP (100)	Chemical and mechanical	*Eragrostis teff* (Zucc.) Trotter *Sesamum indicum* L. *Helianthus annuus* L.	Teff Sesame Sunflower
Arable2	Continuous sorghum monocropping field for 2 yr.	Low-input, Urea (50) DAP (50)	Chemical and mechanical	*Sorghum bicolor* L.	Sorghum
Arable3	Continuous maize monocropping field for 3 yr.	Low-input, Urea (100) DAP (100)	Chemical and mechanical	*Zea may* L.	Maize
Arable4	High-input; Sorghum monocropping field site used by DBRC	Mineral high-input ; Urea (100), DAP (150)	Chemical	*Sorghum bicolor* L.	Sorghum
Fruit cropping (FC)	Mixed fruit crops field, adjacent to river and irrigated regularly	Manure, compost and crop residues	Mechanical	*Persea americana* Mill. *Mangifera indica* L. *Coffee arabica* L. *Carica papaya* L. *Musa acuminata* Colla *Lycopersicon esculentum* Mill. *Citrus limonum* Risso.	Avocado Mango Coffee Papaya Banana Tomato Lemon
Natural forest (NF)	Mature forest (30 yr.), mixture of different trees, protected	None	None	*Acacia nilotica* (L.) Delile. *Ficus vasta* Forssk. *Prunus africana* (Hook.f.) kalkman	Acacia Fig tree Red Stinkwood
Acacia Plantation (AP)	Community managed acacia dominated forest, not protected	None	None	*Acacia seyal* Del. *Acacia nilotica* (L.) Delile.	Acacia Acacia

Soil physical and chemical characteristics

Soil particle size of samples from all land use types was determined using the hydrometer method (Gee and Bauder, 1986). Soil organic carbon (OC) was determined by the Walkley-Black dichromate oxidation procedure (Walkley and Black, 1934). Total nitrogen (TN) was determined by the Kjeldahl method (Hinds and Lowe, 1980). Soil available phosphorus was measured according to the method described by Olsen *et al.* (1954). The soil sample analyses were done at the Addis Ababa City Administration Environmental Protection Authority Laboratory. Soil pH was measured at the Department of Microbial Cellular and Molecular Biology, Addis Ababa University, following 1:2.5 (v/v) soil: water suspension with a digital pH meter (HD8602) (**Table 3. 2**).

Table 3. 2: Physical and chemical properties of soil samples from seven land use types at Showa robit, Ethiopia.

Land use type	pH	P (ppm)	T.N %	O.C %	Sand %	Clay %	Silt %	Texture class
Arable1	7.4	9.42	0.1	1.6	56	17	27	Sandy loam
Arable2	7.3	11.92	0.1	1.9	45	27	28	Clay loam
Arable3	7.4	5.98	0.2	1.6	52	22	26	Sandy clay loam
Arable 4	7.3	11.02	0.1	1.7	52	20	28	Loam
Fruit cropping(FC)	7.8	7.5	0.3	2.5	55	16	29	sandy loam
Natural forest (NF)	7.7	34.7	0.26	1.1	61	7	32	Sandy loam
Acacia plantation (AP)	7.9	5.4	0.1	1.3	50	25	25	Sandy loam

P: available phosphorus; T.N: Total nitrogen; O.C: organic carbon; Arable1: Low -input mixed cropping; Arable2: low-input monocropping, sorghum; Arable3: low-input monocropping, maize; Arable4: high-input monocropping, sorghum.

Establishment of trap cultures

Trap cultures in pots were set up in triplicates for all seven land use types in a greenhouse to obtain fresh spores for identification of AM fungi and induce sporulation of species present only as hyphae in field samples. Pots with the size of

15cm were filled with 250 g of soil sub-sample containing root sections from each plot of the field and were thoroughly homogenized with sterile sand (1:1; v/v) according to Morton *et al.* (1993). Maize (*Zea mays* L.), a mycotrophic crop, was selected as an appropriate trap plant for its ability to induce high spore density, diversity and species richness (Yao *et al.*, 2010).

Seeds of maize were surface sterilized by immersing them in a 0.5% sodium hypochlorite solution for 15 minutes. After washing the seeds with sterile water they were sown at 2 cm depth in each plastic pot and covered with sterilized sand. Pots were irrigated daily as needed. No fertilizer was added during the growing period. All seedlings were grown in the greenhouse under natural ambient light and temperature conditions (about 29 °C day/18°C night).

The maize roots were checked for AMF colonization after 45 days. Pots supporting successful mycorrhization were maintained for six months. Watering was reduced during the final three weeks to maximize spore production. At the end of 6 months the plants were cut near the base, and the cultures were air-dried and checked for the presence and identification of spores.

Estimation of mycorrhizal inoculum potential (MIP)

MIP (Mycorrhizal Inoculum Potential) of the different land use types was assessed in a greenhouse bioassay according to Sieverding (1991). A 300g soil sample from each land use type was placed in 450-ml sterile plastic pots. Five seeds of maize were sown per pot, and the seedlings were thinned down to three per pot after emergence. The pots were arranged in a completely randomized design with three replicates. After

five weeks, the trap plants were harvested and their roots were cut into 1-cm sections and stained according to Brundrett *et al.* (1996). The percentage of colonized roots was quantified using the magnified intersection method (McGonigle *et al.*, 1990).

Staining of mycorrhizal roots

Staining of mycorrhizal roots was made according to Brundrett *et al.*, (1996). The root samples were carefully washed several times with tap water. About 0.5 g of root segments were cleared in 10 % (w/v) KOH at 90^0C in a water-bath for 2h, after which they were bleached with alkaline hydrogen peroxide at 10% for 3 minutes at room temperature. The roots were then treated with 1% HCl (v/v) for 15-20 minutes at room temperature and finally stained with 0.05% w/v trypan blue in lactoglycerol (1:1:1; lactic acid, glycerol and water) at 90^0C for 30 minutes in a water-bath.

With the exception of the HCl treatment, samples were drained and washed thoroughly with distilled water at the end of every step. The root samples were then left overnight in the lactoglycerol destaining solution (1:1:1; lactic acid, glycerol and water) in a dark room to remove coloration from root cells. Finally, roots were mounted in PVLG mountant on microscopic slides and covered with 40×22 mm coverslips.

Quantification of AMF root colonization

AM colonization was assessed from cleared and stained roots according to McGonigle *et al.* (1990). A total of 100 intersections were taken for each subsample to estimate percent AM root colonization under a compound microscope (OLYMPUS-BX51) at a magnification of ×200.

Identification and characterization of spores

The AMF spores were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia and Natural Resources Institute Finland (Luke), Laukaa, Finland. About 50-70% of healthy looking spores were picked up with forceps and mounted on slides in polyvinyl-lactic acid-glycerol (PVLG), (Omar *et al.,* 1979) or in PVLG mixed with Melzer's reagent (1:1 v/v) (Morton, 1991). Spores were examined under a compound microscope (OLYMPUS-BX51) at a magnification of ×400 and identified to the species level or to a specific morphotype based on (Schenck and Perez, 1990), online references of species description INVAM *http://invam.caf.wvu.edu,* West Virginia University, USA ,http://www.zor.zut.edu.pl/Glomermycota/, University of Agriculture in Szczecin, Poland ,Schüßler and Walker (2010) and the Schüßler AMF phylogeny website http://www.lrz.de/~schuessler/amphylo/.

Determination of AMF diversity and spore density

The AMF communities on different land use types were detected and calculated based on the following parameters: Spore density (SD) was expressed as the number of AMF spores g^{-1}soil. Species richness (S) was measured as the total number of morphospecies. The Shannon–Wiener index (H') of diversity was calculated using the formula: $H' = -\sum ((n_i/n) \ln (n_i/n))$ where: n_i = number of individuals of species i and n = number of all individuals of all species. The Simpson's dominance index (D) was calculated using the formula $D = \sum (n_i/n)^2$; Evenness (E) was calculated by dividing Shannon–Wiener diversity value by the logarithm of the species richness. These analyses were conducted using the software PAST3 (ver. 3.0).

Isolation frequency (IF) was calculated as (the number of samples in which a given species was isolated/ the total number of samples) ×100%. Relative abundance of spores (RA) was calculated as (the number of spores in a given species / total number of spores) ×100%. The importance value (IV) was used to evaluate the dominance of AMF species based on IF and RA and was calculated as IV = (IF + RA)/2. An IV ≥ 50% indicates that a genus or species is dominant; 10% < IV < 50% applies to common genera or species; an IV ≤ 10% indicates that a genus or species is rare (Chen *et al.*, 2012).

Statistical analysis

Spore abundance data were log(x) transformed and the proportion of root colonization values were arcsine (the inverse sine of the square root of the proportion) transformed prior to analysis to meet assumptions of ANOVA such as normality and homogeneity of variance, but values were expressed as number of spores g^{-1}soil and percentage of root colonization, respectively. ANOVA and correlation analyses were carried out with the SPSS software package (version 21.0).

Significance of differences in AM fungal spore abundance and inoculum potential was tested using Fisher's least significant difference (LSD) at $p < 0.05$ after one-way ANOVA. The relationships between AMF parameters and soil chemical properties (pH, OC, available P, and TN) were determined using Pearson's correlation analysis. The same statistical tests were applied for initial mycorrhizal root colonization and spore formation for the trap cultures inoculated soils from the different sites.

RESULT

AMF spore abundance in the soil and in trap cultures

The AMF spore densities of the different land use types recovered directly from the soil and trap cultures are shown in (**Table 3. 3**). Spore densities in trap cultures were up to twice as high as those recovered from soil. The data also showed differences in the spore counts between soil and trap culture. Accordingly, FC (fruit cropping) and Arable3 land use types showed the highest spore count of 6.1 spores g^{-1} soil from field soil whereas FC and AP displayed the highest spore count of more than 11 spores g^{-1} soil in trap cultures. In general, NF (2.5) and Arable4 (3.8) showed the lowest number of spores per gram of soil in trap culture.

Spore density correlated significantly with species richness and VC%, ($r=0.84$, $P<0.05$; $r=0.94$, $P<0.01$, respectively). Species richness correlated significantly with TN% and O.C %,($r=0.79$, $P<0.05$ and $r=0.76$, $P<0.05$, respectively).

Table 3. 3 AMF spore abundance in soil and trap cultures of the different land use types in a humid lowland sampling area at Showa robit, Ethiopia

Land use type	N	AMF spores g^{-1} of soil	
		Field soil	Trap culture
Arable1	9	5.8 ± 0.8c	7.2 ± 1.7bc
Arable2	3	5.5 ± 1.5bc	9.8± 2c
Arable3	3	6.1 ± 1.4c	6.6± 1.4bc
Arable4	3	3.9 ± 0.5ab	3.8± 0.1ab
Fruit cropping (FC)	21	6.1 ± 0.7c	11.4± 1.4c
Natural forest (NF)	9	3.5 ± 0.2ab	2.5 ± 0.2a
Acacia plantation (AP)	6	2.8 ± 0.5a	11.1± 0.7c

Data are reported as averages and standard errors for the three replicates per land use type. Values followed by different letters denote significant differences between land use types according to Fisher's LSD test at the 5% level after a one-way ANOVA. N: number of replicates; Arable1: Low-input mixed cropping; Arable2: low-input monocropping, sorghum; Arable3: low-input monocropping, maize; Arable4: high-input monocropping, sorghum.

Mycorrhizal inoculum potential

The AM fungal colonization patterns within the roots of maize plants showed that there was considerable heterogeneity between the land use types (**Table 3. 4**). Root colonization occurred with typical structures (arbuscules, vesicles and hyphae) in almost all land use types except in Arable2, where vesicles were not observed.

The highest hyphal colonization of 53.7% and 52.6% were recorded from low-input mixed cropping (Arable1) and fruit cropping systems (FC), respectively, compared with the other land use types that showed mycorrhization rate ranging from 19.9% to 25.8% ($P=0.011$).

Table 3. 4. Percentage of mycorrhizal root colonization in maize after five weeks of growth in soil from seven land use types at Showa robit, Ethiopia.

Land use type	AM colonization (%)		
	Arbuscular Colonization	Vesicular Colonization	Hyphal Colonization
Arable1	8.6 ±3ab	4.1 ±0.6ab	53.7±10.5c
Arable2	2 ± 1.5a	0	21±7.5ab
Arable3	7.4 ±0.3ab	0.9±0.5ab	36±8abc
Arable4	3.3 ±0.1ab	1.2±0.2ab	19.9±1.2ab
Fruit crops	12.6 ± 1.8b	7.5±1.4b	52.6±5.8c
Natural forest	2.6 ± 0.8a	2.2±0.9a	25.8±7.7ab
Acacia plantation	5.5± 1.7ab	4.7±2.1ab	30.2±2.1abc

Data are reported as averages and standard errors for three replicates per land use types. Values followed by different letters denote significant differences among land use types according to Fisher's LSD test at the 5% level after a one-way ANOVA. Arable1: Low-input mixed cropping; Arable2: low-input monocropping, sorghum; Arable3: low-input monocropping, maize; Arable4: high-input monocropping, sorghum.

AMF community composition

A total of 42 and 33 AMF morphospecies, were identified from field soil and trap culture, respectively (**Fig. 3. 2**). Four morphospecies, from *Glomus, Acaulospora* and *Gigaspora,* from field soil samples and five morphospecies, from *Glomus, Acaulospora, Gigaspora* and *Ambispora* were unidentified (data not shown). With a few exceptions of Arable 2, 3, and 4 land use types, field soil revealed more species than trap culture, and mixed cropping (Arable1), fruit cropping (FC), and natural forest (NF) and acacia plantation (AP) harbored more species than did monocrops (Arable2, Arable 3, and Arable 4).

A total of fourteen species, from *Acaulospora, Funneliformis, Glomus, Scutellospora, Claroideoglomus* and *Gigaspora* were detected in field soil samples but not from trap

cultures; whereas four species from *Sclerocystis*, *Racocetra*, *Gigaspora* and *Ambispora* were identified from trap cultures but not detected from soil samples (data not shown).

The genera *Glomus* and *Acaulospora* were the most diversified group represented by the highest number of morpho-species (9 species each), followed by the genera *Funneliformis* and *Gigaspora* (**Table 3. 5**). More species from the dominant genera *Glomus, Funneliformis,* and *Acaulospora* were retrieved from field soil than from trap cultures, but the other genera showed no significant difference in their distribution between the two methods.

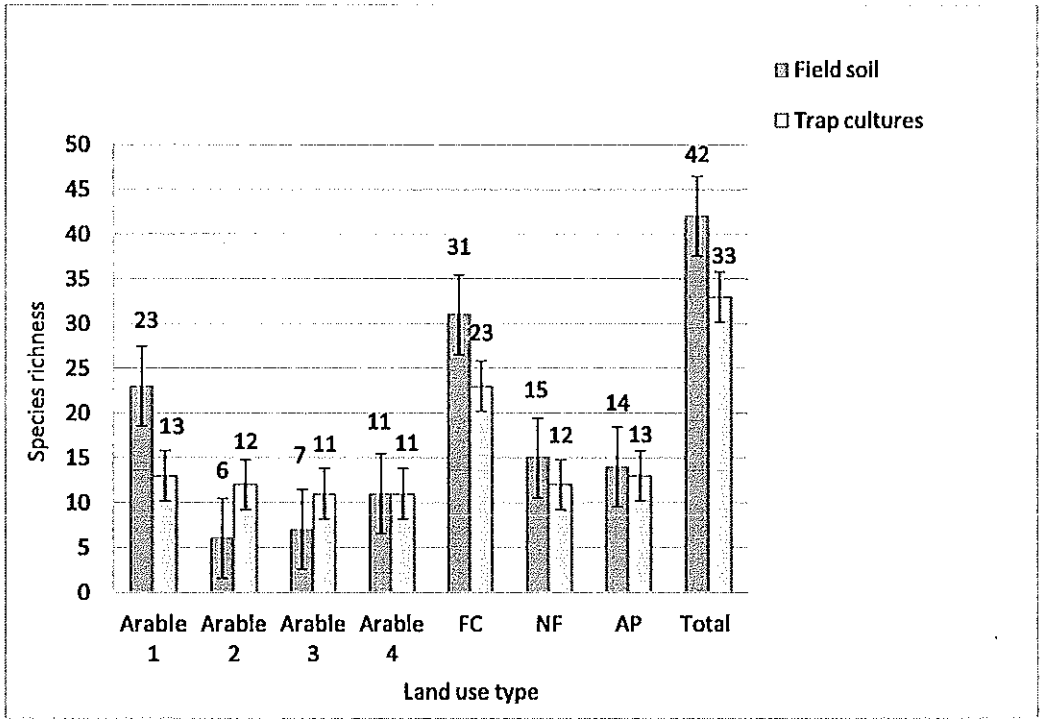

Fig. 3. 2 AMF species richness (numbers on top of bars) in field soil and trap cultures for different land use types at Showa robit, Ethiopia. Vertical bars indicate ± standard errors of means, N= 3. Arable1: Low-input mixed cropping; Arable2: low-input monocropping, sorghum; Arable3: low-input monocropping, maize; Arable4: high-input monocropping, sorghum; FC: fruit cropping; NF: natural forest; AP: acacia plantation.

Isolation frequency, relative abundance and dominant AMF species

The Isolation frequency (IF) and relative abundance (RA) of AMF species varied greatly among land use types **(Table 3. 5)**. The genera *Claroideoglomus* (all species), *Funneliformis* (*F. mossae*, and *F. geosporum*), *Paraglomus* (*P. occultum*), *Rhizophagus* (*R. diaphanus*) were distributed across all land use types. The genera *Entrophospora* and *Ambispora* were recovered only in two of the land use types. *Gigaspora* species were more limited to arable lands (Arable1, 2, 3 & 4) both in field and trap culture soils. Although the genus *Acaulospora* included a relatively large number of species, their distribution was more limited to perennial cropping systems (FC, NF, and AP).

On the basis of IV (important values), the genera *Claroideoglomus* and *Funneliformis* were categorized into the dominant genera with IV 63 and 56, respectively (Chen *et al.*, 2012). However, the different species under the different AMF genera were categorized into "common" and "Rare" groups with $10<IV< 50$ and $IV\leq 10$), respectively.

Table 3. 5: Community structure, isolation frequency (IF), relative abundance (RA) and importance values (IV) of AMF species in soil and trap culture on different land use types from Showa robit, Ethiopia.

AMF genera	No of species	IF (%)	RA (%)	IV (%)	Status	AMF species	IF (%)	RA (%)	IV (%)	Status	Occurrence in land use types
Claroideoglomus	4	92.9	34.7	63.8	Dominant	*Cl. claroideum*	80.5	17.5	49	Common	All
						Cl. etunicatum	61.5	11	36.2	Common	All
						Cl. luteum	58	7.5	32.8	Common	All
Funneliformis	6	91.3	21.6	56.4	Dominant	*Funneliformis mosseae*	71.5	10.5	41	Common	All
						*F. caledonium**	49	9.7	29.4	Common	All except AP
						F. geosporum	47	5.9	26.5	Common	All
Glomus	9	80.2	16.8	48.5	Common	*Glomus sp2*	42.5	7.2	24.9	Common	All except A3 &A4
						G. aggregatum	45	5.2	25.1	Common	All except A4
Paraglomus	*1*	27.3	4.06	15.7	Common	*Paraglomus occultum*	27.5	4	15.7	Common	All
Rhizophagus	*2*	34	3.8	18.9	Common	*Rhizophagus diaphanus*	30.8	3.4	17.1	Common	All
Acaulospora	*9*	21	2.8	11.6	Common	*A. scrobiculata*	6.8	0.7	3.7	Rare	A1, FC, NF, AP
Gigaspora	*5*	35	6.5	20.8	common	*Gigaspora gigantea*	25	4.8	14.9		A1, A2,A3,A4,
Diversispora	*1*	6.8	0.87	3.83	Rare	*Diversipora epigaea*	6.9	0.8	3.8	Rare	A1, FC, NF, AP
Septoglomus	*1*	16.5	3	9.7	Rare	*Septoglomus constrictum*	16.6	3	9.8	Rare	A1, FC, NF, AP
Pacispora	*1*	7.7	0.8	4.3	Rare	*Pacispora scintillans*	8	0.8	4.4	Rare	A1, FC, NF, AP
*Scutellospora**	*2*	12.7	2.8	7.75	Rare	*S. pellucida**	10.3	1.7	6	Rare	A1, NF, AP
Racocetra	*2*	8.3	1.5	4.9	Rare	*Racocetra gregaria*	3.6	0.5	2	Rare	A4, FC, NF
*Sclerocystis***	*1*	15.6	2	8.8	Rare	*Sclerocystis sinuosa***	17.2	2	9.6	Rare	A1, FC, NF
Entrophospora	*1*	8	0.6	4.4	Rare	*E. nevadensis*	8.4	0.65	4.5	Rare	A2, FC
*Ambispora **	*2*	12.9	1.4	7.15	Rare	*Ambispora fennica***	11	1.3	6.15	Rare	A3, FC

*Only in field soil; **only in trap culture

AMF species richness and diversity across land use types

AMF species richness varied among different land use types (**Fig. 3. 2**). It ranged from 6-31 species in field soil samples and from 11-23 species in trap culture, respectively. Species richness was the highest in FC (31) followed by Arable1 (23), NF (15), AP (14) and the others (6-11) in the field soil. Likewise, the greatest species richness from trap cultures was observed in FC (23) but the others were not significantly different from one another (13-11). On average, the species richness recorded in FC, Arable1, and NF in field soil was five, four and three times greater than in the monocropped arable fields Arable2 and Arable3.

AMF diversity, expressed by the Shannon-Wiener diversity index also varied among different land use types (**Table 3. 6**). Values for Shannon–Wiener diversity index, species dominance and species evenness were 2-2.64, 0.1-0.16 and 0.53-0.88, respectively. The highest Shannon-Wiener diversity, the lowest dominance and the lowest evenness were recorded from fruit crops, whereas the lowest diversity index, the highest dominance and the highest evenness were recorded from the monocropped fields (Arable 2, 3 & 4). In general, AMF diversity was lower in the trap cultures than in the field samples, whereas there was no significant difference in species dominance (Simpson's index) between the soil and the trap culture samples (**Table 3. 6**).

Table 3. 6. Diversity indices of AMF community in different land use types of Showa robit, Ethiopia.

	Shannon_H			Dominance_D			Evenness_e^H/S		
Land use type	Field soil	Trap culture	Mean	Field soil	Trap culture	Mean	Field soil	Trap culture	Mean
Arable1	2.54	2.27	2.41	0.12	0.13	0.12	0.55	0.74	0.65
Arable2	1.74	2.28	2.01	0.19	0.12	0.15	0.94	0.81	0.88
Arable3	1.8	2.19	2	0.18	0.14	0.16	0.87	0.81	0.84
Arable4	2.25	2.14	2.2	0.13	0.15	0.14	0.86	0.77	0.82
Fruit cropping	2.81	2.46	2.64	0.09	0.11	0.1	0.54	0.51	0.53
Natural forest	2.61	2.1	2.36	0.08	0.15	0.11	0.91	0.68	0.8
Acacia plantation	2.38	2.21	2.3	0.11	0.13	0.12	0.77	0.7	0.74

Arable1: low-input mixed cropping; Arable2: low-input monocropping, sorghum; Arable3: low-input monocropping, maize; Arable4: high-input monocropping, sorghum.

DISCUSSION

Spore abundance

AMF species diversity and spore abundance were studied in soil from different land use types in a low-land (sub-moist warm) agro-ecosystem, Ethiopia. The spore densities recovered through direct count from soils of all land use types varied between 2.8 spores g $^{-1}$ and 6.1 spores g^{-1} of soil (**Table 3. 3**). Trap cultures established from the same land use types showed higher spore numbers, 2.5-11.4 spores g^{-1} trap culture soil. The numbers of spores recovered from fruit crops and acacia plantation were 2-4 times higher in the trap cultures than when counting directly from the soil. In general, trap culturing enhanced spore abundance but decreased AMF species richness. The monocropped land use types, where the number of species recovered in the trap culture were higher or similar, were an exception from this main rule.

There was also an inverse relation between soil P content and spore density in the different land use types. The highest spore numbers in both field and trap cultures were obtained from FC associated with low P content and the lowest from natural forest and arable 4 characterized by high soil P content. Similarly, other studies in Finnish and Swedish soil showed negative relationship between spore density and P content. This indicates that certain AMF species are induced to sporulate abundantly under low P availability in the soil (Mårtensson and Carlgren, 1994; Kahiluoto *et al.*, 2001).

Mycorrhizal inoculum potential

The MIP bioassay showed that fruit cropping (FC) and mixed cropping in Arable1 were colonized by higher percentage of AMF than the relatively high-input sorghum monocropped field (Arable4), low-input sorghum monocropped field (Arable2) and natural forest (NF) (**Table 3. 4**). In general, there was a slight, but not significant positive correlation between spore density and hyphal colonization both in the soil and trap culture. However, vesicular colonization was strongly correlated with spore density ($r=0.94$, $P<0.01$). This result is consistent with studies from southeast Spain (Azcón-Aguilar *et al.*, 2003) where it was found that the numbers of spores of AM fungal species are the propagule sources which were best correlated with the total mycorrhizal potential in the rhizosphere of the target plant species from Mediterranean shrublands.

The highest values of MIP in low-input mixed cropping and organically managed fruit cropping can be related to higher plant species diversity compared to the monocrops indicating that AMF colonized roots of different plants species are the major sources

of propagules that would result in higher MIP values. Several studies also show higher levels of AMF root colonization under organic management and low input mixed cropping system than in monocropping with maize and other crops (Gosling *et al.,* 2010; Verbruggen *et al.,* 2010; Bedini *et al.,* 2013). However, contrary to this result, Purin *et al.* (2006) obtained no differences in MIP values between conventional and organic apple orchards in Brazil. A study conducted among different cropping systems and land use types in Kenya showed a significantly higher AMF inoculum potential in maize-bean intercropping systems than in maize or wheat monocrops in both dry and wet regions (Muchane *et al.,* 2012).

AMF community composition

A total of 42 and 33 AMF morphospecies belonging to 15 genera and 8 families were identified from soil and trap cultures, respectively (**Fig.3. 2**). This result is quite similar to a study of different cropping systems in Sudan (Abdelhalim *et al.*, 2012), in which 42 AMF species belonging to 12 genera in 8 families were discovered. The genera *Glomus, Funneliformis, Septoglomus, Claroideoglomus, Entrophospora, Acaulospora, Paraglomus, Diversispora, Pacispora,* and *Ambispora* were commonly detected in both studies. However, the genera *Gigaspora, Rhizophagus, Racocetra, Sclerocystis,* and *Scutellospora* were not identified from Sudan, and the genera *Archaeospora* and *Kuklospora,* were not detected in this study.

The AMF species diversity observed in this study was much higher than the 17 species identified in *Acaulosporaceae* (5), *Glomeraceae* (4), *Gigasporaceae* (5) and others (3) from different land use types in Kenya (Jewfa *et al.*, 2009). This may be related to the diversity and the type of plants sampled from the land use types. Other

studies have also showed that coexisting plant species within a habitat are associated with divergent AMF communities, showing that host preference has a strong influence on AMF community composition in soil (Vandenkoornhuyse *et al.*, 2002; Scheublin *et al.*, 2004).

Although trap culturing enhanced spore abundance it reduced AMF species richness compared with the field soil samples. Similarly, Chaturvedi *et al.* (2012) found that AMF diversity in trap cultures of one year was decreased from 50 to 21, although spore abundance was higher. Tchabi *et al.* (2008) reported that out of a total of 59 AMF species detected in soils of different ecological zones of West Africa, only seven had sporulated after 10 and 24 months of trap culturing.

Isolation frequency, relative abundance and dominant AMF species

Claroideoglomus and *Funneliformis* were dominant genera according to Chen *et al.* (2012), because they were found in all land use types. The genera *Glomus*, *Paraglomus, Rhizophagus*, *Acaulospora* and *Gigaspora* were categorized as common. It is interesting to note that more than 50% of the genera were classified as rare. Previous reports have also shown that *Glomus* was dominant in other agroecological regions of Ethiopia (Muleta *et al.*, 2008; Birhane *et al.*, 2010).

The genera *Glomus, Funneliformis,* and *Claroideoglomus* were also reported to be dominant in Cameroon (Snoeck *et al.*, 2010) and other sub-Saharan regions, in North Côte d'Ivoire (Nandjui *et al.*, 2013), in different land use types of Kenya (Jefwa *et al.*, 2009, 2012), in the Namibia desert (Stutz *et al.*, 2000), in natural and cultivated savannas of Benin, West Africa (Tchabi *et al.*, 2008), in selected crops in the White

Nile State, Central Sudan (Abdelhalim *et al.,* 2013) and in temperate agroecosystems in Europe (Oehl *et al.,* 2003). The high incidence of *Glomus* and *Funneliformis* spp. has been associated with their capacity to produce more spores in a shorter time than genera such as *Gigaspora* and *Scutellospora* (Bever *et al.*, 1996: Oehl *et al.,* 2009). These species could therefore, be selected for future studies as AMF inocula after testing their compatibility with different crops and checking their persistence in the field.

AMF species richness and diversity

The number of AMF morphospecies recovered from the fruit cropping system and the mixed cropping system (Arable1) was almost double the number of morphospecies collected from each of the other land use types from monocrop fields (Arable 2, Arable 3, and arable 4) and woody vegetation (natural forest, and acacia plantation) (**Fig.3. 2**). In general, lower AMF species diversity was recorded in high–input (Arable 4) and low-input monocropped fields compared to organically managed fruit crops or low-input mixed cropping (Arable1).

Our study also showed that the AMF species diversity (23 species) of the mixed cropping system (Arable 1) was much higher than the 12 AMF species reported from similar maize and sesbania intercrops from Southern Malawi (Jefwa *et al.,* 2006). However, the AMF diversity of monocrops (Arable 2 and Arable 3) was almost similar to the 12 AMF species collected from indigenous forest to croplands in Southern Kenya (Jefwa *et al.,* 2012) and from maize monocrops in Southern Malawi (Jefwa *et al.,* 2006). Similar pattern of AMF diversity (15-17morphospecies) was also reported from crop land, fallow land, natural forest and tree plantations in the high

altitude regions of Kenya (Jefwa *et al.,* 2009) and from grassland, woodland and intensified monocropping systems in Maasai Mara ecosystems in Kenya (Muchane *et al.,* 2012).

This study showed no clear impact of soil P on the diversity of AM fungi. Accordingly, Gosling *et al.* (2013) suggested that host species is more important than soil P for determining AM diversity, except at the highest P concentration. We observed a strong positive correlation between AMF species richness and spore density ($r=0.84$, $P<0.05$). AMF species richness also correlated strongly positively with soil organic carbon and total nitrogen both in field soil and trap cultures ($P<0.05$). Other studies have also shown that spore density and species richness are usually positively correlated with soil organic carbon contents and soil pH (Tchabi *et al.,* 2008).

AMF diversity indices

The highest diversity index value was recorded from the FC land use type both in field soil and trap cultures. This result is similar to previous reports which show that organic systems have higher AMF community diversity indices than conventional or monocropping systems (Helgason *et al.,* 1998; Oehl *et al.,* 2003; Verbruggen *et al.,* 2010). It has been suggested that conventional farming systems may select for a small set of generalist AMF species (Helgason *et al.,* 2007; Verbruggen *et al.,* 2010), while organic farming systems are characterized by high species diversity (Mäder *et al.,* 2002).

CONCLUSION AND RECOMMENDATION

This study showed high AM fungal diversity, but also high variation in AM fungal community composition among seven land use types in the humid and semi-arid soil of Showa robit, Ethiopia. The difference in AMF community structure was most closely related to a variety of biotic and abiotic factors, including various aspects of agricultural management practice and land use. Markedly higher numbers of AMF species and higher rate of mycorrhizal infectivity potential were obtained in FC and Arable1 compared with the other land use types. This clearly implies that organic farming and diversification of crops in agriculture is a more sustainable land use system for enhancing biological soil qualities, including maintenance of AMF diversity, than highly fertilized monocropping systems.

The study also showed that *Claroideoglomus* and *Funneliformis* were the dominant genera in all land use types in both trap culture and field soil. It also shown that some AMF species could be missed when studying either soil or trap cultures implying that there is a need to use both methods for getting a full picture of the AMF species diversity in a study area. Future studies should be focused on the dominant species for further selection of AMF inocula for enhancing productivity in different cropping system.

In this study, the AM fungal diversity and community composition analyses relied on an assessment of spore morphotypes for identification. If it is complemented with molecular identification of AMF species directly from plant roots it can fully show the heterogeneity of the organisms in relation to land use types. Except the AMF genera and species that dominated all land use types and plants, the occurrence of

some rare species in specific land use types should also be studied to fully realize their role in nutrient and water uptake and protection against plant pathogens and in improvement of crop productivity.

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REFRENCES

Abdelhalim, T. S., Finckh, M. R., Babiker, A. G. and Oehl, F. (2013). Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. *Arch. Ag. Soil Sci.* 60:377-391.

Azcón-Aguilar, C. and Barea, J. M. (1997). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – a n overview of the mechanisms involved. *Mycorrhiza.* 6:457 – 464.

Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R. and Barea, J. M. (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Appl. Soil Ecol.* 22:29-37.

Bedini, S., Avio, L., Sbrana, C., Turrini, A., Migliorini, P., Vazzana, C. and Giovannetti, M. (2013). Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. *Biol. Fertil. Soils.* 49:781-790.

Bever, J. D., Morton, J. B., Antonovics, J. and Schultz, P. A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in mown grassland. *The J. Ecol.* 84:71-82.

Birhane, E., Kuyper, T. W., Sterck, F. J. and Bongers, F. (2010). Arbuscular mycorrhizal associations in *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *Forest Ecol.Manag.* 260:2160-2169.

Bossio, D. A., Girvan, M. S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K. M., Ball, A. S., Pretty, J. N. and Osborn, A. M. (2005). Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microb. Ecol.* 49:50-62.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture.* ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra.

Burrows, R. L. and Pfleger, F. L. (2002). Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Can. J. Bot.* 80:120-130.

Chaturvedi, S., Tewari, V., Sharma, S., Oehl, F., Wiemken, A., Prakash, A. and Sharma, A. K. (2012). Diversity of arbuscular mycorrhizal fungi in oak-pine forests and agricultural land prevalent in the Kumaon Himalayan hills, Uttarakhand, India. *Br. Microbiol. Res. J.* 2:82-96.

Chen, K., Liu, W., Guo, S., Liu, R. and Li, M. (2012). Diversity of arbuscular mycorrhizal fungi in continuous cropping soils used for pepper production. *Afri J Micro Res.* 6:2469 – 2474.

Chifflot, V., Rivest, D., Olivier, A., Cogliastro, A. and Khasa, D. (2009). Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems. *Agricult. Ecosyst. Environ.* 131:32-39.

Douds, D. D., Janke, R. R., and Peters, S. E. (1993). VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. *Agricult. Ecosyst.. Environ.* 43:325-335.

Estaún, V., Savé, R. and Biel, C. (1997). AM inoculation as a biological tool to improve plant revegetation of a disturbed soil with *Rosmarinus officinalis* under semi-arid conditions. *Appl. Soil Ecol.* 6:223-229.

FAO. (2007). State of the World's Forests Report.

Garedew, E., Sandewall, M., Söderberg, U. and Campbell, B. M. (2009). Land-use and land-cover dynamics in the central Rift Valley of Ethiopia. *Environ. Manag.* 44:683-694.

Gee, G. W., and Bauder, J. W. (1986). Particle size analysis. In: *Method of soil analysis, part 1: Physical and mineralogical methods,* (Klute A. ed.). Soil Science Society of America, Madison, Wisconsin USA. 383- 411.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46:235-244.

Getachew, F., Abdulkadir, A., Lemenih, M. and Fetene, A. (2012). Effects of different land uses on soil physical and chemical properties in Wondo Genet area, Ethiopia. *New York Sci. J.* 5:110-118.

Girmay, G., Singh, B. R., Mitiku, H., Borresen T. and Lal, R. (2008). Carbon stocks in Ethiopian soils in relation to land use and soil management. *Land Degrad. Dev.* 19:351-367.

Gosling, P., Mead, A., Proctor, M., Hammond, J. P. and Bending, G. D. (2013). Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. *New Phytol.* 198:546-556.

Gosling, P., Ozaki, A., Jones, J., Turner, M., Rayns, F. and Bending, G. D. (2010). Organic management of agricultural systems results in a rapid increase in colonization potential and spore populations of arbuscular mycorrhizal fungi. *Agricult. Ecosys. Environ.* 139:273-279.

Hartemink, A. E., Veldkamp, T. and Bai, Z. (2008). Land cover change and soil fertility decline in tropical regions. *Turk. J. Agric.For.* 32:195-213.

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H. and Young, J. P. W. (1998). Ploughing up the wood-wide web? *Nature.* 394:431.

Helgason, T., Merryweather, J. W., Young, J. P. W. and Fitter, A. H. (2007). Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *J. Ecol.* 95:623 -630.

Hinds, A. and Lowe, L. E. (1980). Ammonium-N determination. Soil nitrogen. Berthelot reaction. *Soil Sci. and Plant Anal.* 11: 469– 475.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I. R. and Frossard, E. (2002). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza.* 12:225-234.

Jefwa, J. M., Mung'atu, J., Okoth, P., Muya, E., Roimen, H. and Njuguini, S. (2009). Influence of land use types on occurrence of arbuscular mycorrhiza fungi in the high altitude regions of Mt. Kenya. *Trop. Subtrop. Agroecosyst.* 11:277-290.

Jefwa, J. M., Okoth, S., Wachira, P., Karanja, N., Kahindi, J., Njuguini, S., Ichami, S., Mung'atu, J., Okoth, P. and Huising, J. (2012). Impact of land use types and farming practices on occurrence of arbuscular mycorrhizal fungi (AMF) Taita-Taveta district in Kenya. *Agricult. Ecosys. Environ.* 157:32-39.

Jefwa, J. M., Sinclair, R. and Maghembe, J. A. (2006). Diversity of glomale mycorrhizal fungi in maize/sesbania intercrops and maize monocrop systems in southern Malawi. *Agroforest. Syst.* 67:107-114.

Johnson, D., Vandenkoornhuyse, P. J., Leake, J. R., Gilbert, L., Booth, R. E., Grime, J. P., Young J. P. W. and Read, D. J. (2003). Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytol.* 161:503-515.

Kahiluoto, H., Ketoja, E., Vestberg, M. and Saarela, I. (2001). Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant and Soil.* 231:65-79.

Lemenih, M., Karltun, E., Olsson, M. 2005. Soil organic matter dynamics after deforestation along a farm field chronosequence in southern highlands of Ethiopia. *Agricult. Ecosys. Environ.* 109:9-19.

Li, L.-F., Li, T. and Zhao, Z.-W. (2007). Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. *Mycorrhiza.* 17:655-665.

Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science.* 296:1694-1697.

Mårtensson, A. M. and Carlgren, K. (1994). Impact of phosphorus fertilization on VAM diaspores in 2 Swedish long-term field experiment. *Agricult. Ecosys.Environ.*. 47:327-334.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.*. 115:495-501.

Morton, J. B. (1991). INVAM newsletters. vol 1-5. West Virginia University, Morgantown.

Morton, J. B., Bentivenga, S. P. and Wheeler, W. W. (1993). Germ plasm in the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon.* 48:491-528.

Muchane, M. N., Muchane, M., Mugoya, C. and Masiga, C. W. (2012). Effect of land use system on arbuscular mycorrhiza fungi in Maasai Mara ecosystem, Kenya. *Afr. J. Microbiol. Res.* 6:3904-3916.

Muleta, D., Assefa, F., Nemomissa, S. and Granhall, U. (2008). Distribution of arbuscular mycorrhizal fungi spores in soil of southwestern Ethiopia. *Biol. Fertil. Soils.* 44:653-659.

Nair, P. K. R. (1993). An introduction to agroforestry. Kluwer Academic Publishers, pp.21

Nandjui, J., Voko, D. R. R., Kouadio, A. N. M.-S., Fotso, B., Tano, Y. and Zeze, A. (2013). Assessment of the occurrence and abundance of mycorrhizal fungal communities in soils from yam (*Dioscorea* spp.) cropping fields in Dabakala, North Côte d'Ivoire. *Afr. J. Agricult. Res.* 8:5572-5584.

NMA (National Metrological Agency, Ethiopia) (2002-2010). Annual climate bulletin for year 2002-2010.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T. and Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Appl. Environ. Microbiol.* 69:2816-2824.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Wiemken, A. and Boller, T. (2009). Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long term microcosms. Agriculture, *Ecosys. Environ.* 134:257-268.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T. and Wiemken, A. (2004). Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia.* 138:574-583.

Omar, M. B., Bolland, L. and Heather, W. A. (1979). PVA (polyvinyl alcohol). A permanent mounting medium for fungi. *Bull. Bri. Mycol. Soc.* 13:31-32.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939.

Purin, S., Filho, O. K. and Stürmer, S. L. (2006). Mycorrhizae activity and diversity in conventional and organic apple orchards from Brazil. *Soil Biol. Biochem.* 38:1831-1839.

Schenck, N. C. and Perez, Y. (1990). Manual for the identification of VA mycorrhizal Fungi, 3rd edn. Synergistic publications, Gainesville, Fla.

Scheublin, T. R., Ridgway, K. P., Young, J. P. W. and van der Heijden, M. G. A. (2004). Non legumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Appl. Environ. Microbiol.* 70:6240-6246.

Schüßler, A. and Walker, C. (2010). The Glomeromycota. A species list with new families and new genera. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Electronic version freely available at www.amf-phylogeny.com.

Sieverding, E. (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems. GTZ, Eschborn and Hartmut Bremer Verlag, Friedland, 365 pp.

Smith, S. E. and Read, D. J. (2008). Mycorrhial symbiosis, 3rd Ed. Elsevier Ltd.

Snoeck, D., Abolo, D and Jagoret, P. (2010). Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. *Agroforest. Syst.* 78:323-328.

Stürmer, S. L. and Siqueira, J. O. (2011). Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. *Mycorrhiza.* 21:255-267.

Stutz, J. C., Copeman, R., Martin, C. A. and Morton, J. B. (2000). Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. *Can. J. Bot.* 78:237-245.

Sýkorová, Z., Wiemken, A. and Redecker, D. (2007). Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. *Appl. Environ. Microbiol.* 73:5426-5434.

Tchabi, A., Coyne, D., Hountondji, F., Lawouin, L., Wiemken, A. and Oehl, F. (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. *Mycorrhiza.* 18:181-195.

Vandenkoornhuyse, P., Husband, R., Daniell, T. J., Watson, I. J., Duck, J. M., Fitter, A. H. and Young, J. P. W. (2002). Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Mol. Ecol.* 11:1555-1564.

Verbruggen, E., Röling, W. F. M., Gamper, H. A., Kowalchuk, G. A., Verhoef, H. A. and van der Heijden, M. G. A. (2010). Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol.* 186:968-979.

Walkley, A. and Black, I. A. (1934). An examination of the Degtareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* 37:29–38.

Wardle, D. A. and Lavelle, P. (1997). Linkages between soil biota, plant litter quality and decomposition. In: *Driven by Nature: Plant Litter Quality and Decomposition*. (Cadisch, G., Giller, K. E, eds.). CAB International, Wallingford, pp. 107-125.

Yamato, M., Ikeda, S. and Iwase, K. (2009). Community of arbuscular mycorrhizal fungi in drought-resistant plants, Moringa spp., in semiarid regions in Madagascar and Uganda. *Mycoscience.* 50:100 – 105.

Yao, Q., Gao, J.-L., Zhu, H.-H., Long, L.-K., Xing, Q.-X. and Chen, J.-Z. (2010). Evaluation of the potential of trap plants to detect arbuscular mycorrhizal fungi using polymerase chain reaction-denaturing gradient gel electrophoresis analysis. *Soil Sci. Plant Nutr.* 56:205-211.

Chapter Four

Mycorrhizal status and AMF community structure of fruit crops from low-input cropping system of Showa Robit

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Mycorrhizal status and AMF community structure of fruit crops from low-input cropping system in Showa Robit, Ethiopia

ABSTRACT

Arbuscular mycorrhizal (AM) fungi association of six fruit plant species [*Mangifera indica* L. (mango), *Musa acuminate* Colla. (banana), *Carica papaya* L. (papaya), *Citrus limon* Risso. (lemon), *Persea americana* Mill. (avocado), and *Psidium guajava* L. (guava)] was investigated from a low land area of Showa Robit. Percentage root colonization, spore abundance, species richness and diversity were examined. The result showed that fruit crops fell into higher spore density group of (7.2-8.8 spores g^{-1} of soil) and low spore density group (3.7-5.3 spores g^{-1} of soil). Accordingly, mango (*Mangifera indica*), avocado (*Persea americana*), banana (*Musa acuminate),* and lemon (*Citrus limon*) were from the high density spore group; whereas, papaya (*Carica papaya*), and guava (*Psidium guajava*) fell into the low spore density group at $P < 0.05$. The AM colonization also showed that mango and lemon have high mycorrhization (71.7%), which guava has a low mycorrhization of 27.3%. A total of 32 morphospecies into 12 genera were characterized from all the fruit crops. The highest AMF species richness was in mango (18 species) followed by banana (16 species) and guava (14 species). The species *Claroideoglomus claroideum* and *Glomus aggregatum* were the dominant species ("generalists") among the fruit trees. A total of 13 AMF species were detected in only one of the tested fruit trees, out of which four species were recovered from mango indicating that the crops are selective to specific mycorrhizal fungi.

Key words: Arbuscular mycorrhizal fungi (AMF); *Glomus*; Root colonization; Spore abundances

INTRODUCTION

Banana, papaya, mango, guava, orange, avocado, and lemon are important fruit crops for both domestic consumption, export markets and industrial processing in Ethiopia. According to Central Statistics Agency (CSA), the total area under fruit trees in the previous years was about 61,973 hectares which was 0.46% of the total land area under cultivation in the country (CSA, 2012/13).

Although the land cover for fruits is small compared to cereals, they are mostly produced in low-input intercropping and agroforestry systems and good sources for cash for many small-scale farmers. The major constraints for less area coverage of these crops is low production due to low soil fertility, low agricultural inputs and increased pressure of pests and diseases (Seyoum *et al.,* 2011; Gaidashova *et al.*, 2012).

The problems of soil fertility and pest infestation disease can be tackled using integrated soil fertility management (ISFM) and integrated pest management (IPM) packaged in cultural, chemical and biological systems in order to enhance production of crops in general, and that of fruits in particular (Lovato *et al.*,1995).

Arbuscular mycorrhizal fungi (AMF) are one of the biological components that can be applied together with other methods that could help to boost production of crops. They are soil organisms known to penetrate deep into the cortical cells of roots and extend their extraradical hyphae outside the root so as to serve as extensions of plant roots system. In so doing they increase the absorptive surface area of the root system for nutrient and water uptake particularly phosphorus (P) (Smith and Read, 2008),

nitrogen (N) and other immobile nutrients (Sawers, *et al.*, 2008). Many studies showed that AMF associations with plants increase shoot and root biomass as well as plant tissue nutrient concentrations, enhance tolerance for drought conditions (Auge', 2001), and increase resistance to soil borne pathogens (Wehner *et al.*, 2009).

Fruit crops are one of the vascular plants that are associated with arbuscular mycorrhizal fungi (AMF) (Khade and Rodrigues, 2009). These crops are known to harbor diverse group of AMF spores and that cover up to 50% of the plant roots (mycorrhization) depending upon the contents of P and other nutrients in the soil (Gaidashova *et al.*, 2012; Abdellhalim *et al.*, 2013). There are also suggestions that different management and land use systems influence the density and diversity of mycorrhiza in the rhizosphere of fruit crops (Alarcón *et al.*, 2012).

A few of the hitherto reports in Ethiopia revealed that species composition and community structure of AMF vary depending upon the type of plants in acacia woodland (Yohannes and Assefa, 2007) and diversity of shed trees in coffee agroforestry system (Muleta *et al.*, 2008; Chanie and Assefa, 2013). However, studies on the role of AMF in fruit crops are very limited.

Recently, the urgent need for low-input organic horticultural plant production and reduction of chemical inputs in the sector necessitated alternative approaches that involve soil microorganisms. These alternatives should take in to consideration the role of AMF a beneficial soil organism, to protect crops from pests and diseases, and boost production.

The objective of this study was to evaluate AMF root colonization, spore density, and AMF community structure of different fruit crops under low input fruit production in Showa robit, Ethiopia.

MATERIALS AND METHODS

Study site

The study site was undertaken in Showa Robit, Amhara Regional State, Ethiopia, located at 09^0 57' (N), and 039^0 51' (E), and an altitude of 1305 m above sea level. The area is a low land or Erteb Kola (sub-moist warm) with an average annual maximum temperature of 32^0C, minimum temperature of 16^0C and precipitation of 968 mm (NMA, 2002-2010). The area is characterized by agroforestry practices such as agrisilvicultural (crops and shrubs/trees) and agropastoral systems (crops and pastures/animals and trees). In the agrisilvicultural annual fruits, vegetables and garden cash crops are intercropped with perennial crops such as banana, papaya, mango, lemon, avocado, guava, tomato, sugar cane and coffee plantations (Nair, 1993). The management practice of the area is low input system, in which manures, compost and crop residues are applied.

Collection of soil and root samples

The sampling was done in November, 2011 during the dry season of the year. Six different fruit crops were selected for the survey. They were *Mangifera indica* L. (mango), *Musa acuminate* Colla. (banana), *Carica papaya* L. (papaya), *Citrus limon* Risso. (lemon), *Persea americana* Mill.(avocado), and *Psidium guajava* L. (guava). Voucher specimens of the crops were brought to the National Herbarium for identification/verification.

Five hundred grams of rhizosphere soil were taken from a depth of 0-30 cm and subsequently pooled into one composite sample per location and plant species. The sampling locations were determined with GPS for the selection of the sampling area. The samples were taken from 10m×10m transects in three replicates of sampling locations from approximately 1sqkm of the agricultural fields.

From each of the three locations three individual fruit trees were randomly selected from which a total of 18 samples were composited and collected in alcohol sterilized plastic containers. They were air dried and stored at room temperature for further analysis. Fine root samples from each individual crop were also collected and stored in 50% alcohol at 4°C for determination of root colonization by AM fungi (Brundrett et al., 1996). The physico-chemical properties of the soil samples are given in **Table 4.1**

Table 4.1 Physical and chemical properties of soil samples from rhizosphere soil of six fruit plants

Fruit species	Common name	pH	P (ppm)	T.N %	O.C %	Sand %	Clay %	Silt %	Texture class
Mangifera indica L.	Mango	7.7	13.84	0.28	2.32	54	14	32	Sandy loam
Musa acuminate Colla.	Banana	7.7	21.61	0.22	2.62	57	12	31	Sandy loam
Carica papaya L.	Papaya	7.7	17.87	0.15	1.36	66	16	18	Sandy loam
Citrus limon Risso.	Lemon	7.6	26.31	0.23	4.69	55	16	29	Sandy loam
Persea americana Mill.	Avocado	7.8	8.98	0.4	4.69	44	38	18	clay
Psidium guajava L.	Guava	7.5	9.64	0.16	1.3	67	10	23	sandy loam

P: available phosphorus; T.N: total nitrogen; O.C: organic carbon; ppm: parts per million

Spore extraction and identification

Soil samples were air-dried before extraction, counting and identification of AM fungal spores. AMF spores from the soil samples were extracted by the wet sieving and

decanting method (Gerdemann and Nicolson, 1963), followed by centrifugation in water and in 50% sucrose solution (Brundrett *et al.,* 1996). Sieves of size of 500, 250 and 50µm were used for the wet sieving procedure.

Spores, spore clusters and sporocarps obtained from 250 and 50 µm sieves were counted and observed by using a dissecting microscope. The spores were then mounted on slides in polyvinyl-lactic acid-glycerol (PVLG) (Omar *et al.,* 1979) or in PVLG mixed with Melzer's reagent (1:1 v/v). Spores were examined under a compound microscope and identified to the species level or attributed to a specific morphotype. The AMF spores present were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia and Natural Resources Institute Finland (Luke), Laukaa, Finland. Identification and classification were based on a current species description and identification manual (Schenck and Perez, 1990), online references of species description INVAM http://invam.caf.wvu.edu, University of Agriculture in Szczecin, Poland http://www.zor.zut.edu.pl/Glomermycota/, Schüßler and Walker (2010) and the Schüßler AMF phylogeny website http://www.lrz.de/~schuessler/amphylo/.

Assessment of AMF root colonization

The stored root samples were washed carefully with tap water and cut into segments about 1 cm long. About 0.5 g of root segments were cleared in 10% (w/v) KOH at 90°C in a water bath for 2 to 3 h depending on the structure of the root and its pigmentation (Brundrett *et al.,* 1996). Dark roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3 min at room temperature.

The roots were treated with 10% HCl (v/v) for 15 to 20 min at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90°C for 30 min in a water bath. With the exception of the HCl treatment, samples were drained and washed thoroughly with distilled water at the end of every action. Fungal colonization was quantified using the magnified intersection method of McGonigle *et al.* (1990) under a compound-light microscope (OLYMPUS-BX51) at a magnification ×200. Accordingly, 150 intersections were observed for each sub-sample. The presence of arbuscular mycorrhizal hyphae, vesicles and arbuscules were recorded.

Determination of spore density and diversity of AMF

Spore density (SD) was expressed as the number of AMF spores g^{-1}soil. Species richness (S) was measured as the total number of morphospecies. The Shannon–Wiener index (H^{l}) of diversity was calculated using the formula: $H^{l} = -\sum ((n_i /n) \ln (n_i/n))$ where: n_i = number of individuals of species i and n = number of all individuals of all species. The dominance index (Ds) was calculated using the formula $D = \sum (n_i/n)^2$; Evenness (E) was calculated by dividing Shannon–Wiener diversity value by the logarithm of the species richnes . These analyses were conducted using the software PAST3 (ver. 3.0).

Isolation frequency (IF) was calculated as (the number of samples in which a given species was present/ the total number of samples) ×100%. Relative abundance of spores (RA) was calculated as (the number of spores in a given species / total number of spores) ×100%. Dominant AMF species were determined by the importance value (IV) based on IF and RA and was calculated as IV = (IF + RA)/2. An IV ≥ 50% indicates

that a genus or species is dominant; 10% < IV < 50% applies to common genera or species; an IV ≤ 10% indicates that a genus or species is rare (Chen *et al.*, 2012).

Statistical analysis

Spore abundance data were log(x) transformed and the proportion of root colonization were square root $[(x+0.5)^{1/2}]$ and arcsine (the inverse sine of the square root of the proportion) transformed prior to analysis to meet assumptions of ANOVA such as normality and homogeneity of variance, but values were expressed as number of spores g^{-1}soil and percentage of root colonization, respectively.

Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 21.0). Significance differences in AM fungal spore abundance and percentage of root colonization between the plants were tested using Fisher's least significant difference (LSD) at $p < 0.05$ after one-way analysis of variance (ANOVA). The relationship between AMF parameters and soil chemical properties (pH, OC, available P, and TN) was determined by Pearson's correlation analysis.

RESULTS AND DISCUSSION

Spore abundance

Spore abundance (density) from the soils of the different fruit crops showed variation between the higher spore density group of (7.2-8.8 spores g^{-1}) and low spore density group (3.7-5.3 spores g^{-1} soil). Accordingly, mango (*Mangifera indica*), avocado (*Persea americana*), banana (*Musa acuminate),* and lemon (*Citrus limon*) were characterized by high density spore group whereas, papaya (*Carica papaya*), and guava (*Psidium guajava*) fell into the low spore density group at $P < 0.05$ (Table 4.2).

Table 4.2 AMF spore abundance (g^{-1} soil) of different fruit crops from different parts of the world.

Fruit crops	Ethiopia (Showa robit)	Sudan (Abdelhalim *et al.*,2013)	Bangladesh (Khanam, 2007)	Brazil (Trindade *et al.*, 2006)
Mango	8.8±0.9c	4	2.8	-
Banana	7.3±1bc	12	-	-
Papaya	3.7±0.1a	-	-	7.8
Lemon	7.2±0.6bc	7	0.7	-
Avocado	7.8±1c	-	-	-
Guava	5.3±0.6ab	-	3.2	-

Data are reported as averages and standard errors for three replicates per plant type. Values followed by different letters denote significant differences among fruit crops according to Fisher's LSD test at the 5% level after a one-way ANOVA.

The pattern of spore densities of the fruits in this study was also compared to other studies in different countries (**Table 4.2**). Although the spore density from the rhizosphere of mango was almost twice and three times more abundant than the ones recorded from Central Sudan (4.0 spores g^{-1} of soil) (Abdellhalim *et al.*, 2013) and Bangladesh (2.8 spores g^{-1} of soil) (Khanam, 2007), the spore density under banana was much less than the spore number recorded from the Sudan Abdellhalim *et al.* (2013). However, the spore density under lemon was similar to the finding of Abdellhalim *et al.* (2013), but ten times higher than spore density recorded from Bangladesh (Khanam, 2007) (Table 4.2).

The spore density recorded from Guava (5.3 spores g^{-1} of soil) was higher than the spore number recorded from Bangladesh (3.2 spores g^{-1} of soil). Even if lemon showed the same number of spore with samples from the Sudan, the spore density of papaya in this study (3.7 spores g^{-1} of soil) was more than twice lower than the spore density recorded from Brazil (7.8 spores g^{-1} of soil) (Trinidade *et. al.*, 2006).

In general, relatively higher spore densities were recorded in this study, except from papaya and guava. Different studies showed that variation in spore density among different fruit crops could be attributed to host preference (Bever *et al.* 1996; Mathimaran *et al.* (2007), and the inconsistency in spore abundance among the same fruit crops in different areas might be related to the difference in their responses to specific environmental factors (Muthukumar and Udaiyan, 2002). Khanam (2007) counted more than 10 times higher spore densities from the same fruits and sampling sites in Bangladesh within three years.

AMF root colonization

The roots of the different fruits were colonized by all AM fungal structures, i.e. arbuscules, vesicles and hyphae with different pattern of percentage of colonization (Fig. 4.1; Table 4. 3). The mean percentage of hyphal colonization across all fruit crops was within the range of 27.3 % and 71.7 %. Hyphal colonization in mango, lemon and avocado roots were 71.7, 71 and 66.3 %, respectively which was significantly higher compared to papaya (54.7 %), banana (46 %) and guava (27.3 %). Similarly, the arbuscule and vesicle colonization of the different crops was (1.3%-10%) and (0.3-20%), respectively.

The data also showed that hyphal colonization in banana, lemon and avocado almost concurred with colonization of arbuscular and vesicular structures, except in mango. This was contrary to the report of Khanam (2007), where no vesicles and arbuscules were detected from the roots of lemon, mango, and guava. The percentage hyphal colonization recorded in this study was higher than the ones recorded for mango (45%),

banana (40%), and lemon (30) in White Nile State, Central Sudan (Abdelhalim *et al.*, 2013), and the percentage colonization of the roots of mango (30%), lemon (23%) but similar with root colonization of guava (27%) from horticultural farm in Bangladesh (Khanam,2007). In general, the high-mycorrhization fruits; mango and lemon displayed more than twice the root colonization of the same fruits reported from the Sudan (Abdellhalim *et al,* 2013) and Bangladesh (Khanam, 2007). It is interesting to note that the pattern of colonization on papaya (54.7%) was similar to the study in India (50 %) (Khade and Rodrigues, 2009) but much higher than in Brazil (31%) (Trinidade *et al*, 2006). Likewise guava (27.3%) in this study showed the same pattern of hyphal colonization reported from Bangladesh (26.7%) (Khanam, 2007). The significant variation observed in the colonization of AM fungi among different fruit plants and within the same species might be due to differences in root structure, climatic and soil factors (Khade and Rodrigues, 2009; Gaidashova *et al.*, 2012) and AMF diversity and species composition (Jansa *et al.*, 2007).

Table 4. 3 Pe rcentage of AM fungal root colonization in six fruit crops at Showa robit, Ethiopia.

	AM colonization (%)		
Plant species	Arbuscular Colonization	Vesicular Colonization	Hyphal Colonization
Mango	1.3±0a	8.2±3ab	71.7±1.7c
Banana	3.7±1ab	0.3±0.1a	46±5.1b
Papaya	1.7±0.5a	2.3±1.3ab	54.7±5.2b
Lemon	10±4b	20±3.6c	71±3c
Avocado	10±3b	15.7±4bc	66.3±0.1c
Guava	2.3±1ab	2.3±1.2ab	27.3±3.1a

Data are reported as averages and standard errors for three replicates per land use types. Values followed by different letters denote significant differences among fruit crops according to Fisher's LSD test at the 5% level after a one-way ANOVA.

In the present study, slight positive correlation (r=0.56) was observed between AMF root colonization and spore density of AM fungi. This is because the two parameters are influenced by many biotic and abiotic factors such as the type of species, plant host and soil nutrients (Stutz and Morton, 1996). The high mycorrhization of fruits indicates that the fruits, in general, induce high levels of infective propagules in the rhizosphere soil. It has been reported that the numbers of infective propagules are positively correlated with root colonization levels (Azcón-Aguilar *et al.*, 2003).

The relationships between the distribution of AMF and soil chemical properties were not significant except that the organic carbon was significantly positively correlated with the percentage of root colonization by arbuscules and vesicles ($r=0.95$, $P<0.01$; $r=0.89$, $P<0.05$, respectively) (data not shown). A similar trend was reported in Brazil by Trindade *et al.* (2006), that showed a positive correlation between AM colonization and organic carbon in papaya plantations ($r= 0.32$, $P < 0.01$).

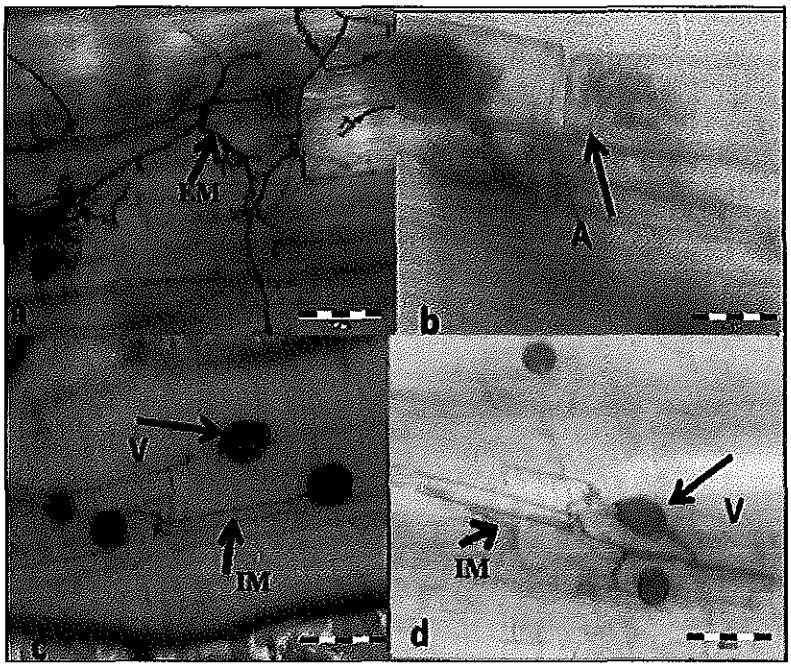


Fig.4.1: Arbuscular mycorrhizal colonization in the roots of fruit crops: (a) network of extraradical mycelium (EM); (b) a typical arbuscule (A) in a cortical cell and (c) & (d) vesicle (V) and intracellular mycelium (IM) in root cell.

AMF species richness and diversity

AMF species richness was comparatively variable between the fruit trees. The highest AMF species richness was in mango (18), banana (16), guava (14) and papaya, lemon and avocado (12) (Fig. 4.2). The number of AM fungal species isolated from banana and lemon was slightly higher than previous investigations for banana (14) and for lemon (10) but similar for mango (18) from White Nile State, Central Sudan (Abdelhalim *et al.*, 2013). This is contrary to the detection of only 1 AMF species from banana and 2 species from lemon grown in the different land use systems of Mexico (Alarcón *et al.*, 2012).

However, the AMF species diversity from avocado and papaya in this study was (2-3 times) lower than the AMF morphotypes isolated from the rhizosphere soil of avocado

(36) in Mexico (Alarcón *et al.*, 2012) and papaya (24) from Brazil (Trindade *et al.*, 2006). On the other hand, the diversity of AMF from papaya (12) is similar to the number of 13 morphotypes identified from India (Khade and Rodrigues, 2009). It is interesting to note that although guava was characterized by low spore density and low root colonization, it harbored diverse species of AMF (14 morpho species) compared to the fruits with higher spore numbers and high mycorrhization such as lemon and avocado. In this study, the number of genera identified from mango, banana, and lemon was similar to the ones reported from the Sudan (Abdelhalm et al, 2007).

A total of 32 species representing 12 genera were detected from field soil samples under the rhizosphere of the fruit crops (Fig.4.2). The dominant genera were *Acaulospora, Funneliformis* and *Glomus* which were diversified into 8 species, 6 species, and 6 species of AMF, respectively. They were followed by the genera *Claroideoglomus* and *Gigaspora* with 3 and 2 representative species, respectively. These genera represented almost 80 % of the species recovered from the fruit crops.

In general, the data showed that only 43% of the species were found in the rhizosphere of the half and more of the fruit crops (**Table 4.4**). The AMF species community distribution based on importance value (IVs) showed that (2 species) *Claroideoglomus claroideum* and *Glomus aggregatum* were distributed in almost all fruits and categorized as dominant species with IVs of (53-60) (Chen *et al.*, 2012).

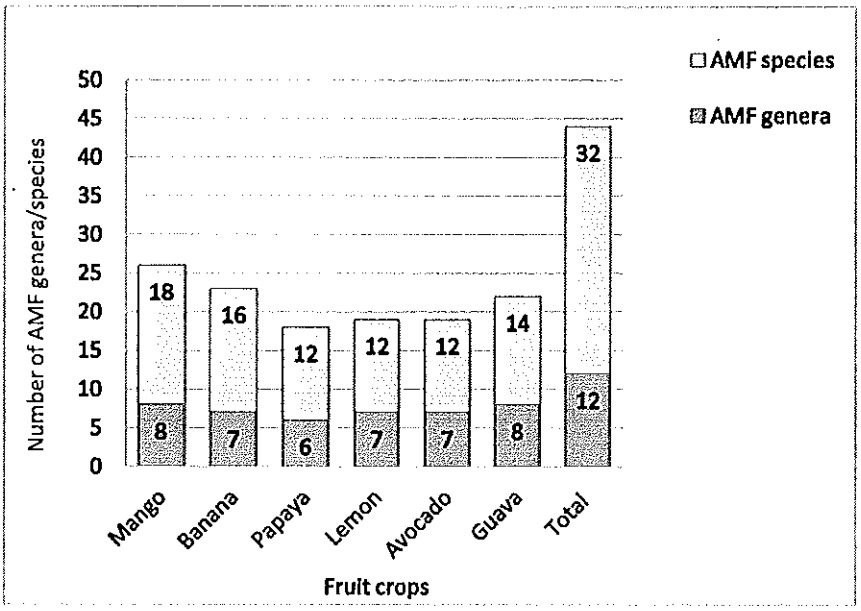


Fig. 4. 2: AMF genera and species richness in the six species of fruit crops

The dominant AMF species detected among the fruit trees appear to be generalist fungi, because they were detected in roots of a wide range of plant species (Stutz *et al.*, 2000; Zhao and Zhao, 2007). Likewise 17 species were categorized into the "common group" represented by the high IV representative of AMF; with *Claroideoglomus etunicatum, Cl. luteum, Funneliformis mosseae, F. caledonium* and *Glomus microcarpum* that were found in all but one fruit crop (IV 42-46) (**Table 4.4**). It is interesting to note that 41% of the species recovered from fruit crops were classified in the "rare category" according to the definition of Chen *et al.* (2012). These "Rare" AMF species were clustered into one or the other fruit crops indicating that they have strong affiliation for them. Consequently, mango fruit harbored the maximum number of four species that were not found elsewhere whereas, lemon harbored three species, and two species were distributed each in banana and guava. Avocado and papaya harbored one species each that did not occur in any of the other fruits (**Table 4.4**).

It is interesting to note that although *Acaulospora* w as represented by the highest number of species (8 species), the distribution was limited to specific fruits as opposed to the genus *Clareoideoglomus* that was represented by a few species and yet was distributed across the majority of the fruits. Most species from *Acaulospora* together with two species from the genus *Gigaspora* was categorized into the "rare" cluster (**Table 4.4).**

On the contrary, the genera *Septoglomus, Pacispora, Entrophospora, Diversispora* and *Rhizophagus* were represented by single species; they were categorized into a commonly occurring group. Moreover these genera were distributed in more than half of the fruits tested indicating that they were relatively widely distributed amidst their limitation in the number of species they contained.

Table 4.4 List, isolation frequency (IF), relative abundance (RA) and important values (IVs) of AMF species recovered from the rhizosphere of fruit crops at Showa robit.

Species	Mango	Banana	Papaya	Lemon	Avocado	Guava	IF (%)	RA (%)	IV (%)	Status
Claroideoglomus										
*Cl. Claroideum (*Schenck & Sm.) Walker & Schuessler	x	x	x	x	x	x	100	18.89	59.4	Dominant
Cl. etunicatum (Becker & Gerd.) Walker & Schuessler	x	x		x	x	x	83.3	8.75	46	Common
Cl. luteum (Kenn, Stutz & Morton) Walker & Schuessler		x	x	x	x	x	83.3	8.75	46	Common
Funneliformis										
F. badium (Oehl, Redecker & Sieverd.) Walker & Schuessler				x			16.6	0.46	8.53	Rare
*F. caledonium (*Nicolson & Gerd.) Walker & Schuessler	x	x	x	x		x	83.3	4.14	43.7	Common
F. coronatum (Giovann.) Walker & Schuessler	x				x		33.3	1.38	17.3	Common
F. geosporum (Nicolson & Gerd.) Walker & Schuessler	x	x	x		x		66.6	7.37	37	Common
F. mosseae (Oehl, Redecker & Sieverd.)Walker & Schuessler	x	x	x	x		x	83.3	12.44	47.9	Common
F. verruculosusm (Błaszk.) C. Walker & Schuessler	x						16.6	0.46	8.53	Rare
Glomus										
Gl. aggregatum Schenck & Sm.	x	x	x	x	x	x	100	5.99	53	Dominant
Gl. albidum N.C. Schenck & G.S. Sm.	x		x				33.3	0.92	17.1	Common
Gl. microaggregatum Koske, Gemma & Olexia						x	16.6	0.46	8.53	Rare
Gl. microcarpum Tul. & Tul.	x	x	x	x		x	83.3	2.3	42.8	Common
Glomus sp.1 (#2) sporocarpic, thick wall, smooth	x						16.6	0.46	8.53	Rare
Glomus sp.2 (#3) red brown geosporum like	x	x	x				33.3	1.38	17.3	Common
Rhizophagus										
R. fasciculatus (Thaxt.) Walker & Schuessler	x			x		x	50	1.38	25.7	Common

Septoglomus										
S. constrictum (Trappe) Sieverd., Silva& Oehl	x	x			x	x	66.6	5.53	36.1	Common
Acaulospora										
A. denticulata Sieverd. & S. Toro		x			x	x	50	1.38	25.7	Common
A. faveata Trappe & Janos					x		16.6	0.46	8.53	Rare
A. kentinesis (Wu & Liu) Kaonongbua, Morton &Bever	x						16.6	0.92	8.76	Rare
A. rehmii Sieverd. & S. Toro				x			16.6	0.46	8.53	Rare
A. scrobiculata Trappe		x			x		33.3	2.76	18	Common
A. spinosa Walker & Trappe		x	x			x	50	2.3	26.2	Common
A. tuberculata Janos & Trappe	x						16.6	0.46	8.53	Rare
Acaulospora sp.		x					16.6	0.92	8.76	Rare
Pacispora										
Pacispora scintillans (Rose & Trappe) Walker, Vestberg & Schuessler				x	x	x	50	4.6	27.3	Common
Diversispora										
D. epigaea (Daniels & Trappe) Walker & Schuessler	x	x	x				50	2.3	26.2	Common
Entrophospora										
E. nevadensis J. Palenzuela, N. Ferrol, Azcón-A	x				x		33.3	4.6	19	Common
Racocetra										
R. gregaria (Schenck & Nicolson) Oehl. Souza & Sieverd.				x			16.6	0.46	8.53	Rare
Gigaspora										
Gi. margarita Becker & Hall			x				16.6	0.46	8.53	Rare
Gigaspora sp.		x					16.6	0.92	8.76	Rare
Paraglomus										
Paraglomus occultum (Walker) Morton & Redecker						x	16.6	0.46	8.53	Rare

In all cases the generalist *Glomus, Funneliformis* and *Claroideoglomus* were identified from these fruits. However, the genera *Rhizophagus, Gigaspora* and *Racocerta* were missed from these fruit crops from the Sudan, whereas the genera *Ambispora* and *Archeospora* were not detected from this study.

In the present study, the diversity of AMF communities based on the Shannon-Wiener diversity index (**Table 4.5**) was the highest in banana ($H^{!}$=2.57) and the lowest in papaya ($H^{!}$=2.02). AMF species evenness ranged between 0.82 and 0.63 and the lowest even distribution was found in mango and papaya, the highest in banana. The species dominance (D) was the highest in papaya (D = 0.18) and the lowest in banana (D = 0.09).

Table 4. 5. Diversity indices of AMF community in six fruit crops

	Shannon_H	Dominance_D	Evenness_e^H/S
Mango	2.46	0.13	0.63
Banana	2.57	0.09	0.82
Papaya	2.02	0.18	0.63
Lemon	2.18	0.14	0.74
Avocado	2.27	0.12	0.81
Guava	2.34	0.12	0.74

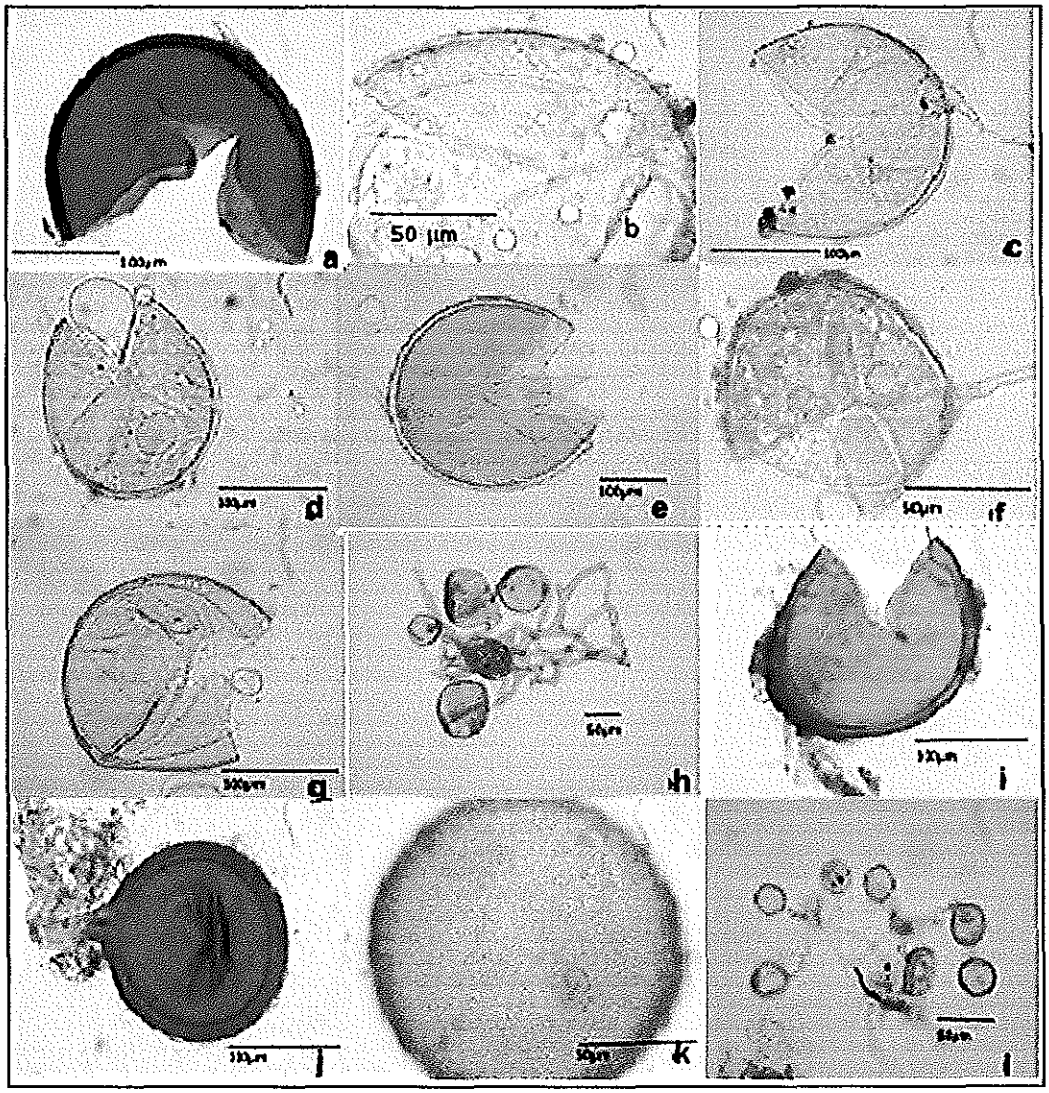


Fig. 4. 3: Some AMF species identified from rhizosphere soil samples of fruit plants in Ethiopia. All photos are from slides made in PVLG. a) *Septoglomus constrictum,* b) *Acaulospora kentinesis* c) *Funneliformis mosseae,* d) *Cl. luteum,* e) *F. caledonium,* f) *Paraglomus occultum,* g) *Acaulospora scrobiculata* h) *Glomus aggregatum,* i) *Glomus* sp.2, j) *Glomus* sp.1, k) *Ac. denticulate* l) *Gl. microaggregatum*

CONCLUSION AND RECOMMENDATION

In the present study, the association of AM fungi with six fruit tree species was evaluated in low-input cropping system of Showa Robit. The spore abundance and the species diversity of AMF identified from the fruit crops were relatively high. Highest

percentage of AMF root colonization and spore density was recorded from mango whereas lowest was from papaya and guava.

Generally, AMF species richness, root colonization and spore density recorded in the fruit crops were comparatively variable between the fruit crops which emphasize the fact that, the variation might be associated with host plant species and edaphic factors. However, further steps to be undertaken to study the functional diversity of AM fungi associated with the roots of these plants to determine their relative contribution to different mycorrhizal functions.

REFERENCES

Abdelhalim, T. S., Finckh, M. R., Babiker, A. G. and Oehl, F. (2013). Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. *Arch. Ag. Soil Sci.* DOI: 10.1080/03650340.

Alarcón, A. Hernández-Cuevas, L. V. Ferrera-Cerrato, R. and Franco-Ramírez , A. (2012). Diversity and agricultural applications of arbuscular mycorrhizal fungi in Mexico. *J. Biofertil. Biopestici.* 3:1

Augé, R. M. (2001). Water relations, drought and vesicular- arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3 – 42. doi:10.1007/s005720100097.

Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R. and Barea, J. M. (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Applied Soil Ecology*. 22:29-37.

Bever, J. D., Morton, J. B., Antonovics, J. and Schultz, P. A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in Mown grassland. *J. Ecol.* 84:71-82.

Brundrett, M., Bougher N., Dell, B., Grove, T. and Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra.

Chanie, T. and Assefa, F. (2013). Arbuscular mycorrhizal fungi associated with shade trees and *Coffea arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afrika focus* 26:11-131.

CSA, (2012). Central Statistics Agency agricultural sample survey, report on area and production of major crops. *Statistical bulletin,* V, 1: 532

Gaidashova, S., Nsabimana, A., Karamura, D., Asten, P. and Declerck, S. (2012). Mycorrhizal colonization of major banana genotypes in six East African environments. *Agricult. Ecosys. Environ.* 157: 40–46.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Bri. Mycol. Soc.* 46:235-244.

Jansa, J., Smith, J. F. and Smith, S. E. (2007). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol.* 177: 779–789.

Khade, S. W. and Rodrigues, B.F. (2009). Arbuscular mycorrhizal fungi associated with varieties of *Carica papaya* L. in tropical agro-based ecosystem of Goa, India. *Trop. Subtrop. Agroecosyst.* 10: 369 - 381

Khanam, D. (2007). Assessment of arbuscular mycorrhizal association in some fruit plants in Bangladesh. *Bangladesh J. Microbiol.* 24:34-37.

Lovato, P. E., Schüepp, H., Trouvelot, A. and Gianinazzi, S. (1995). Application of Arbuscular Mycorrhizal Fungi (AMF) in Orchard and Ornamental Plants. In: *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology.* (Varma, A. and Hock, B. eds.). Springer Berlin Heidelberg pp. 443-467.

Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E. and Jansa, J. (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agricult. Ecosys. Environ.* 119:22-32.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 115:495-501.

Muleta, D., Assefa, F., Nemomissa, S., Granhall, U. (2008). Distribution of arbuscular mycorrhizal fungi spores in soil of southwestern Ethiopia. *Biol. Fertil. Soils.* 44:653–659.

Muthukumar, T. and Udaiyan, K. (2002). Seasonality of vesicular- arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecologica* 23:337-347.

Muthukumar, T., Sha, L., Yang, X., Cao, M., Tang, J. and Zheng, Z. (2003). Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. *Mycorrhiza,* 13:289–297

Nair, P. K. R. (1993). An introduction to agroforestry. Kluwer Academic Publishers, pp.21

NMA (National Meteorology Agency of Ethiopia) (2002-2010). Annual climate bulletin for the year 2002-2010.

Omar, M. B., Bolland, L. and Heather, W. A. (1979). PVA (polyvinyl alcohol). A permanent mounting medium for fungi. *Bull. Bri. Mycol. Soc.* 13:31–32.

Sawers, R. J. H., Yang, S-Y., Gutjahr, C., and Paszkowski, U. (2008). The molecular components of nutrient exchange in arbuscular mycorrhizal interactions. In: *Mycorrhizae: sustainable agriculture and forestry.* (Siddiqui, Z. A., Akhtar, M. S., and Kazuyoshi Futai, K., Eds). Springer Science + Business Media B.V. pp. 37-59.

Schenck, N. C. and Perez, Y. (1990). Manual for the identification of VA mycorrhizal Fungi, 3rd edn. Synergistic publications, Gainesville, Fla.

Schüßler, A. and Walker, C. (2010). The Glomeromycota. A species list with new families and new genera. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische

Staatssammlung Munich, and Oregon State University. Electronic version freely available at www.amf-phylogeny.com.

Seyoum, A., Dorosh, P. and Asrat, S. (2011). Crop Production in Ethiopia: Regional Patterns and Trends. ESSP II Working Paper 16.

Smith, S. E., and Read, D. J. (2008). *Mycorrhial Symbiosis* 3rd Ed. Elsevier, Oxford, UK.

Stutz, J. C. and Morton, J. B. (1996). Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Can. J. Bot.* 74:1883-1889.

Stutz, J. C., Copeman, R., Martin, C. A. and Morton, J. B. (2000). Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. *Can. J. Bot.* 78: 237-245.

Trindade, A. V., Siqueira, J. O. and Stürmer, S. L. (2006). Arbuscular mycorrrhizal fungi in papaya plantations of Espirito Santo and Bahia, Brazil. *Braz. J. Microbiol.* 37:283-289.

Wehner, J., Antunes, P. M., Powell, J. R., Mazukatow, J. and Rillig, M. C. (2009). Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? *Pedobiologia*, doi:10.1016/j.pedobi.2009.10.002.

Zhao, D. D. and Zhao, Z. W. (2007). Biodiversity of arbuscular mycorrhizal fungi in the hot-dry valley of the Jinsha River, Southwest China. *Appl. Soil Ecol.* 37:118-128.

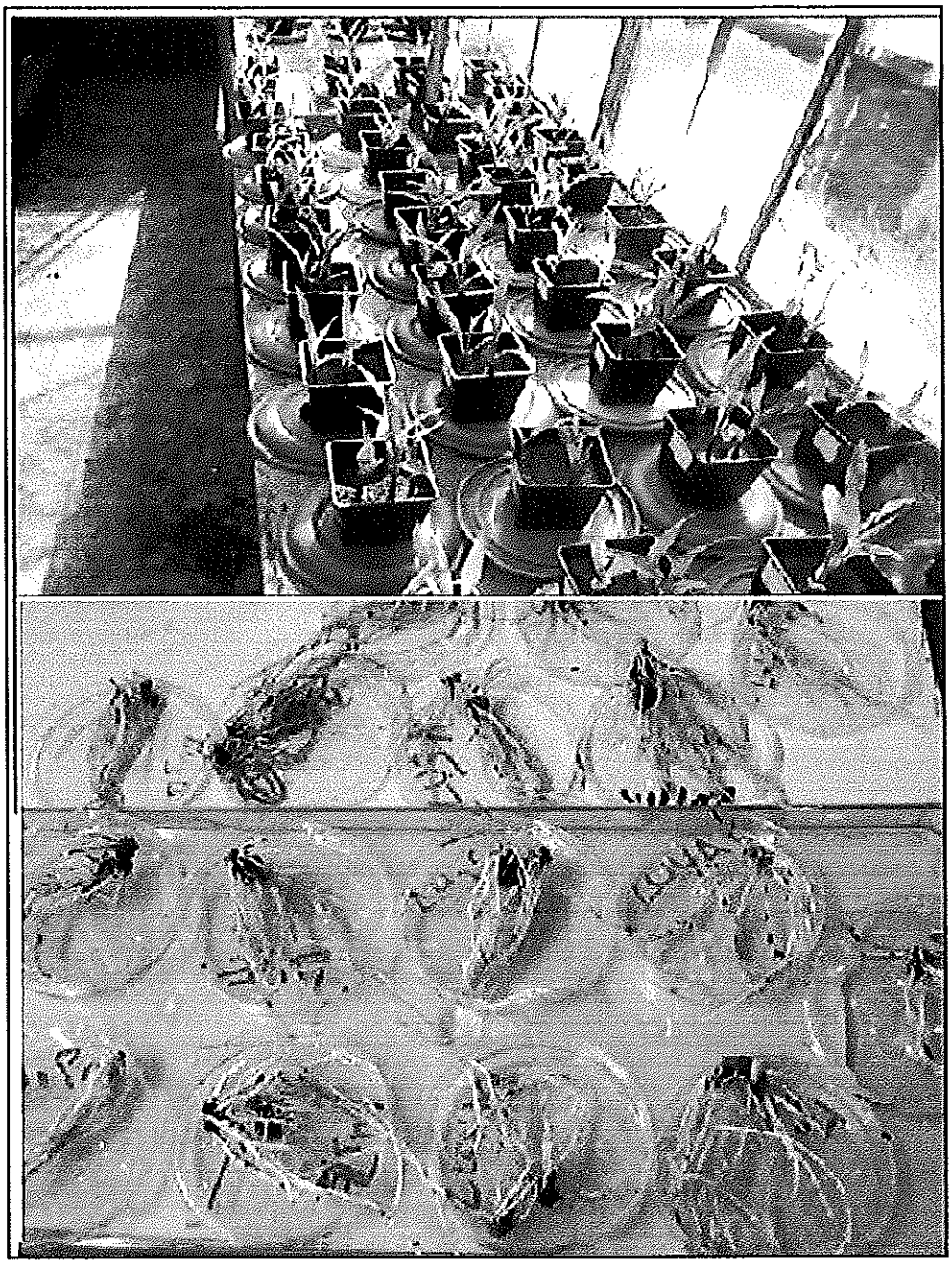
5. Appendices

Appendix A. Pearson's correlation coefficients between AM structural colonization, spore density, species richness and selected physico-chemical properties of soil associated with acacia trees from different land use systems in Ethiopia

Variable	SD	SR	AC	VC	HC	TN	OC	AP	pH	Clay	Silt	Sand
SD	1	.275	.523	.342	.489	.047	-.262	-.728**	-.027	-.710**	.649*	.604*
SR		1	-.088	-.148	-.192	-.013	-.293	.108	.142	.177	-.190	-.132
AC			1	.819**	.853**	.034	.341	-.263	-.266	-.319	.458	.163
VC				1	.730**	.173	.370	-.240	-.280	-.193	.338	.059
HC					1	.274	.536	-.454	-.149	-.563	.628*	.405
TN						1	.657*	-.263	-.178	-.380	.445	.259
OC							1	.068	-.259	-.126	.337	-.037
AP								1	-.135	.773**	-.843**	-.569
pH									1	.212	-.106	-.237
Clay										1	-.804**	-.922**
Silt											1	.512
Sand												1

SD: spore density; SR: spore richness; AC: arbuscular colonization; VC: vesicular colonization; HC: hyphal colonization; TN: total nitrogen; AP: available phosphorus;
*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Appendix B. Photos of greenhouse bioassay



Appendix c. Pearson's correlation coefficients between AM structural colonization, spore density, species richness and selected physico-chemical properties of field soil samples from Showa robit, Ethiopia

Variable	AC	VC	HC	SD	SR	pH	Avil. P	TN	OC	sand	clay	silt
AC	1	.783*	.441	.670	.602	.225	-.365	.601	.502	.230	-.052	-.357
VC		1	.032	.942**	.749	.618	.033	.504	.322	.499	-.366	-.033
HC			1	.141	.256	-.538	-.152	.432	.626	-.089	.062	.016
SD				1	.843*	.513	.227	.559	.474	.529	-.475	.224
SR					1	.538	.154	.789*	.762*	.188	-.204	.183
pH						1	.270	.405	-.076	.343	-.283	.071
P							1	.269	-.198	.696	-.853*	.963**
TN								1	.602	.338	-.350	.280
OC									1	-.280	.218	-.021
sand										1	-.963**	.613
clay											1	-.803*
silt												1

SD: spore density; SR: spore richness; AC: arbuscular colonization; VC: vesicular colonization; HC: hyphal colonization; TN: total nitrogen; AP: available phosphorus;
*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Appendix D. Pearson's correlation coefficients between AM structural colonization, spore density, species richness and selected physico-chemical properties of trap culture from Showa robit, Ethiopia

Variable	AC	VC	HC	SD	SR	pH	avail.P	TN	OC	sand	clay	silt
AC	1	.783*	.938**	.400	.602	.225	-.365	.601	.502	.230	-.052	-.357
VC		1	.895**	.292	.749	.618	.033	.504	.322	.499	-.366	-.033
HC			1	.250	.575	.319	-.156	.516	.321	.472	-.292	-.189
SD				1	.434	.215	-.673	.127	.530	-.650	.720	-.675
SR					1	.538	.154	.789*	.762*	.188	-.204	.183
pH						1	.270	.405	-.076	.343	-.283	.071
avail.P							1	.269	-.198	.696	-.853*	.963**
TN								1	.602	.338	-.350	.280
OC									1	-.280	.218	-.021
sand										1	-.963**	.613
clay											1	-.803*
silt												1

SD: spore density; SR: spore richness; AC: arbuscular colonization; VC: vesicular colonization; HC: hyphal colonization; TN: total nitrogen; AP: available phosphorus;
*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Appendix E. List, isolation frequency (IF %) and relative spore abundance (RA) of the AMF species identified in field soil

	IF%								RA%									
AMF genera and species	A1	A2	A3	A4	FC	NF	AP	Mean	A1	A2	A3	A4	FC	NF	AP	Mean	IV	Status
Acaulospora	33.3	-	-	33	71.4	11.1	50	28.4	3	-	-	5	9.4	3.1	8.6	4.16	16	Common
A. denticulata Sieverd. & S. Toro	11.1	-	-	33	9.5	-	-	7.7	1	-	-	5	0.9	-	-	0.99	4.3	Rare
A. faveata Trappe & Janos	-	-	-	-	4.7	-	-	0.67	-	-	-	-	0.4	-	-	0.06	0.4	Rare
A. kentinesis (Wu & Liu) Kaonongbua, Morton & Bever	-	-	-	-	9.5	-	-	1.36	-	-	-	-	0.9	-	-	0.13	0.7	Rare
A. rehmii Sieverd. & S. Toro	11.1	-	-	-	4.7	11.1	-	3.84	1	-	-	-	0.4	3.1	-	0.64	2.2	Rare
A. scrobiculata Trappe	-	-	-	-	28.6	-	16.7	6.47	-	-	-	-	2.9	-	2.8	0.81	3.6	Rare
A. spinosa Walker & Trappe	-	-	-	-	19	-	-	2.71	-	-	-	-	1.9	-	-	0.27	1.5	Rare
A. splendida Sieverd, Chaverri & Rojas	11.1	-	-	-	-	-		1.59	1	-	-	-	-	-	-	0.14	0.9	Rare
A. tuberculata Janos & Trappe	-	-	-	-	4.7	-	33.3	5.43	-	-	-	-	0.4	-	5.7	0.87	3.2	Rare
Acaulospora sp1	-	-	-	-	9.5	-	-	1.36	-	-	-	-	0.9	-	-	0.9	1.1	Rare
Claroideoglomus	100	66.7	33.3	100	100	100	100	85.7	26.3	22.2	7.6	35	31	28.1	25.7	25.1	55	Dominant
C. claroideum (Schenck & Sm.) Walker & Schuessler	100	33.3	33.3	100	100	44.4	16.7	61.1	15.2	16.6	7.6	25	20.7	12.5	2.8	14.3	38	Common
C. etunicatum (Becker & Gerd.) Walker & Schuessler	22.2	-	-	67	42.8	33.3	83.3	35.5	2	-	-	10	4.4	9.3	14.3	5.7	21	Common
C. lamellosum (Dalpé, Koske & Tews) Walker & Schuessler	22.2	-	-	-	-	-	-	3.17	2	-	-	-	-	-	-	0.29	1.7	Rare
C. luteum (Kenn, Stutz & Morton) Walker & Schuessler	66.6	33.3	-	-	57.1	22.2	50	32.7	6	16.6	-	-	5.9	6.2	8.6	6.19	19	Common
Diversispora	11.1	-	-	-	28.6	22.2	-	8.84	1	-	-	-	2.9	6.2	-	1.44	5.1	Rare
D. epigaea (Daniels & Trappe) Walker & Schuessler	11.1	-	-	-	28.6	22.2	-	8.84	1	-	-	-	2.9	6.2	-	1.44	5.1	Rare
Entrophospora	-	-	-	-	47.6	-	-	6.8	-	-	-	-	4.9	-	-	0.7	3.8	Rare
E. nevadensis J. Palenzuela, N. Ferrol, Azcón-Aguilar & Oehl	-	-	-	-	47.6	-	-	6.8	-	-	-	-	4.9	-	-	0.7	3.8	Rare
Funneliformis	100	100	100	100	100	66.7	50	88.1	39.4	33.3	38.5	25	30	18.7	11.4	28	58	Dominant
F. badium (Oehl, Redecker & Sieverd.) Walker & Schuessler	-	-	-	-	4.7	-	-	0.67	-	-	-	-	0.4	-	-	0.06	0.4	Rare
F. caledonium (Nicolson & Gerd.) Walker & Schuessler	55.5	66.6	100	67	42.8	11.1	-	49	5	22.2	23	10	4.4	3.1	-	9.67	29	Common
F. coronatum (Giovann.) Walker & Schuessler	-	-	-	-	14.3	-	-	2.04	-	-	-	-	1.5	-	-	0.21	1.1	Rare
F. geosporum (Nicolson & Gerd.) Walker & Schuessler	88.8	-	33.3	33	80.9	22.2	50	44.1	8.1	-	7.6	5	8.4	6.2	11.4	6.67	25	Common
F. mosseae (Oehl, Redecker & Sieverd.)Walker & Schuessler	100	33.3	33.3	67	100	33.3	-	52.4	26.3	11.1	7.6	10	14.8	9.3	-	11.3	32	Common

F. verruculosusm (Blaszk.) C. Walker & Schuessler	-	-	-	-	4.7	-	-	0.67	-	-	-	-	0.4	-	-	0.06	0.4	Rare
Glomus	100	66.7	66.7	67	100	55.6	100	79.4	17.2	22.2	23	15	10.8	15.6	25.7	18.5	49	Common
Gl. aggregatum Schenck & Sm.	44.4	-	-	-	57.1	11.1	16.7	18.5	4	-	-	-	5.9	3.1	2.8	2.26	10	Common
Gl. albidum N.C. Schenck & G.S. Sm.	-	-	-	-	9.5	-	-	1.36	-	-	-	-	0.9	-	-	0.13	0.7	Rare
Gl. hoi Berch & Trappe	11.1	-	-	-	-	-	-	1.59	1	-	-	-	-	-	-	0.14	0.9	Rare
Gl. microaggregatum Koske, Gemma & Olexia	-	-	-	-	-	-	16.7	2.39	-	-	-	-	-	-	2.8	0.4	1.4	Rare
Gl. microcarpum Tul. & Tul.	-	-	-	-	19	-	-	2.71	-	-	-	-	1.9	-	-	0.27	1.5	Rare
Gl. monosporum Gerd. & Trappe	11.1	-	-	33	-	-	-	6.34	1	-	-	5	-	-	-	0.86	3.6	Rare
Gl. tortuosum N.C. Schenck & G.S. Sm.	11.1	-	-	-	-	-	-	1.59	1	-	-	-	-	-	-	0.14	0.9	Rare
Glomus sp1(#2) sporocarpic , thick wall , smooth (80-110µm)	-	-	-	-	4.7	-	100	15	-	-	-	-	0.4	-	17.1	2.5	8.8	Rare
Glomus sp2(#3) red brown geosporum like	100	66.7	66.7	67	14.3	44.4	16.7	53.6	10	22.2	23	10	1.5	12.5	2.8	11.71	33	Common
Rhizophagus	11.1	-	-	67	14.3	22.2	33.3	21.1	1	-	-	10	1.5	6.2	5.7	3.49	12	Common
R. diaphanus (Morton & Walker) Walker & Schuessler	11.1	-	-	67	4.5	22.2	33.3	19.7	1	-	-	10	0.4	6.2	5.7	3.33	12	Common
R. fasciculatus (Thaxt.) Walker & Schuessler	-	-	-	-	9.5	-	-	1.36	-	-	-	-	0.9	-	-	0.13	0.7	Rare
Septoglomus	22.2	-	-	-	46.7	11.1	50	18.6	2	-	-	-	4.9	6.2	17.1	4.31	11	Common
S. constrictum (Trappe) Sieverd., Silva& Oehl	22.2	-	-	-	46.6	11.1	50	18.6	2	-	-	-	4.9	6.2	17.1	4.31	11	Common
Racocetra	-	-	-	-	4.7	11.1	-	2.26	-	-	-	-	0.4	3.1	-	0.5	1.4	Rare
R. gregaria (Schenck & Nicolson) Oehl, Souza & Sieverd.	-	-	-	-	4.7	11.1	-	2.26	-	-	-	-	0.4	3.1	-	0.5	1.4	Rare
Gigaspora	66.7	66.7	-	67	14.3	-	-	30.6	6	22.2	-	10	1.5	-	-	5.67	18	Common
Gi. albida Schenck & Sm	22.2	-	-	-	-	-	-	3.17	2	-	-	-	-	-	-	0.29	1.7	Rare
Gi. gigantea (Nicolson & Gerd.) Gerd. & Trappe	44.4	66.7	-	33	-	-	-	20.6	4	22.2	-	5	-	-	-	4.46	13	Common
Gi. margarita Becker & Hall	-	-	-	33	4.7	-	-	5.43	-	-	-	5	0.4	-	-	0.77	3.1	Rare
Gigaspora sp.	-	-	-	-	9.5	-	-	1.36	-	-	-	-	0.9	-	-	0.13	0.7	Rare
Scutellospora	33.3	-	33.3	-	-	22.2	16.7	12.7	3	-	7.6	-	-	6.2	2.8	2.8	7.8	Rare
S. cerradensis Spain & Miranda	-	-	33.3	-	-	-	-	4.7	-	-	7.6	-	-	-	-	1.09	2.9	Rare
S. pellucida (Nicolson & Schenck) Walker & Sanders	33.3	-	-	-	-	22.2	16.7	10.3	3	-	-	-	-	6.2	2.8	1.71	6	Rare
Pacispora	11.1	-	-	-	23.8	-	-	4.99	1	-	-	-	2.5	-	-	0.5	2.7	Rare
Pacispora scintillans (Rose & Trappe) Walker, Vestberg & Schuessler	11.1	-	-	-	23.8	-	-	4.99	1	-	-	-	2.5	-	-	0.5	2.7	Rare
Paraglomus	11.1	-	66.7	-	-	22.2	16.7	16.7	1	-	23	-	-	6.2	2.8	4.71	11	Common
Paraglomus occultum (Walker) Morton & Redecker	11.1	-	66.7	-	-	22.2	16.7	16.7	1	-	23	-	-	6.2	2.8	4.71	11	Common

Appendix F. List, isolation frequency (IF %) and relative spore abundance (RA) of the AMF species identified in trap cultures of soil from seven land use types at Showa Robit, Ethiopia.

	IF%								RA%									
AMF genera and species	A1	A2	A3	A4	FC	NF	AP	Mean	A1	A2	A3	A4	FC	NF	AP	Mean	IV	Status
Acaulospora Trappe & Gerd.	33.3	-	33.3	-	14.3	11.1	-	**13.1**	2.4	-	4.2	-	1	1.8	-	**1.343**	**7.2**	**Rare**
A. denticulata Sieverd. & S. Toro	-	-	33.3	-	-	-	-	**4.76**	-	-	4.2	-	-	-	-	**0.6**	**2.7**	**Rare**
A. rehmii Sieverd. & S. Toro	-	-	-	-	4.7	-	-	**0.67**	-	-	-	-	0.3	-	-	**0.043**	**0.4**	**Rare**
A. scrobiculata Trappe	33.3	-	-	-	4.7	11.1	-	**7.01**	2.4	-	-	-	0.3	1.8	-	**0.643**	**3.8**	**Rare**
Acaulospora sp.	-	-	-	-	4.7	-	-	**0.67**	-	-	-	-	0.3	-	-	**0.043**	**0.4**	**Rare**
Claroideoglomus	100	100	100	100	100	100	100	**100**	51.2	45.6	56.5	20.7	47.1	46.3	42.7	**44.3**	**72**	**Dominant**
C. claroideum (Schenck & Sm.) Walker & Schuessler	100	100	100	100	100	100	100	**100**	21.4	17.5	25	13.8	21.6	25.5	21.3	**20.87**	**60**	**Dominant**
C. etunicatum (Becker & Gerd.) Walker & Schuessler	100	100	100	66.7	100	44.4	100	**87.3**	21.4	17.5	16.7	6.9	17	17.7	17.9	**15.73**	**52**	**Dominant**
C. luteum (Kenn, Stutz & Morton) Walker & Schuessler	100	100	100	100	100	33.3	50	**83.3**	7.1	10.5	12.5	10.3	8	7.3	3.4	**8.443**	**46**	**Common**
Diversispora	-	-	-	-	-	-	33.3	**4.76**	-	-	-	-	-	-	2.2	**0.314**	**2.5**	**Rare**
D. epigaea (Daniels & Trappe) Walker & Schuessler	-	-	-	-	-	-	33.3	**4.76**	-	-	-	-	-	-	2.2	**0.314**	**2.5**	**Rare**
Entrophospora	-	33.3	-	-	33.3	-	-	**9.51**	-	1.7	-	-	2.6	-	-	**0.614**	**5.1**	**Rare**
E. nevadensis Błaszk., Madej & Tadych: J. Palenzuela, . N. Ferrol, Azcón-Aguilar & Oehl	-	33.3	-	-	33.3	-	-	**9.51**	-	1.7	-	-	2.6	-	-	**0.614**	**5.1**	**Rare**
Funneliformis	100	100	100	100	100	88.8	100	**98.4**	9.7	15.8	13	13.8	14.5	14.8	24.7	**15.19**	**57**	**Dominant**
F. badium (Oehl, Redecker & Sieverd.) Walker & Schuessler	-	-	-	-	-	11.1	-	**1.59**	-	-	-	-	-	1.8	-	**0.257**	**0.9**	**Rare**
F. geosporum (Nicolson & Gerd.) Walker & Schuessler	33.3	100	33.3	33.3	38.1	11.1	100	**49.9**	2.4	5.3	4.2	3.4	2.6	1.8	15.7	**5.057**	**27**	**Common**
F. mosseae (Oehl, Redecker & Sieverd.)Walker & Schuessler	100	100	66.7	100	100	66.6	100	**90.5**	7.1	10.5	8.3	10.3	11.7	10.9	8.9	**9.671**	**50**	**Common**
Glomus	100	100	33.3	33.3	100	100	100	**80.9**	14.6	21	4.2	3.4	19.5	27.7	14.6	**15**	**48**	**Common**
Gl. aggregatum Schenck & Sm.	100	66.6	33.3	-	100	100	100	**71.4**	7.1	3.5	4.2	-	11	21.8	8.9	**8.071**	**40**	**Common**
Gl. hoi Berch & Trappe	-	100	-	33.3	52.4	-	-	**26.5**	-	12.3	-	3.4	4.3	-	-	**2.857**	**15**	**Common**
Gl. microaggregatum Koske, Gemma & Olexia	-	-	-	-	4.7	-	-	**0.67**	-	-	-	-	0.3	-	-	**0.043**	**0.4**	**Rare**
Gl. microcarpum Tul. & Tul.	-	-	-	-	-	-	16.7	**2.39**	-	-	-	-	-	-	1.1	**0.157**	**1.3**	**Rare**
Gl. tortuosum N.C. Schenck & G.S. Sm.	66.7	-	-	-	19	-	-	**12.2**	4.8	-	-	-	1.3	-	-	**0.871**	**6.5**	**Rare**
Glomus sp1 sporocarpic , thick wall , smooth (80-110 µm)	-	-	-	-	-	-	50	**8.33**	-	-	-	-	-	-	3.4	**0.486**	**4.4**	**Rare**
Glomus sp2 red brown geosporum like	33.3	100	-	-	33.3	33.3	16.7	**30.9**	2.4	5.3	-	-	2.3	5.5	1.1	**2.371**	**17**	**Common**

Rhizophagus	-	100	100	-	28.6	-	100	**46.9**	-	5.3	13	-	2	-	8.9	**4.171**	**26**	**Common**
R. diaphanus (Morton & Walker) Walker & Schuessler	-	100	66.7	-	28.6	-	100	**42.2**	-	5.3	8.3	-	2	-	8.9	**3.5**	**23**	**Common**
R. fasciculatus (Thaxt.) Walker & Schuessler	-	-	33.3	-	-	-	-	**4.76**	-	-	4.2	-	-	-	-	**0.6**	**2.7**	**Rare**
Septoglomus	-	-	-	-	33.3	-	66.6	**14.3**	-	-	-	-	6	-	5.6	**1.657**	**8**	**Rare**
S. constrictum (Trappe) Sieverd., Silva& Oehl	-	-	-	-	33.3	-	66.6	**14.3**	-	-	-	-	6	-	5.6	**1.657**	**8**	**Rare**
Sclerocystis	100	-	-	-	9.5	-	-	**15.6**	11.9	-	-	-	0.7	1.8	-	**2.057**	**8.8**	**Rare**
S. sinuosa (Gerd. & Bakshi)	100	-	-	-	9.5	11.1	-	**17.2**	11.9	-	-	-	0.7	1.8	-	**2.057**	**9.6**	**Rare**
Racocetra	-	-	-	100	-	-	-	**14.3**	-	-	-	17.2	-	-	-	**2.457**	**8.4**	**Rare**
R. alborosea (Ferrer & Herrera) Oehl, Souza & Sieverd.	-	-	-	100	-	-	-	**14.3**	-	-	-	13.8	-	-	-	**1.971**	**8.1**	**Rare**
R. gregaria (Schenck & Nicolson) Oehl, Souza & Sieverd.	-	-	-	33.3	-	-	-	**4.76**	-	-	-	3.4	-	-	-	**0.486**	**2.6**	**Rare**
Gigaspora	100	33.3	33.3	100	9.5	-	-	**39.4**	7.3	1.7	4.2	31	0.7	-	-	**7.367**	**23**	**Rare**
Gi. albida Schenck & Sm	-	-	-	33.3	-	-	-	**4.76**	-	-	-	3.4	-	-	-	**0.486**	**2.6**	**Rare**
Gi. gigantea (Nicolson & Gerd.) Gerd. & Trappe	33.3	33.3	33.3	100	-	-	-	**28.6**	2.4	1.7	4.2	27.6	-	-	-	**5.129**	**17**	**Common**
Gi. rosea Nicolson & Schenck	66.6	-	-	-	4.7	-	-	**10.2**	4.8	-	-	-	0.3	-	-	**0.729**	**5.5**	**Rare**
Gigaspora sp (unidentified)	-	-	-	-	4.7	-	-	**0.67**	-	-	-	-	0.3	-	-	**0.043**	**0.4**	**Rare**
Ambispora	-	-	66.7	-	23.5	-	-	**12.9**	-	-	8.3	-	1.7	-	-	**1.429**	**7.2**	**Rare**
A. fennica Walker, Vestberg & Schuessler	-	-	66.7	-	9.5	-	-	**10.9**	-	-	8.3	-	0.7	-	-	**1.286**	**6.1**	**Rare**
Ambispora sp.	-	-	-	-	14.3	-	-	**2.04**	-	-	-	-	1	-	-	**0.143**	**1.1**	**Rare**
Pacispora	-	-	-	-	23.8	33.3	16.7	**10.5**	-	-	-	-	1.6	5.5	1.1	**1.171**	**5.8**	**Rare**
P. scintillans Oehl & Sieverd	-	-	-	-	23.8	33.3	16.7	**10.5**	-	-	-	-	1.6	5.5	1.1	**1.171**	**5.8**	**Rare**
Paraglomus	66.6	100	-	33.3	42.8	22.2	-	**37.8**	4.8	8.8	-	3.4	3.3	3.7	-	**3.429**	**21**	**Common**
P. occultum (Walker) Morton & Redecker	66.6	100	-	33.3	42.8	22.2	-	**37.8**	4.8	8.8	-	3.4	3.3	3.7	-	**3.429**	**21**	**Common**

(A1)Arable1: low-input mixed cropping; (A2) Arable2: low-input monocropping, sorghum; (A3)Arable3: low-input monocropping, maize; (A4)Arable4: high-input monocropping, sorghum; FC: fruit cropping; NF: natural forest; AP: acacia plantation

Appendix G. The research team at Showa robit during the sampling period

Appendix I. The research team during the laboratory works in Ethiopia and Finland