#### **ABUNDANCE AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN DIFFERENT LAND USE TYPES IN JABI TEHNAN WOREDA, WESTERN GOJAM, ETHIOPIA**

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**ABSTRACT:** Arbuscular mycorrhizal fungi (AMF) community structure and function are affected by changes induced by severe deforestation and land degradation. In order to evaluate this, a site was selected at Jabi Tehnan Woreda with eight land use types, grouped into four monocrops (annuals), one mono tree (perennial), two mixed crop, and one mixed tree-crop (perennial) to study AMF spore density, root colonization and identification. Spore density of the different cropping systems varied significantly both within and between land use types ranging from 104 spores/100 g soil from eucalyptus (*E*. *globulus*) to 929 spores/100 g soil for mixed crop I (cabbage+sunflower+maize). All plants formed AM symbiosis with different types of structures, except cabbage (*Brassica oleraceae*). The AM fungal colonization pattern showed variations ranging from 22% (teff and eucalyptus) up to 73.4% from maize in mixed crop I. A total of 12 AMF genera and 42 morphospecies were identified from the different cropping systems, of which the highest number of species was recorded under the genus *Acaulospora* (15 species), followed by 4 each to genera *Claroideoglomus*, *Diversispora* and *Funneliformis*, 3 each to *Dentiscutata*, *Gigaspora* and *Rhizophagus*, 2 to *Scuttelospora* and 1 each to *Cetraspora*, *Paraglomus*, *Racocetra* and *Septoglomus*. Based on important value (IV), 47% of the AMF species were found to be common and none of them were dominant. In this study, AMF species diversity was much lower in tree-based cropping system than in the annual cropping system suggesting that the rapid root dynamics and turnover in the short seasoned crops may enhance the maintenance of AMF community.

**Key words/phrases**: Arbuscular mycorrhizal fungi, Crops, Diversity, Land use, Trees.

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#### **INTRODUCTION**

Arbuscular mycorrhizal fungi (AMF) are one of the mycorrhizal groups that colonize roots of the majority of higher plants. It is estimated that more than 80% of all terrestrial plants form this type of association, that includes many agriculturally and horticulturally important crop species (Smith and Read, 2008). Olsson *et al*. (1999) had estimated that they are probably the most ubiquitous fungi in agricultural soils, accounting for 5–36% of the total biomass in the soil and 9–55% of the biomass of soil micro-organisms.

The mycorrhizal symbiosis enhances plant uptake of immobile soil nutrients, in particular phosphorus, and nitrogen because of hyphal competition for these nutrients, reduce damage caused by pathogenic fungi, bacteria, and nematodes through direct and indirect effects (Azcón-Aguilar and Barea, 1996; Cardoso and Kuyper, 2006).

In general, AMF can influence the plant diversity, productivity, community structure, and ecosystem processes and is decisive for both plant community structure and ecosystem productivity (Sanders, 1998; Van der Heijden *et al*., 1998). Therefore, the application of AMF is of interest for the reclamation of the vegetation cover of degraded lands (Requena *et al*., 2001). It is as equally important to develop AMF management strategies for sustainable low-input but reasonably productive and ecologically sound agriculture (Tilman *et al*., 2002).

Studies undertaken over the past 30 years have shown that common management practices such as P fertilizer applications (Lu *et al*., 1994), fallow periods (Kabir and Koide, 2002), and intensive tillage (Kabir, 2005) may have negative effects of varying degrees on AMF abundance (Lekberg and Koide, 2005). Modern intensive farming practices are evidently a threat for AMF, as indicated by studies of AMF performance in different agroecosystems (Oehl *et al*., 2003).

In Ethiopia, several researches have been undertaken on the importance of mycorrhiza in improving the staple crop plant, teff (*Eragrostis teff* ) on acidic soil (Tekalign Mamo and Killham, 1987), diversity and spore density of AMF on coffee agroforestry and other Afromontane agroecosystems (Tesfaye Wubet *et al*., 2003; Diriba Muleta *et al*., 2008; Tadesse Chanie and Fassil Assefa, 2013), indicating that the mixed cultivation systems of Ethiopia, such as coffee, have revealed AMF species richness among the coffee and dominant coffee shade tree species. Recently, Zerihun Belay *et al*. (2013; 2015) showed that land use types drastically affected AMF colonization and AMF diversity in a wet agroecosystem at Shoa Robit and dry land agroforestry system in the central parts of Ethiopia.

In Ethiopia, various kinds of intact forest, agroforestry system, and low input cropping land use systems have been practised. Yet, the effect of these land use types on spore density and diversity of AMF were not exhaustively studied. Thus, this study was aimed at investigating how land use system changes have affected AMF species diversity, spore density and root colonization from intact forest to mixed and mono cropping systems in Jabi Tehnan Woreda, western Gojam.

#### **MATERIALS AND METHODS**

### **Study site**

The study site is located in Jabi Tehnan Woreda, western Gojam, in Amhara Regional State located between 10° 39′58.2″ and 10° 40′27.8″ N latitude and 037° 13′57.5″ and 037° 14′27.9″ E longitude. The altitude of the woreda ranges from 1500–2300 m.a.s.l. with temperature ranges between 14°C and 32°C, average annual temperature of 32°C, and average rainfall of 1250 mm per annum. Jabi Tehnan Woreda is known for its agroecosystem dominated by mono and mixed cropping systems growing major cash and food crops such as: sorghum, maize, finger millet, pepper, faba bean, teff, wheat, potato, barley sunflower, niger etc. in crop rotations, and growth of trees like eucalyptus, croton, and Juniper, etc, in agroforestry system (Asresie Hassen *et al.,* 2014).

### **Collection of root and soil samples**

A purposive sampling technique was used to include eight land use types (Table 1). From each sampling site, a 10 m x 10 m transect was established to collect 500 g of composite soil samples from the rhizosphere of crops and trees in sterile plastic bags from October-November 2013. Similarly, twelve (12) fine roots with 10–15 cm length from each crop and tree were collected, washed with water, cut into 4–5 cm pieces, fixed in 50% FAA (formalin-acetic acid-alcohol in 1:1:18 ratio) and stored at 4°C (Zhao *et al*., 2001).



Fig.1. Map of the study area.

Table 1. Different land use types of sampling sites at Jabi Tehnan Woreda.

No.	Land use type	<b>Crops cultivated</b>	Scientific name
	Monocrop I	Pepper	Capsicum annum
2	Monocrop II	Finger millet	Eleusine coracana
3	Monocrop III	Teff	Eragrostis teff
4	Monocrop IV	Niger	Guizotia abyssinica
	Mono tree-crop I	Eucalyptus	Eucalyptus globulus
6	Mixed crop I	$Cabbage + sunflower + maize$	Brassica oleraceae + Helianthus annuus + Zea mays
	Mixed crop II	Cabbage + faba bean	B. oleraceae + Vicia faba
8	Mixed tree-crop II	$Croton + juniperus$	Croton macrostachyus $+$ Juniperous procera

### **Physico-chemical analysis of the soil**

The physical and chemical properties of soils were analysed by using the methods described by Tan (1996). Soil pH was measured in de-ionized water (1:2.5 soils to water) by potentiometric method, organic carbon by the K2CrO<sup>7</sup> wet-combustion method; total N was determined by the Kjeldahl method; available P and available K were extracted with  $NH_4CO_3 + DTPA$  (Diethylene tri-amine penta-acetic acid) and measured by ICP-AES at National Soil Testing Centre, Addis Ababa (Table 1).





AVP: Available phosphorus; TN: Total nitrogen; OC: organic carbon; AVK: Available K.

Data are reported as averages and standard errors for three replicates per plant type. Values followed by different letters denote significant differences among plants according to Duncan's multiple range test at the 0.05 level of probability after one way ANOVA test

### **Spore extraction and enumeration**

AM fungal spores were extracted from soil by wet sieving and decanting, as described by Gerdemann and Nicolson (1963), and by sucrose centrifugation, as described by Brundrett *et al*. (1996). The soil sample (100 g) was suspended in 1 L water by gentle stirring. Heavier particles were then allowed to settle for a few seconds and the suspension was decanted through a 500 μm sieve to remove large particles and allow the spores to pass through. The suspension was passed through a 212 μm sieve and finally through a 45 μm sieve. The contents of 212 μm and 45 μm were then poured into a centrifuge tube containing water and centrifuged for 5 minutes at 2000 rpm. After pouring off the upper solution, 50% sucrose was added to the debris at the bottom and the mixture was then centrifuged for 1 min at 2000 rpm (Wagtech international 3000 systems). The supernatant was carefully poured through a 45 µm sieve and rinsed in tap water to remove the sucrose and poured into plastic Petri-dish for examination under the stereoscopic microscope (ISO 9001). The spore density was enumerated according to INVAM http://invam.caf.wvu.edu. Finally, healthy looking spores were collected from the plastic Petri-dish by fine tweezers or micro pipette, and then used 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 solution at 90 $^{\circ}$ C in a water bath for 2–3 h, and treated with 10% HCl (v/v) for 15–20 minutes at room temperature, and stained in

0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90°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 of 200x to identify and measure percent colonization of hyphae, vesicles and arbuscules.

# **Identification and characterization of AMF**

The AMF spores were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia. Healthy looking spores were picked 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 400x and identified to the species level or to a specific morphotype based on a current species description and identification manual (Schenck and Perez, 1990), online references of species description INVAM http://invam.caf.wvu.edu, West Virginia University, USA, 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/.](http://www.lrz.de/~schuessler/amphylo/) Isolation frequencies (IF), relative abundance (RA) of spores and the importance value (IV) were calculated according to Chen *et al*. (2012).

# **Data analysis**

Data analysis was carried out with SPSS software (version 21). Data on percentage of AM colonization was transformed by arcsine  $X^{\frac{1}{2}}$  and spore densities were transformed by  $log(x+1)$  to fulfil the assumption of normality and homogeneity of variances before analysis of variance (Li *et al*., 2007). Means given in tables were subject to one-way ANOVA to test the differences in AM colonization and spore density. Mean separation was done by Duncan's multiple range test at the 0.05 level of probability. The relationship between the AMF parameters and soil chemical properties (pH, OC, available P, and TN) was determined by Pearson's correlation analysis.

#### **RESULTS AND DISCUSSION**

### **Physico-chemical parameters**

The physico-chemical property of the soil samples of the study area is shown in Table 2. No significant difference  $(p>0.05)$  was observed in pH value between the land use types. However, higher percentage of OC was

recorded among the land uses with woody plants than the annual cropping systems. Total nitrogen was slightly, but not significantly (p>0.05) higher in soils of mixed cropping systems than the other land use types. Variations in available K and available P were significant  $(p<0.05)$  amongst the land use types (Table 2).

### **Spore density and root colonization by arbuscular mycorrhizal fungi**

The spore density of the different cropping systems is shown in Table 3. Accordingly, spore density varied greatly  $(p<0.05)$  both within and between land use types ranging from 104 spores/100 g soil from Eucalyptus (*E. globulus*) mono (tree) cropping to 929 spores/100 g soil for mixed cropping system (cabbage  $+$  sunflower  $+$  maize). The spore density from maize (929) spores/100 g) was slightly lower than the spore density of  $1100-1150$ spores/100 g reported from maize monocrop in Spain (Sasvari and Posta, 2010).

In general, the data indicated that spore density was significantly  $(p<0.05)$ higher in mixed crops and monocrops than the perennials (Eucalyptus) and (Juniperus + Croton) (Table 3). However, the mean spore densities recorded in the present study, in general, were lower than the counts (370–880 spores/100 g soil) reported from different crops of Showa Robit, Ethiopia (Zerihun Belay *et al*., 2014) and also less than Picone (2000) and Zhao *et al*. (2001) who reported total numbers of spores which ranged from 100 to 10,000 spores 100g-1 dry soil from tropical forest and pasture and 55 to 1908 spores  $100g^{-1}$  of dry soil from the tropical rain forest of Xishuanagbanna, southwest China, respectively. Different studies showed that variation in spore density among different crops/trees/cropping systems could be attributed to host preference and different abiotic factors (Bever *et al*. 1996; Muthukumar and Udaiyan, 2002; Mathimaran *et al*., 2007).

All plants formed AM symbiosis with all structures (arbuscules and vesicles, hyphal colonization), except cabbage (*Brassica oleraceae*). Members of the Brassicaceae do not host AMF for they produce toxic chemicals with broad spectrum biocidal properties (Vierheilig *et al*., 2000). The colonization of different AM structures varied greatly among plant species both within and between land use types based on results of one-way ANOVA. The AM fungal colonization pattern showed heterogeneity among the roots of the cropping types (Table 3). The highest hyphal colonization of 73.4% was recorded from maize (mixed crop I) followed by faba bean (mixed crop II) and pepper (monocrop I) with hyphal colonization of 63.4% and 60.3%, respectively. The least root colonization was recorded from

eucalyptus (*Eucalyptus globulus*) and teff (*Eragrostis teff*) monocrops with root colonization of 22.4  $(\%)$  and 21.7  $(\%)$ , respectively (p=0.011).

Table 3. Spore density and mycorrhization of different land use types from Jabi Tehan Woreda, West Gojam.

<b>Land use</b>	<b>Crop</b>	$SD/100$ g of soil	<b>RLA</b> $(\%)$	$RLV$ $(\% )$	$RLH$ $(\frac{9}{6})$
Monocrop I	Pepper	523c	6.1a	2.3 <sub>bc</sub>	60.3 <sub>b</sub>
Monocrop II	Finger millet	581c	8.2a	0.5d	45.6c
Monocrop III	Teff	479cd	0.9c	3.1 <sub>b</sub>	21.7e
Monocrop IV	Niger	554c	1.2c	4.2a	35.7d
	$Maize +$		3.5 <sub>b</sub>	0.3d	73.4a
Mixed crop I	$S$ unflower +	929a	3b	0.1d	30d
	Cabbage		$\mathbf{0}$	$\mathbf{0}$	$\mathbf{0}$
Mixed crop II	Faba bean +	823b	0.5c	1.2c	63.4b
	Cabbage		$\mathbf{0}$	$\Omega$	$\Omega$
Monocrop/tree I	Eucalyptus	104e	2.1 <sub>b</sub>	1.1c	22.4e
Mixed Tree-crop	$Croton +$	431d	7.5a	2.1 <sub>bc</sub>	55.7b
П	Juniperus		7a	2c	53b

SD: spore density; RLA: Root Length Arbuscules; RLV: Root Length Vesicles; RLH: Root Length with Hyphae. Data are reported as averages and standard errors for three replicates per land use type. Values followed by different letters in a column denote significant differences among land use types according to Fisher's LSD test at 5% level of significance.

On the contrary, other studies showed that eucalyptus tree and teff had higher mycorrhization rate of 34.8–55.9% (Jones *et al*., 1998), 20–80% (dos Santos *et al*., 2001) and 31–60% (Tekalign Mamo and Killhalm, 1987), respectively. Similarly, lower rate of root infection of pepper (10–20%) (Castillo *et al*., 2013) and higher rate of mycorrhization of 72.7% were detected in southern Ethiopia (Beyene Dobo *et al*., 2016). The root colonization of maize (73.4%) in this study was much higher than the one (36.5–37.5%) reported by Sasvari and Posta (2010), but lower than 80.6% reported by Beyene Dobo *et al.* (2016).

In general, AMF colonization levels in mixed cropping systems were high compared to single cropping systems. There was no statistically significant correlation between RLA, RLV, RLH, pH, TN, OC, AVP and AVK in either land use types (data not shown). However, a significant positive correlation ( $r=0.76$ ;  $p=0.027$ ) between AMF colonization and spore density was observed in this study which is consistent with the reports of Zerihun Belay *et al*. (2013; 2014). However, Beyene Dobo *et al*. (2016) found no relationship between the percentage of root colonization and spore density. It is suggested that the biotic and abiotic factors such as the type of fungal species, plant host and soil nutrients influenced the parameters (Stutz and Morton, 1996).

# **Species diversity of arbuscular mycorrhizal fungi**

In this study, a total of 12 AMF genera and 42 morphospecies were identified from the different cropping systems (Table 4). This is similar to a total number of more 41 and 42 morphospecies in 14 and 15 genera recorded from the different land use types in a dry and humid agroecosystem of central Rift Valley Ethiopia (Zerihun Belay *et al*., 2013) and Showa Robit, Ethiopia (Zerihun Belay *et al*., 2015), respectively. Although the number of morphospecies were similar with the one reported from the Sudan (42 AMF species), the number of genera were lower than the same report (12 genera) made by Abdelhalim *et al*. (2013).

Amongst the AMF genera, the highest number of species was recorded from the genus *Acaulospora* (15 species), followed by the genera *Clariodeoglomus*, *Funneliformis* and *Rhizophagus* (4 species each) (Table 4). 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 (Jefwa *et al*., 2009). The dominance of *Acaulospora* was also recorded from previous agroecosystem studies where the two genera *Acaulospora* and *Glomus* were represented by 9 species each (Zerihun Belay *et al*., 2015) and *Acaulospora* (9 species) and *Funniliformis* (6 species) were identified (Zerihun Belay *et al*., 2013).

In general, the genera *Acaulospora*, *Glomus*, and *Funniliformis* were widespread irrespective of the species number and the agroecosystems. The dominance of these genera may be related to their sporogenous characteristics, i.e., the production of relatively small spores within a short period of time compared to the large spores of the genera *Gigaspora* and *Scutellospora* (Hepper, 1984; Bever *et al*., 1996).

Based upon the IV value, the different AMF genera were generally categorized into "Commonly distributed" and "Rarely distributed" species across the different land use types (Table 4). None of the identified AMF species found to be dominant (IV≥50%) according to Chen *et al*. (2012). This is different from the report of Zerihun Belay *et al*. (2014), where the genera *Claroideoglomus* and *Glomus* were categorized into the dominant genera with IV of 59.4% and 53%, respectively. Furthermore, all species from *Rhizophagus* were categorized into the "Common" group (with IV 10 %<X<50%) together with many species of *Claroideoglomus* (75%) *Acaulospora* (60%), and *Funniliformis* and *Diversispora* (50% each) distributed in many of the cropping systems. However, all species from *Gigaspora* and *Scuttelospora* were "Rare (IV<10%)" and distributed in one or the other land use types (Table 4).

With regard to AMF species richness among the cropping systems, the mixed crops (annuals) harboured the majority of the species 36 (85.7%) followed by monocrops 21 (50%). Likewise, the mixed tree cropping (Croton + Podocarpus) harboured 8 species (19%) compared to 4 species (9.5%) from the mono-tree eucalyptus land use type (Table 5). The fact that the maximum number of species detected from mixed cropping system (cabbage + sunflower + maize) (85.7%) compared to the monocrops  $(50%)$ combined together showed that the monocrops, maize, sunflower and faba bean are mycorrhizal without the contribution of cabbage which is nonmycorrhizal (Plenchette *et al*., 1983).

This result is much higher than the 11 species detected from four genera, *Acaulospora*, *Glomus*, *Scuttelospora* and *Gigaspora* reported from a Maize/Sesbania intercrops and maize monocrop systems in southern Malawi (Jefwa *et al*., 2006). It suggested that coexisting plant species within a habitat are associated with divergent AMF communities and host preference has a strong influence on AMF community composition in soil (Vandenkoornhuyse *et al*., 2002; Scheublin *et al*., 2004). Diriba Muleta *et al*. (2008) found higher abundance of AMF spores in agroforestry (mixed cropping) systems especially when legumes served as shade crops than in monocultural systems in south west Ethiopia.

In this study, AMF species diversity was much lower in tree-based cropping system (Eucalyptus) or mixed Croton + Juniperus plantation than in the annual cropping systems (monocrops, mixed crops). Even though mixed cropping is known to enhance more AMF diversity, Jefwa *et al*. (2006) observed lower species diversity of AMF in agroforestry systems with a tree *Sesbania macrantha* and *S. sesban* than in maize monocrops, and suggested that the higher species diversity in the maize fields was due to the short maize cropping season, inducing rapid root dynamics and turnover, as compared to the much longer growth cycles of the agroforestry plots. Although monocrops are considered as diverse in this study, the data showed that individual crops were significantly different in spore counts, mycorrhization and AMF diversity (Table 3, 5). However, several reports showed that maize and faba bean are characterized by high mycorrhization and high diversity of AM fungi (Sasvari and Posta, 2010).



Table 4. The relative distribution of the different AMF genera and morphospecies from the different cropping systems of Jabi Tehnan Woreda, West Gojam.







\*: Genera; MoCL: monocropped land; MiCL: mixed cropped land; MoTL: monotreeland; MiTL: mixed treeland, IF: Isolation frequency; RA: Relative abundance; IV: Important value

AMF genera	<b>Monocrops</b>	<b>Mixed crop</b>	<b>Monocrop</b> trees <b>Eucalyptus</b>	<b>Mixed trees</b> Croton+Juniperus
Acaulospora	9	13		3
Clarioideoglomus	2	3		
Cetraspora				
Dentiscutata	$\overline{2}$			
Diversispora	3			
Funneliformis		4		
Gigaspora				
Paraglomus				
Racocetra				
Rhizophagus	3			
Scuttelospora				
Septoglomus				
<b>Total</b>	21	36	4	8
Percentage $(\% )$	50%	85.7%	$9.5\%$	19%

Table 5. Distribution of the different AMF species on the different cropping systems of Jabi Tehnan Woreda, West Gojam.

Among the AMF species, the relatively dominant species were *Di. tortuosa* and *F. geosporum*, that were distributed in all cropping systems, whereas *R. clarus*, *G. luteum*, *A. denticulata* and *A. rehemi* were detected from most of the sites, and the other group (54%) were found across at least two of the cropping systems (data not shown). It was also shown that a little over 42% of the AMF species were restricted to only one of the fields of the land use types.

However, it is interesting to note that the dominant species in the mixed cropping in this study were the genera *Acaulospora* with 36% of species and *Funneliformis* with 11% of species; likewise, the dominant one in Ethiopia was the genus *Acaulospora* and *Funneliformis* each with 13% species under the similar sunflower-tef-sesame mixed cropping system (Zerihun Belay *et al.*, 2015).

In conclusion, this study confirms the low diversity and density of AMF spores in tree-based cropping system than in the annual cropping system suggesting that the rapid root dynamics and turnover in the short seasoned crops may enhance the maintenance of AMF community. Moreover, mixed crops harboured higher AMF diversity than the monocrops showing that the coexisting plant species have a strong influence on AMF community.

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