RESEARCH ARTICLE

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Diversity of arbuscular mycorrhizal fungi under the rhizosphere soil of different cropping systems

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ABSTRACT
This study has been conducted to evaluate the diversity of Arbuscular Mycorrhizal Fungi (AMF) under the rhizosphere soil of different monocrops in the grazing and croplands. A descriptive cross-sectional survey was employed to understand the rate of root colonization and diversity of AMF. 23 different types of AMF morphospecies in 12 AMF genera were isolated across 12 different types of land covers. The AMF under the rhizosphere soil of Eucalyptus tree has shown the highest biomass compared to all other land uses, with spore density of 1907.4±0.404 $100g^{-1}$ of dry soil. The lowest AMF biomass has been recorded in the rhizosphere soil of the Mango tree, with a spore density of 260.1±0.121 $100g^{-1}$ dry soil. The total root colonization (RLC) of the various land covers by AMF ranges from 11.15-85.41%. Finally, further study on the implication of agricultural inputs on the microbial community under different cropping systems is recommended.

Key words: Arbuscular Mycorrhizal Fungi, Colonization, Diversity, Hawassa, Spore density

Introduction

Arbuscular Mycorrhizal Fungi (AMF) is a group of obligate biotrophs, to the extent that they must develop a close symbiotic association with the roots of a living host plant to grow and complete their life cycle (Wahab et al., 2023). The term "mycorrhiza" literally derives from the Greek 'mykes' and 'rhiza', meaning fungus and root, respectively. AMF can symbiotically interact with more than 80% of the plants on the Earth. Molecular DNA sequencing-based analyses have recently contributed to a great extent by shedding light on a previously unseen and profound diversity within this phylum (Schüßler & Walker, 2011). AMF is found in the roots of about 80-90% of plant species (mainly grasses, crops, and herbs) and exchange benefits with their partners, as is typical of all mutual symbiotic relationships (Wang & Qiu, 2006). They represent an interface between plants and soil, growing their mycelia both inside and outside the plant roots. AMF provides the plant with water, soil mineral nutrients (mainly phosphorus and nitrogen), and protection from pathogens. In exchange, photosynthetic compounds are transferred to the fungus (Shi et al., 2023; Bonfante and Genre, 2010). AMF 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 microorganisms (Olsson et al., 1999).

The populations of AM fungi are greatest in plant communities with high diversity such as tropical rainforests and temperate grasslands where they have many potential host plants and can take advantage of their ability to colonize a broad host range (Smith & Read, 2002). There is a lower incidence of mycorrhizal colonization in very arid or nutrient-rich soils. Mycorrhizas have been observed in aquatic habitats; however, waterlogged soils have been shown to decrease colonization in some species (Smith & Read, 2002).

However, the effects of factors such as plant community in the AMF community composition are less clear (Horn et al 2017; Zobel & OÈ pik, 2014). In general, the infectivity and diversity of AMF communities are often reduced in disturbed habitats such as agro-ecosystems or post-mining sites (PuÈschel et al., 2008). The specificity, host range, and degree of colonization of mycorrhizal fungi are difficult to analyze in the field due to the complexity of interactions between the fungi within a root and the system. There is no clear evidence to suggest that arbuscular mycorrhizal fungi exhibit specificity for colonization of potential AM host plant species as do fungal pathogens for their host plants (Smith & Read, 2002).

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Though they are few, scholars in Ethiopia indicated that the difference in the cropping system affected the diversity of AMF. For instance, according to Zerihun et al. (2013), land use types drastically affected AMF colonization and AMF diversity in a dry land agroforestry system in the central part of Ethiopia. Besides, the study carried out by Mengsteab et al.*(*2013) and Diriba et al. (2008) revealed that the number of spore counts was significantly higher under the canopy of trees than outside the canopy. Meanwhile, the unpublished study conducted in the Northern part of Ethiopia, Jabi Tehnan woreda western Gojam *by* Moges (2014) reported that spore density of the different cropping systems varied significantly within and between land-use types. Moreover, the study findings of Tesfaye et al. (2009) confirm distinct fungal communities associated with the diverse tree species.

Studies done by Beyene et al. (2016b), in Sidama region, South Ethiopia, revealed the role of the physicochemical nature of the soil in AMF abundance and density. In that, the soil with pH of 6.18-6.28 and texture of clay loam and sandy loam favored mycorrhizal development. Besides, Beyene et al. (2016b) reported favorable AMF growth at moderate to low concentrations of P and N.

Information regarding the diversity of AMF under the rhizosphere soil of various cropping systems in Ethiopia is very minimal. Particularly, very little or nothing is known regarding the AMF diversity associated with the cropping system and soil in the Hawassa area. Nonetheless, trends of AMF diversity particularly associated with the use of inorganic fertilizers are also seeking attention in this specific area.

This kind of study would attribute to the food security and agricultural development agendas of the nation. By 2050, global agriculture will have the task of doubling food production to feed the world (Ray et al., 2013). At the same time, dependence on inorganic fertilizers and pesticides must be reduced. For these reasons, significant advances in AMF research are needed to allow their stable use in agriculture. Their application and synergistic combination with other functionally efficient microbial consortia that include PGPR (Plant Growth Promoting Rhizobacteria), saprophytic fungi, and other helper microorganisms will help farmers develop a more sustainable cropping system (Castiglione et al., 2021; Dodd & Ruiz-Lozano, 2012). Hence, the findings of the study substantiate knowledge regarding the diversity and abundance of AMF across the different cropping systems around Hawassa city, Sidama regional state of Ethiopia.

Materials and Methods

Study area Description

The study has been undertaken at randomly selected agricultural plots and open grassland allotted for livestock grazing. All the farm plots and the grassland are located at the outskirt of the Hawassa area. Hawassa is located in the north-eastern part of the SNNP region and is bounded by the Oromiya region in the North, Shebedeno district in the south Wondogenet district in the east, and Lake Hawassa in the west. Its geographic location lies between $6^0 91'$ and $7^0 10'$ North latitude and $38^0 41'$ and $38^0 55'$East longitude, with altitude ranges from 1501-2500 m.a.s.l. The total area of Hawassa City is about 271 Km^2. As per the 2014/15 statistical abstract of the SNNPR, the population of Hawassa city administration reached 355,610. The average population density of the city is 1,312 people per/ km^2 of which 35% of the population represents the rural inhabitants, who mainly rely on agricultural activity to support their livelihood (SNNPR-BoFED, 2014/15).

The rural portion of the Hawassa administration covers about 11,940 hectares. The land use of this rural area is characterized as annual crops(2,409 ha), permanent crops (3,360 ha), grazing land (850 ha), forest (1,156 ha), potentially cultivable (275 ha), uncultivable (165 ha), and others (3,725 ha). The mean annual rainfall of the area ranges from 801-1400 mm. The mean annual temperature of the area ranges between 17-22.5 ^{0}C (SNNPR-BoFED, 2014/15).

Study design

A descriptive cross-sectional field survey is employed to understand the rate of root colonization and diversity of AMF under the rhizosphere soils of permanent crops, annual major crops, forest lands, and open grazing fields.

Field Sample Collection

Soil and root samples were collected to determine the AMF diversity and evaluate root colonization respectively. Sampling was carried out in randomly selected croplands (organically cultivated tomato, inorganically cultivated tomato, Enset, Coffee, Maize, Eucalyptus, Wanza, Banana, Shewshewe (Oak), Mango, Pepper, and an open grazing field. Sample collection was made from Feb-April 2018.

Soil and Root Sample Collection

A composite soil of 300 g, within a depth of 0-20 cm, was collected from the rhizosphere of each studied plant (Table 1). The soil samples were air-dried and packed individually in sterile polythene bags and stored at 4º C until processing for extraction and enumeration of AM fungal spores. A total of (12x3) soil samples were collected. The soils samples collected from each site were divided into three portions to enable three times replication and transported to the Microbiology laboratory at Hawassa teacher training center. Recovery of very fine roots of AMF, from the rhizosphere of each studied plant, was realized through the collection of fine root samples of 0.5 mg (12x3) and it has been cut into 1 cm pieces, washed with tap water, put in a test tube and

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preserved in 50% ethanol and stored at 4 ^{0}C for further analysis (Zhao et al., 2001).

intersection) respectively. All were quantified by examining 100-150 intersections per sample.

Table 1: *Georeferenced rhizosphere soil and root sampling points, Hawassa, Ethiopia*

Scientific Name	Local (Common) Name	Altitude	Latitude	Longitude
Solanum lycopersicum	Tomato Inorganic	1679	07^{0}00' 371"	038^0 27' 670"
Zea mays	Maize	1675	07^{0}00' 375"	038^0 27' 692"
Ensete ventricosum	Enset	1677	07^{0}00' 369"	038^0 27' 694"
Solanum lycopersicum	Tomato (Organic)	1677	07^{0}00' 369"	038^0 27' 694"
Coffea arabica	Coffee	1678	07^{0}00' 370"	038^0 27' 690"
Eucalyptus globulus	Eucalyptus	1687	07^{0}05' 48.9"	038^{0}29' 16.6"
Cordia africana	Wanza	1683	07^{0}05' 48.0"	038^{0}29' 16.3"
Musa acuminata	Banana	1686	07^{0}50' 48.1"	038^{0}25' 16.5"
Casuarina equisetifolia	Shewshewe (Oak)	1685	07^{0}05' 48.7"	038^{0}29' 15.8"
Mangifera indica	Mango	1687	07^{0}05' 48.9"	038^{0}29' 16.6"
Capsicum annuum 'Serrano'	Pepper	1690	07^{0}50' 48.1"	038^{0}25' 16.5"

NB: Agricultural input use is classified as organic and inorganic. It is based on the information received from private farm owners and personal observation in the field. Organic: Refers to the use of no industrially processed agrochemicals and/or fertilizers as an input to help hasten plant growth and protection from disease. Inorganic refers to use of industrially processed agrochemicals and /or fertilizers

Sample Analysis

Analysis of the rhizosphere soil and roots were made to investigate AMF diversity and estimate the rate of root colonization. It was an engagement from May-Aug, 2018.

Investigation of AMF root colonization of study plants

AMF root colonization was assessed according to Phillips & Hayman (1970). Root samples were washed several times with tap water and cleared in 10% (w/v) KOH by heating in a water bath at 90°C for 1-2 hrs and cooled at room temperature. After cooling, the root samples were washed 3-5 times with tap water, acidified in 1% HCl for 1hr and stained with 0.05% trypan blue, and finally disdained in acidic glycerol. The AM fungal structures were observed under a compound light microscope (Olympus-bx 51) at 200 fold magnification. Fungal colonization was estimated using the magnified intersection method of (McGonigle et al.,1990) as total root length colonization RLC=100 [(G-N)/G], the percentage of root length colonized by arbuscules, arbuscular colonization AC = 100 (A/G), and the percentage of root length colonized by mycorrhizal vesicles, vesicular colonization VC = 100 (V/G). RLC, N, A, V, and G are designated as RLC (total root length colonization), N (no fungal structure), A (arbuscules), V (vesicles), and G (total

Identification and quantification of AMF spores within the rhizosphere soil of study plants

AMF spore diversity (species range and enumeration) work was determined by rendering methodology by Brundrett et al. (1996). Accordingly, 100g of each soil sample were suspended into a 2-liter container and mixed vigorously to free spores from the soil and roots. The supernatant was subsequently decanted through standard sieves (480, 106, 50 & 38 μm) after having been intermittently centrifuged at 2000 rpm for 5 minutes. The last pellet (38 μm) was suspended in 60% sucrose solution and thoroughly mixed and centrifuged at 2000 rpm for 1 minute to collect the spores. The spores and sporocarps, from the upper solution of the centrifuged tube, were then be rinsed with tap water and transferred into plastic Petri-dishes. Counting has been made under 4x stereomicroscope according to http://invam.caf.wvu.edu, and spore density was expressed as the number of spores and sporocarps per 100 g of dry soil. Healthy looking spores were collected and mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) to identify them into the representative morpho-species based on the descriptions of the International Culture Collection of Vesicular/Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu; 2006) and following descriptions

by (Schenck & Perez,1990) using a compound light microscope (Olympus-bx 51) at X200 magnification.

Quantitative study or spore enumeration work was done according to INVAM, http://invam.caf.wvu.edu.as follow. First using a fine ruler, the diameter of the ocular field of the stereomicroscope at a magnification of 40X was determined (where spores can be easily distinguished from mineral particles and organic debris). The area of the spherical field was calculated at that magnification (40X). It was having a diameter of 5 mm and the area of the field was 19.6 mm2 (radius 2.5mm). Plastic Petri dishes were used to count spores because the base of the plate is flat. Because the dish also is hydrophobic, enough water is added to have complete coverage of the base. Those dishes which were 85 mm across were used. That is the area of the base that was calculated to be 5672 mm2. From this datum and the area of the ocular field (19.5 mm 2), and the total number of fields in the dish was: 5672/19.6. After that, the extracted spore suspension was added to a petri dish and then the dish was rotated randomly to spread out spores as evenly as possible. Finally, spores were counted in 40 fields randomly chosen over the area of the dish. An average number of spores per field was calculated and multiplied by 289 (#fields/dish).

Determination of AMF diversity

The AMF communities from(12x3) sampling areas were detected and calculated based on the following parameters: Spore density (SD) was expressed as the number of AMF spores 100 g-1 soil. Species richness (S) was measured as the total number of morpho-species. The Shannon–Wiener index (H′) of diversity was calculated using the formula: H'= -Σ ((ni /n) ln (ni/n)) where: ni = 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 = Σ (ni/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).

Data Analysis

Data analysis was carried out with SPSS software (version 21). Data on the percentage of AM colonization was transformed by arcsine X½ and spore densities were transformed by log (x+1)to fulfill 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 among the cropping plants of the fields. Mean separation was done by Duncan's multiple range test at the 0.05 level of probability.

Results and Discussion

Results of studied soil physicochemical properties are given in Table 2 below. The positive association between spore density and percentage of OC and total Nitrogen was identified, yet the association was not statistically significant (r= 0.514, P<0.088 and r=0.513, P< 0.088 respectively). Similarly, the spore density of AMF was associated positively with that of OC and total Nitrogen (r=0.411 P<0.185 and r=0.409, P<0.187 respectively), nonetheless it was not a statistically significant association.

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Table 2. *Physicochemical results of rhizosphere soil sampled from the study area, Hawassa Ethiopia*

Crop	pH	EC(dS/m)	C (%)	AvN (%)	Av P(ppm)	Texture			Texture Class
						Sand (%)	Clay (%)	Silt (%)	
Coffee	7.58	553	1.56	0.134	10.4	59	22	19	Sand Clay Loam
Mango	7.34	565	1.17	0.101	11.6	55	24	21	Sand Clay Loam
Wanza	7.04	992	3.90	0.336	15.8	51	26	23	Sand Clay Loam
Maize	5.05	1413	1.95	0.168	11.2	69	20	11	Sandy Loam
Shewshewe	6.41	332	1.17	0.101	10.2	37	46	17	Clay
Banana	5.11	1449	2.34	0.202	14.2	65	26	9	Sand Clay Loam
Tomato (org)	7.24	392	1.56	0.134	9.2	67	24	9	Sand Clay Loam
Eucalyptus	6.26	715	1.56	0.134	9.0	69	24	7	Sand Clay Loam
Open Grass Land	7.32	210	0.78	0.067	8.8	53	32	15	Sand Clay Loam
Pepper	6.76	1843	1.56	0.134	9.6	57	28	15	Sand Clay Loam
Tomato (inorganic)	6.55	602	1.36	0.118	8.2	59	22	19	Sand Clay Loam

Key: EC=Electrical conductivity, C=Carbon, AvN= Availabele Nitrogen, AvP= Available phosphorus

AMF Community abundance

This study which has been conducted in Hawassa area districts of Sidama region state uncovered the presence of 1046 spores of 23 different types of AMF morpho-species (Table 3) in 12 AMF genera (Table 4). The genera are *Acaulospora, Cetraspora, Claroideoglomus, Dentiscutata, Diversispora, Funneliformis, Gigaspora, Glomus, Racocetra, Rhizophagus, Sclerocystis, and Scutellospora*. Correspondingly, the study conducted in South Ethiopia, Sidama area at agroforestry practicing lands of Shebedino & Wensho districts by Beyene et al. (2016b) had indicated the presence of about 29 morpho-species belonging to 9 genera (*Acaulospora, Glomus, Claroideoglomus*, *Funneliformis*, *Pacispora, Septoglomus, Rhizophagus, Scutellospora,* and *Gigaspora*). Here, we could appreciate about 77.78% of similarity in the type of AMF genera abundant in the two study outputs. It might be attributed due to the similarity in physicochemical (moderately acidic and loam soil) feature of the studied areas, regardless of the difference in the type of crops studied.

In another study done in Ethiopia by Zerihun et al. (2015) a total of 42 AMF morpho-species belonging to 15 genera were identified. Similarly, the AMF diversity study in13 selected crops in Sudan (Abdelhalim et al., 2013), in which 42 AMF species belonging to 12 genera were discovered. As we compare the similarity of the current study in the abundance of AMF genera, there is about 83.33% of resemblance with that of Zerihun et al. (2015), it most probably attributed due to the similarity in some important soil physicochemical characteristics (Similarity in soil pH, available phosphorus, and total nitrogen level). However, the land cover of the study by Zerihun et al. *(*2015) and that of the present one have shown few similarities; nonetheless in both studies land covers such as maize, coffee, tomato, Open Grass Lad were observed.

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Table 3. *Morphological features used to identify AMF spores (INVAM, http://invam.caf.wvu.edu.)*

Sn	Shape	Color	Diameter Range(µm)	Spore Wall Layers	Germinal Wall	Subtending Hyphae	Species Identified
1	Globose to Sub globose	Pale yellow-brown	100-130	Three layers (L1, L2, and L3	Two walls (GW1,GW2)	Hyphae are coiled mostly near entry points	*Acaulospora dilatata*
2	Globose to sub globose, sometimes irregular.	Red-orange Dark red-brown	240-360	Three layers (L1, L2, and L3)	Two walls (GW1,GW2)	Funnel-shaped	*Acaulospora foveata*
3	sub globose or globose	dark brown or black	150-175	Two layers (L1, L2)		Characteristic dark swelling	*Acaulospora sporocarpia*
4	Globose, subglobose	Hyaline/white - yellow-brown	120-240	Three layers (L1, L2, and L3)	Two walls(GW1,GW2)	Densely coiled near the entry point	*Cetraspora pellucida*
5	Globose, subglobose	Pale yellow-Pale orange	60-160	Two layers (L1, L2)		Interconnected Hyphae	*Claroideoglomus etunicatum*
6	Mostly globose.	Dark red-brown	240-520	Two layers (L1, L2)	Two walls(GW1,GW2)		*Dentiscutata nigra*
7	Globose to sub globose	yellow-brown	160-320	Three layers (L1, L2, and L3)		coenocytic or sparsely septate, thin-walled hyphae	*Diversispora globifera*
8	Globose to sub globose	Pale orange-brown	120-220	One Layer (L1)	-	Cylindrical to slightly flared	*Diversispora tortuosa*
9	Globose to sub globose	Straw to dark orange-brown	100-260	Three layers (L1, L2, and L3)	-	Flared to funnel-shaped	*Funneliformis mosseae*
10	Globose to sub globose	pale yellow-brown	160-280	Three layers (L1, L2, and L3)	-	Tightly coiled hyaline hyphae	*Gigaspora rosea*
11	Globose to sub globose	Bright greenish-yellow to bright yellow-green	240-400	Three layers (L1, L2, and L3)	-	Tightly coiled hyaline hyphae	*Gigaspora gigantea*
12	Globose to sub globose	Pale Yellow-Light Yellow	280-620	One Layer (L1)	No		*Glomus tortusum*
13	Globose to sub globose	milky white (opaque	150-220	One Layer (L1)		Two subtending hyphae then merge into one	*Glomus lacteum*
14	Sub globose to irregular-	Pale Yellow	150-220	One Layer (L1)	-	Usually cylindrical to slightly flared.	Glomus coremioides
15	Globose to sub globose	Red-brown to dark red-brown	380-520	Two layers (L1, L2)	One wall (GW1)	Coiled hyaline hyphae	Racocetra gregaria
16	Globose to sub globose, ovoid, oblong, or irregular/knobby	Hyaline to yellow-brown	70-165	Three layers (L1, L2, and L3)		Usually cylindrical to slightly flared.	Rhizophagus irregularis
17	Globose, obovate, to irregular	yellow in color	40-120	Two layers (L1, L2)		Usually is single but occasionally double	Rhizophagus aggregatus

Table 3 (Cont'd). *Morphological features used to identify AMF spores (INVAM, http://invam.caf.wvu.edu.)*

Sn	Shape	Color	Diameter Range(µm)	Spore Wall Layers	Germinal Wall	Subtending Hyphae	Species Identified
18	Globose to sub globose, some irregular	Hyaline to white.	60-120	Three layers (L1, L2, and L3)		Almost exclusively of intraradical hyphae	Rhizophagus diaphanous
19	Globose, subglobose	White, pale cream to yellow-brown	40-140	Three layers (L1, L2, and L3)		Cylindrical to flared with 3 layers	Rhizophagus intraradices
20	Globose, subglobose	white to very pale yellow	100-260	Three layers (L1, L2, and L3)		Cylindrical to flared with 3 layers	Rhizophagus clarus
21	Globose, subglobose	white to yellow-brown/yellow	100-260	Three layers (L1, L2, and L3)		Cylindrical to flared with 3 layers	Rhizophagus manihotis
22	Globose, subglobose	orange-brown to dark orange-brown	200-360	One Layer (L1)		Cylindrical to flared with 1 layer	Sclerocystis sinuosum
23	Globose, subglobose	Hyaline/white to yellow-brown	340-640	Three layers (L1, L2, and L3) but L1 & L2 are more conspicuous	Three walls(GW 1,GW2,G W3)	two-layered brownish-yellow in color	Scutellospora scutata

Table 4. *Recovered AMF genera from the rhizosphere soils of different land covers, Hawassa Ethiopia*

No	Genus	Monocrops
1	*Acaulospora*	Maize, Enset, Open Grass Land
2	*Cetraspora*	Maize
3	*Claroideoglomus*	Eucalyptus, Open Grass Land, Wanza, Mango, Pepper
4	*Dentiscutata*	Enset, Tomato Organic
5	*Diversispora*	Maize, Coffee
6	*Funneliformis*	Open Grass Land, Eucalyptus, Wanza, Pepper
7	*Gigaspora*	Tomato Inorganic Enset, Tomato Organic, Wanza, Shewshewe
8	*Glomus*	Eucalyptus, Wanza, Banana, Mango, Pepper
9	*Racocetra*	Coffee
10	*Rhizophagus*	Maize, Coffee, Enset, Wanza, Tomato Organic, Banana, Shewshewe, Mango
11	*Sclerocystis*	Tomato Inorganic, Maize, Enset, Tomato Organic
12	*Scutellospora*	Open Grass Land, Eucalyptus, Wanza, Mango, Pepper

The genera *Glomus, Funneliformis, Claroideoglomus, Acaulospora, Diversispora, Gigaspora, Rhizophagus, Racocetra, Sclerocystis,* and *Scutellospora* were detected in this study and that of Zerihun et al. (2015). However, the similarity in AMF genera abundance of this study with the findings in Sudan White Nile State was only 41.67%. It may be attributed due to the difference in the soil physicochemical feature and the studied crop types. The texture of Hawassa vicinity soil of the specified studied site had not more than 46% clay, whereas that of the White Nile in Sudan had 60% of Clay, pH of the current study soil was neutral, with very low available phosphorus & total nitrogen unlike that of the Sudanese, which was alkaline, very low in available phosphorus, yet highly enriched with total nitrogen. Moreover, out of the 13 studied crops in Sudan, only mango and banana are similar to the present study, whereas the remaining 11 croplands are not similar to that of the current study. Common genera *Glomus, Funneliformis,*

Table 5. *AMF species composition per cropping system, Hawassa, SNNPR*

	AMF Species Identified											
Crop	***G.ro***	**G.*gi***	**S.*si***	**C.*pe***	**A.*di***	**R.*ir***	**D.*gl***	**R.*ag***	**R.*di***	**A.*sp***	**D.*ni***	**R.*in***
S.lyc*	+	+	+	-	-	-	-	-	-	-	-	-
Z..may	-	-	+	+	+	+	+	+	+	+	-	-
E.ven	+	-	+	-	-	+	-	-	-	+	+	+
OGL	-	-		-	-	-	-	-	-	+	-	-
S.lyc**	+	-	+	-	-	-	-	-	+		+	-
C.ara	-	-	-	-	-	-	-	-	+	+	-	+
E.glo	-	-	-	-	-	-	-	-	-	-	-	
C.afr	+	-	-	-	-	-	-	-	-	-	-	
M.acu	-	-	-	-	-	-	-	+	-	+	-	+
C.equ	-	+	-	-	-	-	-	-	-	+	-	-
M.ind	-	-	-	-	-	-	-	-	-	+	-	+
C.ann	-	-	-	-	-	-	-	-	-	+	-	
	AMF Species Identified											
Crop	**A.*fo***	**F.*mo***	**S.*sc***	**C.*et***	**D.*to***	**R.*cl***	**R.*gr***	**G.*to***	**G.*co***	**G.*la***	**R.*ma***	
S.lyc*	-	-	-	-	-	-	-	-	-	-	-	
Z..may	-	-	-	-	-	-	-	-	-	-	-	
E.ven	+	-	-	-	-	-	-	-	-	-	-	
OGL	-	+	+	+	-	-	-	-	-	-	-	
S.lyc**	-	-	-	-	-	-	-	-	-	-	-	
C.ara					+	+	+	-	-	-	-	
E.glo		+	+	+	-	-	-	+	-	-	-	
C.afr		+	+	+		+	-	+	-	-	-	
M.acu	+	-	-	-	-	-	-	-	+	+	+	
C.equ	+	-	-	-	-	-	-	-	-	-	+	
M.ind	-	-	+	+	-	-	-	+	-	-	-	
C.ann	-	+	+	+	-	-	-	+	-	-	-	

Key: Plant species: S.lyc*: Solanum lycopersicum Inorganic; M.acu: Musa acuminata; OGL: Open Grass Land; C.ann: Capsicum annuum 'Serrano'; Z..may :Zea mays; C.equ: Casuarina equisetifolia; E.ven: Ensete ventricosum; M.ind: Mangifera indica: S.lyc**: Solanum lycopersicum Organic C.ara: Coffea arabica; E.glo: Eucalyptus globules; C.afr: Cordia Africana

Key: AMF species:

G.*ro*: *Gigaspora rosea*
G.*gi*: *Gigaspora gigantea*
S.*si*: *Sclerocystis sinuosum*
C.*pe: Cetraspora pellucida*
A.*di*: *Acaulospora dilatata*
R.ir: Rhizophagus irregularis
S.*sc: Scutellospora scutata*
R.*ma: Rhizophagus manihoti*
D.*gl: Diversispora globifera*
R.*ag*: *Rhizophagus aggregatus*
R.*di: Rhizophagus diaphanus*
A.*sp: Acaulospora sporocarpia*
A.*fo*: *Acaulospora foveata*
D.*ni: Dentiscutata nigra*
R.*in: Rhizophagus intraradices*
F.mo: *Funneliformis mosseae*
C.*et: Claroideoglomus etunicatum*
D.*to*: *Diversispora tortuosa*
R.*cl*: *Rhizophagus clarus*
R.*gr*: *Racocetra gregaria*
G.*to*: *Glomus tortusum*
G.*co*: *Glomus coremioides*
G.*la*: *Glomus lacteum*

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Claroideoglomus, Acaulospora, and Diversispora have been discovered in the study output of Abdelhalim et al. (2013)
and that of the present one. In general, we may conclude that AMF crop association may not always be specific as there is a need to consider other governing parameters such as the physicochemical nature of the soil.

Species richness and diversity of AMF

Regarding the occurrence of a particular AMF species in a certain particular land cover, which is merely termed as spore richness, the monocrop maize is the predominant major perennial annual crop of all the studied land covers (Table 5). Under the rhizosphere soil of Maize, 8 different types of AMF species were discovered. Following maize permanent crop Enset has taken the second dominant place with 7 different types of AMF species; it again followed by coffee, Wanza, and Banana with 6 different types of AMF species.

The least species richness has been recorded in tomato plants growing in inorganic means, with species richness of only 3 different types of AMF species. Extensive use of agrochemicals might be the cause for such compromised species richness in inorganically cultivated tomatoes. As this is beyond the scope of this paper, there might be a need to further study the implication of agricultural inputs on the microbial community underneath the soil.

In general, extensive uses of inorganic agricultural inputs harm AMF association. Soils in the conventional agricultural system are AM fungi-impoverished, particularly with regards to several species (Tsiafouli et al., 2015; Eom et al., 2004). Management practices typical of conventional high input systems, particularly P fertilizer application and the use of biocides, are known to be deleterious to AM fungal symbiosis (de Novais et al., 2019; Spagnoletti et al., 2018). This study output corresponds with the findings of Moges (2014), that stated AMF species diversity was much lower in tree-based cropping systems (Eucalyptus) or mixed croton+juniperus plantation than in the annual cropping systems (monocrops, mixed crops). Similarly, study findings of Zerihun et al. (2015) had reported that higher species richness of about 31 was obtained in mixed fruit crops (Avocado, Mango, Coffee, Papaya, Banana, Tomato & Lemon) and 23 mixed Arable lands (Teff, sesame, sunflower) compared with the other systems, such as mono-crops with high agricultural inputs of Sorghum, Maize& Natural forest. The results denote that organic management and diversification of crops enhance AMF diversity in low-input agricultural systems.

The diversity of AMF species across the different land-use types

The diversity of AMF species across the different land-use types has also been determined. In this regard, the diversity of AMF in the rhizosphere soil of the monocrop maize was found to be tremendous of all the crops, with diversity Shannon Weiner Index (H') of 0.793 (Table 6) followed by Enset (0.739). However, the diversity of AMF in Eucalyptus, Open Grass Land, and Shewshewe has shown a similar diversity pattern, with Shannon Weiner Index (H') of 0.678. The rhizosphere soil under the maize crop has demonstrated a difference of about 80.30% more diverse species of AMF compared to the inorganic input using tomato plantation. The diversity of AMF in inorganically cultivated tomatoes was recorded to be the lowest of all sorts of land uses; this study has indicated that it was even lower than that of organically growing tomatoes by about 20.47%. In general infectivity and diversity of AMF Communities are often reduced in disturbed habitats, where there is extensive use of agrochemicals and various agricultural inputs or post-mining sites (PuÈschel et al., 2008). In the current study, the lowest diversity index recorded (H'=0.440) was recorded in inorganically cultivated tomatoes. This finding has been substantiated by other studies. Oehl et al. (2005) have described that agronomic practices such as monoculture cropping, plowing, or fertilization have frequently been observed to harm the amount as well as the diversity of AM fungi present in soils.

Table 6. *AMF species richness and Shannon Weiner index(H') across different land covers*

Land Cover	Species Richness	H'(Shannon Weiner Index)
Solanum lycopersicum(Ing)	3	0.44
Zea mays	8	0.793
Ensete ventricosum	7	0.739
OGL	4	0.530
Solanum lycopersicum (Org))	4	0.530
Coffea arabica	6	0.678
Eucalyptus globules	4	0.530
Cordia africana	6	0.678
Musa acuminate	6	0.678
Casuarina equisetifolia	4	0.530
Mangifera indica	5	0.609
Capsicum annuum 'Serrano'	5	0.609

Ing- *High Biocide using tomato cultivation;* **Org-** *No biocides using tomato;* **OGL-** *Open Grass Land*

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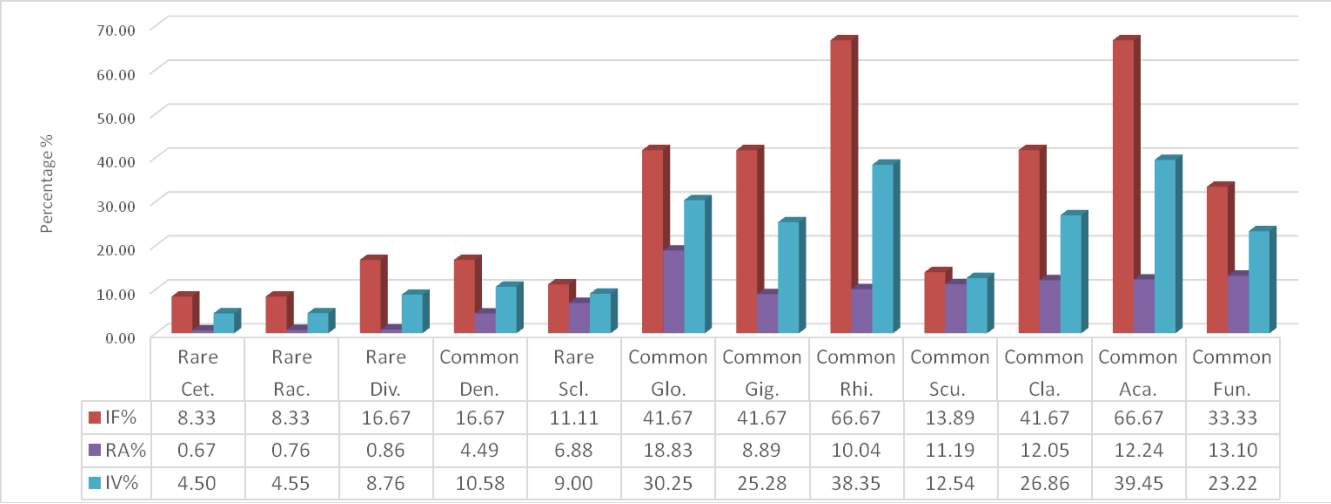
Biomass of AMF in the rhizosphere soil

The biomass of AMF species in the rhizosphere soil across the various land use types has also been determined by measuring the spore density per 100 gm of the sampled soils. In that, the AMF under the rhizosphere soil of Eucalyptus tree has shown the highest biomass compared to all other land uses, with spore density of 1907.4±0.404 spores $100g^{-1}$ of soil. The lowest AMF spore density has been recorded in the rhizosphere soil of inorganically cultivated tomatoes, with a spore density of 375.7±0.000 spores $100g^{-1}$ dry soil. Correspondingly, the study by Cesra et al. (2009) had described that spore density of the different cropping systems varied significantly within and between land-use types ranging from104 spores/100gm soil from Eucalyptus *(E. globulus)* mono (tree) cropping to 929 spores/100gm soil for

mixed cropping system (cabbage+sunflower+maize). Study findings in the Southern Ethiopia of Sidama area has indicated that the highest number of AM spore population was recorded in rhizosphere soils of *Croton macrostachyus (Bisana)* (1066±19.33) and *Catha edulis (*Khat)(1054±53.12) and the lowest spore density was recorded for *Dioscorea alata (*Boyna)(100.00±2.89) spore per 100 g of dry soil. Omitting the probable similarities and differences in the edaphic factor of the two study areas (The current findings and that of Beyene *et al.*, 2016a), the spore density of the similar land covers can be compared. In that, the output of this study area has shown that; *Ensete ventricosum* (Enset), *Coffea arabica* (Coffee), *Zea mays* (Maize), *Mangifera indica* (Mango) & *Cordia africana* has spore densities of 867, 404.6, 876.63, 549.1, and 1522.07/100 gm of soil respectively. Likewise, that of Beyene et al. (2016a) recorded the spore density in the aforementioned land covers as 630, 995,700,580, and 880/100 gm of soil respectively; this, in general, has shown discrepancies between the study outputs, which may be attributed to the physicochemical and management factors of the crops. Unlike the findings of Tadesse & Fasil (2013), who reported the number of spores produced by AMF in all rhizosphere soils of coffee forests as ranging from 578 to 1313 spores/100 g of dry soil the output of this study has indicated that the spore density of coffee to stand at 404.6/100 gm of soil. The result of the present study has revealed that mono-cropping and the use of biocides do negatively attribute to the reduction in the AMF biomass and limitation of AMF diversity in the soil. Moreover, the physicochemical features of the soils do play a role in the density & diversity of AMF; as the AMF biomass and diversity have been demonstrated to differ even within similar monocrops.

Relative abundance (RA), Isolation frequency (IF), and Importance value (IV)

The relative abundance (RA) refers to the spore production potential of AMF. In this regard, the current study reported that the top six genera in declining order of spore production from the total recorded 1046 spores as *Glomus* (18.83%), *Funneliformis* (13.10%), *Acaulospora* (12.24%), *Claroideoglomus* (12.05%), *Scutellospora* (11.7%), and *Rhizophagus* (10.04%) (Figure 1). The output of the present study was substantiated by the study findings by Bever et al. (1996) & Oehl et al. *(*2009) in that *Glomus and Funneliformis spp*. had been indicated to have a high spore-producing capacity in a shorter time than other genera such as



	Rare Cet.	Rare Rac.	Rare Div.	Common Den.	Rare Scl.	Common Glo.	Common Gig.	Common Rhi.	Common Scu.	Common Cla.	Common Aca.	Common Fun.
IF%	8.33	8.33	16.67	16.67	11.11	41.67	41.67	66.67	13.89	41.67	66.67	33.33
RA%	0.67	0.76	0.86	4.49	6.88	18.83	8.89	10.04	11.19	12.05	12.24	13.10
IV%	4.50	4.55	8.76	10.58	9.00	30.25	25.28	38.35	12.54	26.86	39.45	23.22

Figure 1. *AMF Community Structure (genera level) in the rhizosphere soils of the studied Land Covers, Hawassa Ethiopia*

Key: *Aca. = Acaulospora; Cet. = Cetraspora; Cla. =Claroideoglomus; Den. =Dentiscutata; Div. = Diversispora; Fun. = Funneliformis; Gig. =Gigaspora; Glo. =Glomus; Rac. =Racocetra; Rhi. =Rhizophagus; Sci. =Sclerocystis; Scu. =Scutellospora*

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Gigaspora and *Scutellospora.* These species could, therefore, be selected for future studies as AMF inocula - after conducting further testing in their compatibility with different crops and checking their persistence in the field. These AMF genera would be an alternative microbiological technology input to high cost requiring and environmentally unfriendly inorganic input crop production, particularly of horticulture production in Ethiopia.

Meanwhile, a study by Beyene et al. (2016b) showed that spores of four genera *Rhizophugus, Glomus, Funneliformis, and Acaulospora* had higher spore production, accounting for 36.22%, 21.20%, 19.39%, 17.54%, and 11.74% of the total number of spores respectively. The high spore-producing AMF generalists of Beyene et al. (2016b) have showed the general similarity with the present one; except variation in proportion, as the total spore produced and AMF genera identified are also different. Another study, by Zerihun et al. (2015), in humid low land areas of Ethiopia across the various land uses had indicated the occupancy of larger spore production potential to be concurred by the three genera as; *Claroideoglomus (34.7%) Funneliformis (21.60%) and Glomus (16.80%);* which has a similarity with the current study except for the issue of proportional share and order of capacity. In a nutshell, there is a need to investigate these large spore-producing AMF genera; *Glomus, Funneliformis, Acaulospora, Claroideoglomus, Scutellospora,* and *Rhizophagus;* for their AMF inoculum potential; carrying out a thorough compatibility study against different soil physicochemical feature and crop types. This finding may contribute a bit to shade light for Integrated Pest Management (IPM) endeavors to Ethiopian agricultural system, through using microbiological techniques. As AMF inoculum has multifaceted returns in the strive of crop production; increasing yield and its counteraction against biotic and abiotic factors.

Regarding the isolation frequency of AMF genera, *Acaulospora* and *Rhizophagus* were found to be the most abundantly recovered genera of AMF, which were isolated in 66.67% of the rhizosphere soils investigated (Figure 1). These two dominantly occurring genera were recovered in the rhizosphere soils of *Zea mays, Coffea arabica, Ensete ventricosum, Cordia africana,* Tomato (organically growing), Banana, Shewshewe, Mango and Open Grass Land. The second most abundantly isolated genera of AMF were *Claroideoglomus, Gigaspora, and Glomus*, with an isolation frequency (IF) of 41.67%. These were recorded from soils of Eucalyptus, Open Grass Land, Wanza, Mango, Pepper, Tomato (Inorganically & organically growing), Enset, Shewshewe, and Banana. Meanwhile, the AMF genera *Funneliformis* has been the third most isolated one, with a frequency of encounter in 33.33% of the rhizosphere soils examined. *Funneliformis* were recovered in soils from Open Grass Land, Eucalyptus, Wanza, and Pepper. *Dentiscutata* and *Diversispora* were AMF genera isolated in rhizosphere soils of Enset, Tomato (Organically growing), Coffee, and Maize; with an isolation frequency of 16.67%. *Racocetra* and *Cetraspora* have been the least frequently isolated AMF genera, which have been encountered in only 8.33% of the soil samples of Maize and Coffee.Similarly, the diversity study of AMF in humid low land areas of Ethiopia by Zerihun et al. (2015) has informed that *Claroideoglomus* (IF=92.9%), *Funneliformis (*IF=91.3%), *and Glomus* (IF=80.2%) had represented the top three AMF genera which encountered frequently from the rhizosphere soils of the various land covers (Teff, Sesame, Maize, Sorghum, Coffee, Acacia & Fruit Crops) studied. Meanwhile, the same study of Zerihun et al. (2015) had reported that *Racocetra* (IF=8.3%),

Entrophospora (IF=8%), and *Pacispora* (IF=7.7%) as genera with less frequency of encounter. *Claroideoglomus* and *Funneliformis* were dominant genera according to Chen *et al*. (2012) because they were found in all land-use types.

The Importance Value (IV) of the genera entails something about the dominancy of the genera. In this regard the current study notified *Acaulospora, Claroideoglomus, Dentiscutata, Funneliformis, Gigaspora, Glomus, Rhizophagus* and *Scutellospora* were the commonly encountering genera with <10% IV value <50% (Figure 1). In contrast, *Cetraspora, Diversispora, Racocetra,* and

Sclerocystis were found to be species of rare occurrence, with IV value <10%. According to Zerihun et al. (2015) *Claroideoglomus (IV=63.8%), Funneliformis (IV=56.4) are* stated as dominant genera. However, these two genera were found to be commonly occurring once in the current study. Meanwhile, genera belong to *Glomus (IV=48.5%), Paraglomus (IV=15.7%), Rhizophagus (IV=18.9%), Acaulospora (IV=11.6%), and Gigaspora (IV=208%)* were regarded as commonly occurring once. Similar to the current study, *Diversispora, Racocetra* and *Sclerocystis* were found to be species of rare occurrence. However, Zerihun et al. (2015) had also recorded *Septoglomus, Pacispora, Ambispora, Entrophospora, Scutellospora* as rarely occurring AMF genera, unlike the current study which has recorded *Scutellospora* as commonly encountering one, but haven't encountered the other four rarely occurring AMF in total. 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 agro-ecological regions of Ethiopia (Diriba et al., 2008; Emiru 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., 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 current particular study (Figure 1) is in agreement with AMF composition studies done so far in different parts of Africa, hence it entails the consideration of the dominant & common AMF genera during AMF inoculum development schemes.

Root colonization by AMF

The total root colonization (RLC) of various land covers by AMF ranges from 11.15-85.41% (Table 7). The root from Open Grass Land (OGL) was colonized by AMF at a rate of 85.41%; it was the highest of all types of land cover, followed by Pepper (65.93%). It has got quite a strong similarity with the report made by Zerihun et al., (2013), in that the highest AM fungal colonization was found in *Acacia seyal* (67.3%) from open grazing field (OGF) at Zeway. The least AMF colonization numbers in the present study has been recorded in inorganically cultivated Tomato, Mango & Banana, and they were 12.94%, 11.15%, and 12.3%, respectively. A similar study has done by Beyene et al. (2016b) had indicated that the total percentage of mycorrhization (RLC) of the three land-use types was in the range of 55.69% (*Ensete ventricosum*, in mono-cropping) to 90.52% (*Coffea arabica*, in agroforestry).

Table 7. *Root colonization by AMF across the different Land covers, Hawassa, Ethiopia*

Plant species	AC	VC	RLC
Solanum lycopersicum(Inorganic)	4.42± 0.051e	4.96±0.029def	12.94±0.040e
Zea mays	14.82±0.064bc	8±0.003cde	28.53±0.127cde
Ensete ventricosum	3.5±0.020e	2.17±0.044ef	15.7±0.071cde
Open Grass Land	41.64±0.064a	31.74±0.038ab	85.41±0.062a
Solanum lycopersicum(Organic)	7.13±0.089cde	9.25±0.211cdef	28.78±0.115cde
Coffea arabica	52.42±0.208a	41.74±0.228a	81.1±0.226ab
Eucalyptus globulus	22.55±0.070b	10.8±0.082bcd	34.45±0.255cd
Cordia africana	3.12±0.015e	2±0.040ef	14.02±0.063de
Musa acuminate	3.01±0.058e	1.46±0.016f	12.3±0.095e
Casuarina equisetifolia	5.42±0.008de	18.5±0.037bc	35.47±0.108c
Mangifera indica	1.89±0.011e	2.05±0.040ef	11.15±0.054e
Capsicum annuum 'Serrano'	13.02±0.095bcd	5.99±0.026cdef	65.93±0.070b

Key: *AC- Arbuscular Colonization, VC- Vesicular Colonization; RLC-Total Root Length Colonization*

In general, data showed that more mycorrhization occurred in agroforestry (mean mycorrhizal coverage of roots of 71.53%) followed by forest land-use systems (with mean mycorrhizal coverage of roots of 68.63%) and the drastically changed in mono-cropping system (mean mycorrhizal coverage of roots of 53.38%). However, with a few exceptions, annual crops in mono-cropping systems showed higher mycorrhization (>80%) than the woody and perennial plants in other land-use systems. This result has shown similarity with that of the current study, in that mycorrhization in monocrops can go beyond (>80%), nonetheless the lowest mycorrhization range have been recorded to go as low as to the level of 11.15% unlike the one reported in South Ethiopia by Beyene et al. (2016b), which is not less than 55.69%. This could be due to the difference in edaphic factors and crop management approaches used between the two study areas.

As per the AMF diversity study done in India by Kavitha and Nelson (2013), there had been a discrepancy in mycorrhization rate on the same crop but rationalized as the cause to be an edaphic factor. In certain areas, the total root length colonization of the crop (that was sun flower)had been measured to reach 70% but in the other areas, particularly at marginal soil reach 29% and infertile soil to reach 35%. In agreement with the aforementioned rationale, the findings of root colonization in the present study had a considerable discrepancy from that of the study made by Beyene et al. (2016b). To mention the findings in the four similar crops; as per the current study output Wanza, Enset, Coffee & Maize has shown RLC of 14.02; 15.7; 81.1 & 28.53 respectively. Unlike this, the output of Beyene et al. (2016b), had reported that 77.93% in Wanza, 84.71% in Enset, 90.52 in Coffee & 89.19% in Maize.

The AM fungal colonization pattern showed heterogeneity among the roots of the cropping types. As per the study output of Moges (2014) the highest hyphal colonization of 73.4% was recorded from sunflower/maize/ (mixed crops) followed by mixed crop (Faba bean+cabbage) 63.4% and pepper (monocrop) with hyphal colonization of 60.3%, correspondingly the present study has reported root colonization of 65.9 % in Peeper. The least root colonization was recorded from teff (*Eragrostis tef*) and Eucalyptus monocrops with root colonization of 22% (P=0.011) and in the current study the lowest root colonization rate which is 11.15% was recorded in Mango, nonetheless the colonization in Eucalyptus was 34.45%. On the contrary, other studies showed that teff and Eucalyptus trees had a mycorrhization rate of 58-67% (Cesra *et al.*, 2009) 31-60% (Tekalegn & Killhalm, 1987), respectively. The root colonization of Maize was 73.4% in the study by Moges Shenkutie (2014) and 40-44% as per the report by Sasvari & Posta (2010), but in the present study, it was recorded as 28.53%, quite different from both reports.

In all the investigated land covers of the present study, the three important structures of the mycorrhizae (arbuscular, vesicular& hyphal-only) were identified. Nonetheless, according to the report by Zerihun et al. (2015) there was no vesicular mycorrhization in low agricultural input using Sorghum. Moreover as per the study report of Beyene et al. (2016b) there was no Arbuscular and Vesicular colonization in the root length of Wanza, Bisana, Birbira, Korch & Tikurinchet. Meanwhile, as to the report of the current study organically cultivated tomatoes were higher in the rate of colonization by either of the AMF structures (Arbuscules, Vesicles & Hypha-only) and total colonization (RLC) compared to the inorganically growing one. This finding coincided with studies done so far, in that modern agricultural practices such as fertilization, biocide application, and monoculture affect the community composition and diversity of AM fungi (Edlinger et al., 2022; Sallach e al. 2021; Oehl et al., 2004). Additionally, although the effect of biocides on AM symbiosis is complex and not easily predictable, overuse of most biocides reduces AM fungi colonization rates and spore production (Riedo, 2021; Schreiner & Bethlenfalvay, 1997).

As per the output of the current study, arbuscular and vesicular colonization of AMF have shown a strong and statistically significant positive correlation (r=0.821, p<0.001). Correlation analysis showed that arbuscular colonization was positively correlated with hyphal and vesicular colonization (Lingfei et al., 2005). However, the correlation of both arbuscular and vesicular structures to hypha is a slight but not statistically significant positive correlation with (r=0.114 P<0.515) and r=0.060, P<0.748) respectively. There has been spore density and vesicular (r=0.066, P<0.725) as well as arbuscule and spore density (r=0.178, P<0.299) positive correlation, yet it was a slight and not statistically significant one. No significant correlation between AMF colonization and spore density was observed when land-use types were either considered separately or together, which is consistent with several previous reports (Beyene et al., 2016b; Tilal et al., 2013; Uhlmann et al., 2004).

Conclusion

The AMF diversity across the different cropping systems has shown a discrepancy. Annual crops demonstrated the largest association with wide varieties of AMF species, followed by permanent crops. The least AMF-crop association was recorded in the cropping system where there was the use of inorganic agricultural inputs. This signifies the benefit of organic farming to keep the belowground AMF diversity. *Acaulospora* and *Rhizophagus* were found to be the most abundantly recovered genera of AMF, which were isolated in 66.67% of the rhizosphere soils investigated. *Racocetra* and *Cetraspora* have been the least frequently isolated AMF genera, which have been encountered in only 8.33% of the soil samples. Though not statistically significant, it has been discovered that there is a positive association between spore density and species richness with that of the percentage of OC and total Nitrogen in the rhizosphere.

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References

Abdelhalim TS, Finckh MR, Babiker, AG, Oehl, F. 2013. Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. Arc. of agro. and Soil Sci., 60: 377- 391.

Bever JD, Morton JB, Antonovics J,Schultz PA. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in Mown grassland. J. Ecol., 84:71- 82.

Beyene Dobo, Fassil Assefa, Zebene Asfaw. 2016a. Diversity and Abundance of Arbuscular Mycorrhizal Fungi under Different Plant and Soil Properties in Sidama, Southern Ethiopia. Adv. in Biosci. and Bioengine., 4(3):16-24.

Beyene Dobo, Fassil Assefa, Zebene Asfaw. 2016b. Diversity of Arbuscular Mycorrhizal Fungi of Different Plant Species Grown in Three Land Use Types in Wensho and Shebidino Districts of Sidama in Southern Ethiopia. Adv. in Biosci. and Bioengine., 4(4): 25-34.

Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Natu. Communi., 1: 48.

Brundrett M, Melville L, Peterson L. 1996. Practical Methods in Mycorrhiza Research. Mycologue Publications, University of Guelph, Guelph, Ontario, Canada.

Cesra A, Patuicio Pacheco, Gullermo P. 2009. J Soil Sci. Plant Nutr., 9:89:101.

Castiglione AM, Mannino G, Contartese V, Bertea CM, Ertani A. 2021. Microbial Biostimulants as Response to Modern Agriculture Needs: Composition, Role and Application of These Innovative Products. Plants 2021, 10, 1533. https://doi.org/10.3390/ plants10081533.

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

de Novais CB, Avio L, Giovannetti M, de Faria SM, Siqueira JO, Sbrana C. 2019. Interconnectedness, length and viability of arbuscular mycorrhizal mycelium as affected by selected herbicides and fungicides. Appl Soil Ecol 143:144–152.

Diriba Muleta, Fassil Assefa, Sileshi Nemomissa and Granhall U. 2008. Distribution ofArbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monoculture coffee systems in southwestern Ethiopia. Biol. and Ferti. of Soils4, 4 (4): 653–659.

Dodd IC, Ruiz-Lozano JM. 2012. Microbial enhancement of crop resource use efficiency. Curr. Opi. in Biotechnol., 23:236–242.

Edlinger A, Garland G, Hartman K, Banerjee S, Degrune F, García-Palacios P, Hallin S, Valzano-Held A, Herzog C, Jansa J, Kost E, Maestre FT, Pescador DS, Philippot L, Rillig MC, Romdhane S, Saghaï A, Spor A, Frossard E. 2022. Agricultural management and pesticide use reduce the functioning of beneficial plant symbionts. *Nature Ecol. & Evolu.*, *6*(8): 1145. https://doi.org/10.1038/s41559-022-01799-8.

Emiru, Birhanea, Thomas WK, Frank JS, Frans B. 2010. Arbuscular mycorrhizal associations *in Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. Fore. Ecol. and Manage., 260: 2160–2169.

Eom AH, Tae MS, Lee JK.2004. Diversity of arbuscular mycorrhizal fungi in arable and natural soils in Korea. Korean J. Ecol., 27:179–184.

Horn S, Hempel S, Verbruggen E et al. 2017. Linking the community structure of arbuscular mycorrhizal fungi and plants: a story of interdependence?. ISME J., **1:** 1400–1411. https://doi.org/10.1038/ismej.2017.5.

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

Kavitha and Nelson. 2013. Diversity of Arbuscular Mycorrhizal Fungi (AMF) in the Rhizosphere of *Helianthus annuus* L, India. American-Eurasian J. Agric. & Environ. Sci., **13 (7):** 982-987, 2013.

Li LF, Li T, Zhao ZW. 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, 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.

McGonigle TP, Evans DG, Miller RM. 1990.Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. New Phytologist., 116: 629-636.

Mengsteab Hailemariam, Emiru Birhane, Zebene Asfaw,Solomon Zewdie. 2013. Arbuscular mycorrhizal association of indigenous agroforestry tree species and their infective potential with maize in the rift valley, Ethiopia. Agrofore. Syst., 87(6):1261–1272.

Moges Shenkutie. 2014. Arbuscular Mycorrhizal Fungi abundance And diversity across different land-use types In Jabi Tehnan woreda Western Gojam, Ethiopia. MSc thesis In Microbiology, Haramaya University.

Nandjui J, Voko DRR, Kouadio ANMS, Fotso B, Tano Y, 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. Afri. J of Agri. Resear., **8:** 5572-5584.

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

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytol**.,** 165:273–283.

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

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

Olsson PA, Thingsturb I, Jakobsen I, Baath E. 1999. Estimation of the biomass of Arbuscular Mycorrhizal fungi in a linseed field. Soil Biol. Biochem. 31:1879-1887.

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

Wahab A, Muhammad M, Munir A, Abdi G, Zaman W, Ayaz A, Khizar C, Reddy SPP. **2023**. Role of Arbuscular Mycorrhizal Fungi in Regulating Growth, Enhancing Productivity, and. Potentially Influencing Ecosystems under Abiotic and Biotic Stresses. Plants, 12, 3102. https://doi.org/ 10.3390/plants12173102.

Phillips JM, Hayman DS. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc., 55: 58-161.

PuÈschel D, RydlovaÂ J, VosaÂtka M. 2008. Does the sequence of plant dominants affect mycorrhizal development in simulated succession on spoil banks? Plant Soil. **302(1-2):**273-282.

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield Trends Are Insufficient to Double Global Crop Production by 2050. On

PLoS ONE tenth anniversary Publication. https://doi.org/10.1371/journal.pone.0066428.

Riedo J, et al. 2021. Widespread Occurrence of Pesticides in Organically Managed Agricultural Soils—the Ghost of a Conventional Agricultural Past? *Environ Sci Technol.* 2021 doi: 10.1021/acs.est.0c06405.

Sallach JB, Thirkell TJ, Field KJ, Carter LJ. 2021. The emerging threat of human-use antifungals in sustainable and circular agriculture schemes. Plants, People, Planet, 3: 685–693.

Sasvari Z, Posta K. 2010. Effect of different plant densities on the diversity of arbuscular mycorrhizal fungi community in a long-term maize monocrop system. Spanish J of Agricul. Resear., 8(S1):123-130.

Schenck NC, Perez, Y. 1990. Manual for the identification of VA mycorrhizal Fungi, 3rd edn. Synergistic publications, Gainesville, Florida.

Schreiner RP, Bethlenfalvay GJ. 1997. Mycorrhizae, biocides, and biocontrol: 3. Effects of three different fungicides on developmental stages of three AM fungi. Biol Fert Soils., 24:18–26.

Schüßler A, Walker C. 2011. Evolution of the "Plant-Symbiotic" Fungal Phylum, Glomeromycota.Pp.163–185. In: Pöggeler S, Wöstemeyer J(eds.). Evolution of Fungi and Fungal-Like Organisms, Springer, Berlin, Heidelberg.

Shi J, Wang X, Wang E, 2023. Mycorrhizal Symbiosis in Plant Growth and Stress Adaptation: From Genes to Ecosystems. Annu. Rev. Plant Biol. 74 (1), 569–607. https://doi.org/10.1146/annurev-arplant-061722-090342

Smith SE, Read DJ. 2002. Mycorrhizal Symbiosis. Academic Press, London.803p.

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

Southern Nations, Nationalities & Peoples' regional State, Bureau of Finance & Economic Development (SNNPR-BoFED) (2014/15). Annual statistical Abstract.

Spagnoletti FN, Leiva M, Chiocchio V, Lavado RS. 2018. Phosphorus fertilization reduces the severity of charcoal rot (*Macrophomina phaseolina*) and the arbuscular mycorrhizal protection in soybean. J Plant Nutr Soil Sci., 181:855–860.

Stutz JC, Copeman R, Martin CA, Morton JB. 2013. Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa.

Tadesse Chanie and Fasil Assefa.2013. Mycorrhizal fungi associated with shade trees and Coffea arabica L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. Afri. focus, 26:111-131.

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

Tekalign Mamo and Killham KS1987. Effect of soil liming and vesicular-arbuscular mycorrhizal inoculation on the growth and macronutrient content of the teff plant. Plant and soil, 102:257-25.

Tesfaye Wubet,Ingrid Kottke, Demel Teketay, and Franz Oberwinkler. 2009. Arbuscular mycorrhizal fungal community structures differ between co-occurring tree species of dry Afromontane tropical forest, and their seedlings exhibit potential to trap isolates suited for reforestation. Mycol. Progre.,8(4):317–328.

Tilal SA, Maria RF, Abdel GB, Fritz O. 2013. Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan, Archives of agronomy and Soil Science.

Tsiafouli MA, Thébault E, Sgardelis SP et al. 2015. Intensive agriculture reduces soil biodiversity across Europe. Glob. Chang. Biol., 21(2): 973–985. https://doi.org/10.1111/gcb.12752.

Uhlmann E, Gorke C, Petersen A, Oberwinkler F. 2004. Arbuscular mycorrhizae from semiarid regions of Namibia. Cana. J of Bot., 82: 45-653.

Wang B, Qiu YL. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16:299–363.

Zerihun Belay, Vestberg, M, Fassil Assefa. 2013. Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land-use systems in Ethiopia. Afri. J of Microbiol. Resear., 7 (48):5503-5515.

Zerihun Belay, Vestberg V, Fassil Assefa. 2015. Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land-use systems in Ethiopia. Tropical and Subtropical Agroecosystems, 18: 47 – 69.

Zhao Z, Xia Y, Qin X, Li X, Cheng L, Sha T, Wang G. 2001. Arbuscular Mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, Southwest China. Mycorrhiza, 11: 159-162.

Zobel M, OÈ pik M. 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities which drive which? J Veg Sci., 25: 1133-1140.