Diverse arbuscular mycorrhizal fungi species associate with indigenous trees in a natural forest

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Research Article

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Abstract

In terrestrial ecosystems, arbuscular mycorrhizal fungi (AMF)-land plant symbiosis helps plant for nutrient uptake and, protection from abiotic and abiotic stresses. It is important to study the AMF-plant relationship to fully realize the potential to exploit for plant growth, maintenance and establishment in different ecosystem. Therefore, the objective of this study was to determine the diversity, abundance and population densities of AMF and quantify root colonization of the indigenous trees in Munessa forest. To this end, composite fine roots and soil samples were collected from seven dominant indigenous trees within 10 m x10 m plots in the forest. AMF spores were extracted for taxonomic identification and AMF root colonization was determined. A total of 39 AMF morphotypes belonging to 7 genera were recovered. Of which, *Glomus* was the most dominant genus followed by *Acaulospora*, *Glomus rubiforme* was the most dominant species followed by *Acaulospora myriocarpa*. The highest genus and species richness was recorded from *Croton macrostachyus* and *Millettia ferruginea* in this study. Mean AMF spore density was significantly different ($p < 0.05$) among indigenous trees, ranging from 859.6 spores/100g of dry soil under *Albizia shimperiana* to2829.52 spores/100g of dry soil under *Pouteria adolfiifriedericii*. The highest hyphal and vesicular colonizations were observed in *Millettia ferruginea* (71%) and *Celtis Africana* (36.37%) respectively. *Albizia shimperiana* was the least colonized tree by all AMF structures. AMF spore density was positively correlated with soil available phosphorus ($p < 0.05$). Extensive studies are required to select AMF for growth promotion and recruitment of tree seedlings for plantation and, restoration of forest vegetation and rehabilitation of degraded lands.

1. Introduction

Forests cover 31% of the Earth's terrestrial biodiversity (FAO, 2022). However, they are continuously decreasing in the tropics due to natural and man-made disasters. The Ethiopian highlands constitute 60% of the Afromontane tropical forest ecosystems of eastern Africa that are rich in plant and animal species richness and endemic plant and animal species (Schmitt et al., 2010). The woody plant species are the important components of forest ecosystems and deliver resources and habitats for the different biological entities.

Ethiopia is a center for biological diversity due to its wide range of altitude and diverse topography with high and rugged mountains (Zegeye *et al.*, 2011). The natural forest coverage of Ethiopia was 35–40% of the land area in 1950s and 1960s which has been dramatically decreased to less than 3% around 1990s (Asfaw, 2014).

Munessa forest is one of the major timber supplier forests in central Ethiopia. Although the forest is rich with indigenous trees, it suffers from deforestation due to the uncontrolled use of timbering, and overgrazing and encroachment due to agricultural activities (Anissa and Eyasu, 2020).Thus, accelerated conversion of forests to agricultural land-use types and overutilization of forest resources have contributed to a decrease and loss of biodiversity.

Mycorrhizal symbiosis is one of the most widespread and ecologically important mutualistic associations that occur between land plants and mycorrhizal fungi. Arbuscular mycorrhizal fungi (AMF) interact with a broad range of vascular plants (~ 80–90%) in tropical forests, grasslands, alpine and crop lands (Smith and Read, 2008). AMF can enhance uptake of poorly mobile soil nutrients such as P, K and Mg to plants (Rodriguez, 2006), increase tolerance to water stress, induce resistance to pathogens and other abiotic stresses in the soil (Siddiqui and Pichtel, 2008).They are also very important for crop production and growth and establishment of perennial plants, particularly woody plants with deep roots. Moreover, they help in a buildup and maintenance of mycorrhizal networks, or hyphal bridge (hyphal linkage) to exchange resources amongst species (Read and Simard *et al*., 1997; Carvalho *et al*., 2010), increase the volume of soil to be exploited and thereby improve the efficiency of P plant access and cycling in the soil (Muleta *et al*., 2008).

It is well established that different plant species have differential growth responses to AMF, so that the composition and diversity of the AMF community in a natural ecosystem could potentially affect the way plant species coexist, and determine plant community structure (Heijden et al., 1998). Moreover, knowledge about AMF diversity may help to establish relationships of species selection, AMF production and inoculation.

In Ethiopia, several research works were undertaken on AMF species diversity and abundance in different land use types. To mention few, mycorrhizal status of different coffee shed trees in agro-forestry systems (Muleta *et al*., 2007; Channie and Assefa, 2013), dual function of AM fungi and rhizobia with *Acacia polyacatha* (Yohannes and Assefa, 2007), species diversity and population density of AMF in Acacia woodland ecosystem and agro-forestry system in central Shewa (Belay *et al*., 2013; Belay *et al*., 2015), species diversity and population density of AMF in Sidama agro-forestry system (Dobo *et al*., 2016), AMF status of *Erythrina brucei* in different land use types in Ethiopia (Berza *et al*., 2021) and species diversity and population density of AMF in the highlands of northern Ethiopia (Birhane *et al*.,2021).

Several studies have been undertaken to investigate AMF species diversity and abundance among different forest ecosystems. To mention few, AMF species diversity and abundance studies have been conducted in planted forest in eastern China (Wang et al., 2019), in natural forest in south west China (Zhang et al., 2003), in tropical rain forest in south west China (Zhao et al., 2003), in Atlantic and Amazon forests in Brazil (Winagraski et al., 2019) and in natural forest in south Ethiopia (Belay et al., 2020)

Although Wubet et al. (2003) have worked on mycorrhizal diversity in dry afromontane forests in Ethiopia; there is dearth of sufficient information on species diversity and population density of arbuscular mycorrhizal fungi in dense and protected forests in Ethiopia. Hence, the objective of this study was to evaluate the AMF species diversity, abundance, population density and root colonization associated with indigenous trees in Munessa Forest.

2. Materials And Methods

2.1. Study area

The study was conducted in Munessa forest, Arsi forest enterprise in south eastern Ethiopia from November to December 2020. It is located at 240 km from Addis Ababa, the capital city of Ethiopia and lies between 7°25´44´´N and 38°51´05´´E along the eastern escarpment of the Rift Valley. The altitude range extends from 2100 m to 2450 m above sea level. The mean annual rainfall is about 1250 mm. The mean annual temperature varies from 15 to 20°C. Munessa forest encompasses 48% of Arsi forest enterprise which include Munessa, Gambo and Shashemene districts. The forest is composed of natural and plantation trees. The natural forest comprised about 8537ha and plantation forest comprised about 2575 ha (Nino *et al.* 2017).

2.2. Vegetation sampling

Tree species were sampled from the natural forest following Transect method according to Buckland et al. (2007). The whole area was preliminarily surveyed to lie down transects into four directions starting from the middle of the forest. Thus, seven plots (10 mx10 m) were selected across each transect at a distance of 100 m and trees were counted within plots. Two most frequently encountered tree species were considered from each plot. Each tree species was sampled from 7 to 14 plots. The local name (Afan Oromo) (Table 1) of trees were recorded, voucher specimens were pressed and brought to the National Herbarium of Addis Ababa University (AAU) to identify the scientific name of indigenous tree species.

Table 1
The list of indigenous trees species in Munesa forest, their Amharic and scientific names collected from November- to December, 2020.

Scientific name	Family	Amharic	Afanoromo
Podocarpus falcatus	*Podocapaceae*	Zigba	Birbirsa
Croton macrostachyus (Hochst.) Dale	*Euphorbaceae*	Bisanna	Bakkanisa
*Albizia shimperiana*oliv.	*Fabaceae*	Sassa	Mukarba (Karchafe)
Millettia ferruginea (Hochst.) Bak.	*Fabaceae*	Birbira	Dhadhattu
Celtis africanaBurm.f	*Ulmaceae*	Kawoot	Amallaqqaa
Pouteria adolfi-friedericii (Engl.) Baehni	*Sapotaceae*	Keraro	Suduba, (keraro)
Polycias fulva (Hiem)Harms	*Araliaceae*	Yezinjerowenber	Harfattu

2.3. Root and soil sampling

Young fine roots and soil samples were collected from the rhizosphere of selected indigenous trees from each plot during dry season in November to December, 2020. The fine root samples of the same tree species from a plot were pooled to composite. About 500 g soil samples were obtained from tree rhizosphere.The soil sample was collected from 10 cm depth. Fine root were traced back to each tree species to confirm that each fine root is from each tree species. The soil samples were divided into two

for spore extraction and identification and for soil analysis. The soil sample used for AMF spore extraction was air dried immediately on arrival to applied microbiology laboratory, Addis Ababa, University. Similarly, the fine roots were washed with tap water and immersed in 50% ethanol and stored under 4°C to determine AMF root colonization.

2.4. Soil chemical analysis

Soil sample analyses were done following standard procedures at Soil, Water and Plant sample Analysis Laboratory of Engineering Corporation of Oromia (ECO), Addis Ababa, Ethiopia. The soil sub samples were ground and sieved in a 2 mm sieve. The soil pH was determined on 1:2.5 (soil: water) suspension method (Ziadin and Tran, 2007). Organic carbon was determined using Walkley and Black method (1934) and Total nitrogen (TN) according to Kjeldahl method (Hinds and Lowe, 1980).Available phosphorus was determined according to Olsen method (Olsen and Sommers, 1982).

2.5. Staining fine roots and quantification of AMF root colonization

The staining of fine roots was conducted according to Vierheilig et al. (1998). Preserved fine roots were carefully washed with tap water and cut into about 1 cm segments. Root segments were cleared in 10% KOH (w/v) at 121°C in an autoclave for 20 min. Then, they were rinsed with tap water and acidified with 10% HCl (v/v) for 3 min at room temperature. Acidified root segments were rinsed three to five times in tap water and stained at 121°C for 15 min in 5% (Dollar blue) ink-vinegar solution (v/v) with pure white household vinegar (5% acetic acid). The roots were then destained by rinsing three times in 5% acetic acid followed by boiling on hot plate. Stained fine root segments were mounted on a slide with polyvinyl-lactic acid-glycerol (PVLG) parallel to length wise in nine replication, then, covered with cover slip. Mounted roots were dried in oven at 70 ^{o}C for 12–24 hours. Slightly dried roots were squashed to see their internal structures under compound microscope. AMF structures (hyphae, vesicles and arbuscules) were observed at X200 magnification (Olympus-BX51, Japan). Totally nine slides each containing 6–10 root segments were considered for each tree species. Root segments were classified under the following categories: negative (no fungal material found in the root), arbuscules, vesicles and hyphae. The percentage (%) of AMF root length colonization was calculated using the following formula: Colonization = Number of positive root segments/Total number of observed segments × 100 (Giovannetti and Mosse, 1980).

2.6. AMF Spore extraction and enumeration (population density)

AMF spores were extracted using wet sieving and decanting method according to Gedermann and Nicolson (1963). A 50 g of air dried soil was suspended in 500 milliliter of water in triplicates and left for about 5 minutes. Coarse soil particles were gently crushed by hand and allowed to settle down. The suspension was passed and washed through series of four sieves with different pore size arranged from top to bottom (500 µm, 212 µm, 90 µm and 45 µm). This process was repeated 3–4 times until the

suspension became clear and clean. Residues from 500 µm sieves were checked for sporocarps and residues from 212 µm and 90 µm sieves were washed and collected to 45 µm sieve. These were transferred to 15 ml falcon tubes and centrifuged at 2000 rpm for 5 min .The supernatant was poured off and pellets were suspended in 50% sucrose solution (w/v) and centrifuged at 2000rpm for 3 min. The supernatant was transferred to 45 µm sieve to remove the sucrose by washing with tap water. The, spores were transferred to 90 mm gridded plastic Petri dish. Enumeration of AMF spores was carried out under stereomicroscope (ISO 1006; NOVEX, HOLLAND) at 40X magnification according to the method described in International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), (http://invam.wu.edu/).

2.7. Identification of arbuscular mycorrhizal fungi (AMF)

AMF spores were identified at Applied Microbiology Laboratory, Addis Ababa University and Federal University of Minas Gerais, Brazil based AMF spore morphological features. Healthy spores were picked under the dissecting microscope with micropipette and mounted permanently on microscope slides with Polyvinyl-Lactic acid-glycerol (PVLG) or mixture of PVLG and Melzer, s reagent (1:1 v/v) prepared as described in (http://www.zor.zut.edu.pl/Glomeromycota/. The spores were crushed and dried in an oven at 70 ^{o}C for 24 hours. Crushed spores were examined under compound microscope (Olympus-BX51, Japan) and pictures were captured. Spores were identified to genus/species level based on spore color, surface ornamentation, wall structure and subtending hyphae with reference to descriptions provided by INVAM (http://invam.wu.edu/) and http://www.zor.zut.edu.pl/Glomeromycota/.

2.8. Evaluation of AMF species diversity

AMF species richness (SR), isolation frequency (IF), spore density (SD), relative abundance (RA), importance value (IV),Shannon (H)and Simpson (D) diversity indexes were used to evaluate the structure of AM fungal communities in the rhizosphere of different indigenous trees Munessa forest. They were calculated by the following formulas:

SR = total number of identified AMF species or genus /soil sample;

SD = number of spores/100g of air dried soil;

IF = number of samples in which a particular AMF species or genus was observed/the total number of samples) × 100;

RA= (the number of spores of a particular species or genus)/total number of identified spores) × 100;

IV = (IF + RA)/2. IV ≥ 50% indicates that a genus or species is dominant, 10% < IV < 50% applies to common genera or species, and IV ≤ 10% indicates that a genus or species is rare (Chen *et al.*, 2012).

2.9. Data analysis

All statistical analyses were performed using SPSS software (version 23). Spore density and log transformed (hyphal and vesicular colonization) data were subjected to One way ANOVA to test the significant difference within spore density in relation to indigenous tree species and AMF root colonization ($p < 0.05$). Mean separations were done using HSD Tukey's post Hoc test at $p < 0.05$.The relationships between hyphal colonization and spore density, soil parameters and spore density were determined using Pearson correlation.

3. Results And Discussions

3.1. Soil physico-chemical characteristics

The mean value of soil pH, Organic carbon, Organic matter, Total nitrogen and Available phosphorus were 7.36, 10.79, 18.56, 1.19 and 3.7, respectively. The highest pH (7.96) was observed in a soil sample under *Celtis africana* and lowest (6.78) was observed in soil of *Albizia schimperiana*. Thus, pH was varied from neutral to alkaline. The rhizosphere soil of *Pouteria adol-fifriedericii* was the highest in organic carbon (14.9%) and the lowest was *Podocarpus falcatus* (8.2%). Similar to organic carbon, total nitrogen was the highest (1.55%) in *Pouteria adol-fifriedericii* and lowest (0.96%) in *Podocarpus falcatus*. Available phosphorus is also varied from the highest (8.46 ppm) in *Millettia ferruginea* to the lowest (1.11 ppm) in *Podocarpus falcatus* (Table 2).

Table 2
The physic-chemical properties of rhizosphere soil under indigenous trees species of Munessa forest

Tree species	Soil chemical parameters				
	PH (H2O)	O.C (%)	O.M (%)	TN (%)	AV.P (ppm)
Podocarpusfalcatus	7.27	8.2	14.1	0.96	1.11
Croton macrostachyus	7.42	11.7	20.1	1.27	4.55
Albizia schimperiana	6.78	9.7	16.6	1.09	1.22
Millettia ferruginea	7.72	14.2	24.4	1.48	8.46
CeltisAfricana	7.96	12.6	21.7	1.35	6.19
Pouteriaadolfifriedericii	7.36	14.9	25.8	1.55	7.67
Polysciasfulva	7.69	9.3	15.9	1.06	1.43

O.C- organic carbon, O.M- organic matter, TN- Total nitrogen, AV.P-Available phosphorus,ppm- parts per million.

3. 2. Arbuscular Mycorrhizal Fungi Root Colonization

All indigenous trees roots were colonized by all structures of AMF (Table 3; online resource 2). *Millettia ferruginea* induced the highest hyphal colonization (71%) followed by 64% colonization by *Podocarpus falcatus*, whereas *Albizia shimperiana* colonized the lowest (44%) of the root length. (Table 3). However, with regard to the vesicular colonization, *Celtis africana* (36.37%) and *Polycias fulva*(33.36%)showed significantly higher vesicular colonization than the others. The indigenous trees displayed variations in arbuscular mycorrhization ranging from 10.18% (*Albizia shimperiana)*to 13.92% *(Croton macrostachyus).*Another study by Dobo *et al.* (2016) have reported higher hyphal colonizationin *Croton macrostachyus* (64.11*%*) and similar arbuscular colonization in *Millettia ferruginea* (11.67%) in Sidama agro forestry systems in southern Ethiopia. However, Channie and Assefa (2013) havealso found higher hyphal colonization in *Millettia ferruginea* (69%) and in *Polycias fulva* (56%) and, they reported low arbuscular colonization (3–9%). The differences in hyphal colonizations in this particular study and the others could be due to the variation in soil physic-chemical properties, agroforestry practices and climatic conditions that can influence both plants and the AMF. Moreover, this reduced root length colonization could also be due to rapid formation of new young fine rootlets (Torti et al., 1997).

Table 3
AMF mean percentage (%) root colonization (arbuscular, vesicular and hyphal) of indigenous trees in Munessa forest (mean ± SE).

Tree species	Root colonization of AMF structures (%)		
	AC	VC	HC
Albizia shimperiana	10.18 ± 4.11a	15 ± 5.65b	44.44 ± 5.50b
Polycias fulva	12.89 ± 3.36a	33.36 ± 3.81a	59.48 ± 6.41ab
Celtis africana	10.92 ± 3.16a	36.37 ± 5.00a	52.78 ± 3.77ab
Pouteria adolfiifriedericii	11.85 ± 3.84a	15 ± 5.65b	46.85 ± 4.40b
Millettia ferruginea	11.68 ± 3.44a	25.69 ± 7.60ab	71.00 ± 8.19a
Podocarpus falcatus	11.24 ± 4.26a	26.57 ± 8.48ab	64.02 ± 8.76a
Croton macrostachyus	13.92 ± 4.47a	17.75 ± 4.61b	59.60 ± 6.11a

AC-arbuscular colonization; VC-vesicular colonization; HC-hyphal colonization; similar letters denote not significant difference at $p < 0.05$.

The hyphal colonization recorded in this study (44–71%) showed similar pattern of compared with the study of AMF colonization of church forests in Northern Ethiopia which was in the range of 31–82% (Birhane *et al*., 2021), and much higher than the hyphal colonization (18.6%-34%) reported from grassland ecosystem in the Middle Awash Basin, Ethiopia (Terefe *et al*., 2021) that could be due to the differences in soil management practices.

In general, pattern of mean root colonization of HC (56.88%) was twice and four times higher than VC (24.25%) and AC (11.81%) colonization. Vesicular colonization was positively correlated with hyphal

colonization ($r = 0.3$; $p < 0.05$). In this study, we did not observe significant correlation between soil parameters and hyphal colonization. These results corroborate with the findings of Berza *et al*. (2021) and Birhane et al. (2021) who reported hyphal colonization more than the other AMF structures. Parniske (2008) reported that colonization by arbuscules is lower than others, which are partly due to the quick degradation of arbuscles within cortical cells.

In general, variation in AMF colonization of tree species in present study might be due to the differences in structure of the tree root system. Plants having less extensive root systems are more dependent on mycorrhizae than those with extensive fibrous roots that have thin roots, long root hairs, and high root hair density in order to enhance absorption of nutrients (Kahiluoto et al., 2001).

3.3. Arbuscular mycorrhizal fungi spore density of indigenous trees

The mean AMF spore density was significantly different among indigenous trees ($p < 0.05$). Thus, the mean AMF spore density extracted and collected under indigenous trees soil varied from 859.6 to 2829.52 with average of 1970 per 100 g of dry soil. The highest mean spore density was recorded from the rhizosphere soil of *Pouteria adol-fifriedericii* followed by *Millettia ferruginea* and the lowest spore number recorded from *Albizia schimperiana* (Fig. 1).

The spore density recorded in this study was almost twice higher than those recorded from *Croton macrostachyus* (1098 per 100 g of soil), four times more than the record on *Millettia ferrugenea* (670 per 100 g of soil), *Pouteria adol-fi-friedericii* (787 per 100 g of soil), and three times more than reported from *Polycias fulva* (638) in Bonga forest south western Ethiopia (Channie and Assefa, 2013). It is also twice higher than the spore density (927 spores per 100g of soil) recorded from *Podocarpus falcatus*, and four times greater 617 spores per 100 g of soil) from *Millettia ferruginea (*Asmelash *et al*., 2020). However, Birhane *et al*. (2017) reported higher spore density from *Croton macrostachyus* (5729 per 100 g of soil) in the highlands of Northern Ethiopia compared to ours. Similarly, Wang et al. (2019) have reported much higher spore density (438 to 7638 spores per 100 gram dry soil) from different plant species in planted forest in eastern China.

This study showed similar pattern of spore densities reported from Central American tropical forest (110–2600 spores per100 g dry soil (Picone, 2000) and Fragmented Church Natural Forest Remnants in Northern Ethiopia (58-5408 spores per 100g of dry soil) (Birhane *et al*., 2021) and much higher than the ones reported from different parts of Ethiopia: coffee shade trees of Bonga natural forest (4–67 spores per 100 g of dry soil) (Muleta *et al*.,2007),from native forests and agro ecosystems in southern Ethiopia (12–35 spores per 100 g of dry soil) (Belay *et al*. ,2020), from different plant species from Sidama agroforestry system in southern Ethiopia (173–280 per 100 g of dry soil) (Dobo *et al*., 2016) and from woody grassland land use types in the middle Awash basin, Ethiopia (319–488 spores per 100 g of soil) (Terefe *et al*.,2021). The AMF spore density can be affected by season of sample collection, soil physic-

chemical properties and agricultural practices. Spore density in disturbed sites is less than undisturbed natural environments (Muleta et al., 2008;Chanie and Assefa, 2013).

This variation in AMF spore density could be due to factors such as sampling season (Oehl *et al.*,2009), soil properties, Vegetation type, climatic condition and disturbances (Husband, 2002). Birhane *et al.* (2010) also suggested that protected fields had positive effect for spore abundances. Most of soil chemical properties analyzed in this study were not significantly correlated with spore density, except available phosphorus that showed significant positive relationship ($p < 0.05$). Muleta *et al.* (2007) and Birhane *et al.* (2017) also reported positive correlation of spore density with available phosphorus in Bonga natural forest and in the highlands of Northern Ethiopia, respectively. However, the available phosphorus reported in this study was below the optimum phosphorus required for plants growth. In contrast, other studies showed the inverse relationship between concentration of available Phosphorus and AMF diversity and abundance (Ceulemans et al., 2019).

3.4. AMF genus and species composition of different indigenous tree species

In this study, 39 AMF morphotypes belonging to 7 genera of Glomeromycota were identified (Table 5;online resource 1).Twenty and nineteen morphotypes were identified to genus and species level, respectively. The results showed that the Genus *Glomus* contained the largest number of morphotypes (16), followed by *Acaulospora* (11) and *Pacispora* (5), *Racocetra* (3), *Funneliformis* (2) and one genus each from the members of *Paraglomus* and *Gigaspora* (Fig. 2).

Table 4
Isolation frequency, relative abundance and importance value of AMF genus isolated from indigenous trees of Munessa forest.

Genus	IF (%)	RA (%)	IV (%)	Status
Glomus	100	45.1	72.6	dominant
Acaulospora	100	30.5	65.3	dominant
Pacispora	57.1	11	34.1	Common
Racocetra	71.4	5.5	38.5	Common
Funneliformis	57.1	4.9	31	Common
Paraglomus	28.6	2.4	15.5	Common
Gigaspora	14.3	0.6	7.5	Rare
IV ≥ 50%: dominant, 10% < IV < 50%: common, IV ≤ 10%: rare (Chen *et al.*, 2012)				

Table 5
Isolation frequency (IF), Relative abundance (RA) and importance value (IV) of AMF species from different indigenous trees of Munessa forest.

S.N	AMF species	IF (%)	RA (%)	IV	Status
1	*Funneliformis geosporum*	57.1	3	30.1	common
2	*Pacispora dominikii*	57.1	7.3	32.2	common
3	*Acaulospora rehmii*	14.3	0.6	7.5	rare
4	*Glomus rubiforme*	100	18.9	59.45	dominant
5	*Acaulospora myriocarpa*	85.7	7.9	46.8	common
6	*Glomus magnicaule*	71.4	4.9	38.2	common
7	*Racocetra castanea*	57.1	3	30.1	common
8	*Acaulospora lacunosa*	57.1	8.5	32.8	common
9	*Acaulospora mellea*	71.4	3	37.2	common
10	*Paragomus bolivianum*	28.6	2.4	15.5	common
11	*Pacispora franciscana*	14.3	0.6	7.6	rare
12	*Pacispora corralloidea*	42.9	1.8	22.4	common
13	*Acaulospora delicata*	14.3	1.2	7.8	rare
14	*Racocetra gregaria*	14.3	1.8	8.1	rare
15	*Acaulospora spinosa*	28.6	2.4	15.5	common
16	*Glomus multicaule*	14.3	1.2	7.8	rare
17	*Glomus melanosporum*	14.3	1.8	8.1	rare
18	*Glomus fulvus*	14.3	0.6	7.5	rare
19	*Funneliformis mossea*	28.6	1.8	15.2	common
20	*Glomus sp1.*	28.6	1.8	15.2	common
21	*Glomus sp2.*	14.3	1.8	8.1	rare
22	*Glomus sp3.*	14.3	1.2	7.8	rare
23	*Glomus sp4*	28.6	1.8	15.2	common
24	*Glomus sp5*	28.6	1.8	15.2	common
25	*Glomus sp6*	28.6	1.8	15.2	common

IV ≥ 50%: dominant, 10% < IV < 50%: common, IV ≤ 10%: rare (Chen *et al.*, 2012)

S.N	AMF species	IF (%)	RA (%)	IV	Status
26	*Glomus sp7*	42.9	1.8	22.4	common
27	*Glomus sp8*	14.3	1.2	7.8	rare
28	*Glomus sp9*	14.3	1.2	7.8	rare
29	*Glomus sp10*	14.3	1.2	7.8	rare
30	*Glomus sp11*	42.9	2.4	22.7	common
31	*Acaulospora sp1*	42.9	3	23	common
32	*Acaulospora sp2*	14.3	1.2	7.8	rare
33	*Acaulospora sp3*	14.3	1.2	7.8	rare
34	*Acaulospora sp4*	14.3	0.6	7.5	rare
35	*Acaulospora sp5*	14.3	0.6	7.5	rare
36	*Pacispora sp1*	14.3	0.6	7.5	rare
37	*Pacispora sp2*	14.3	0.6	7.5	rare
38	*Racocetra sp*	14.3	0.6	7.5	rare
39	*Gigaspora sp*	14.3	0.6	7.5	rare
IV ≥ 50%: dominant, 10% < IV < 50%: common, IV ≤ 10%: rare (Chen *et al*., 2012)					

Thus the two genera, *Glomus* and *Acaulospora* were categorized as dominant; whereas the remaining four genera were considered as common and one was rare (Chen *et al*., 2012).

The number of AMF morphotypes recovered in this study was comparable to Belay *et al*. (2020) and Belay *et al*.(2013). These authors reported 37 morphotypes belonging to 12 genera in southern Ethiopia (Belay *et al*.,2020) and 41 AMF species belonging to 14 genera from Acacia trees in different land use system in Ethiopia (Belay *et al*.,2013). Although the number of morphotypes were similar to the *Erythrina brucei* in different land use type (33AMF species) (Berza *et al*., 2021), the number of genera (7) recorded in this study were less than the number of genera (11) recorded in Berza *et al* (2021). Terefe *et al.* (2021) have reported less (16 AMF morphotypes) belonging to 10 genera in the middle Awash Basin, Ethiopia. The dominance of genus *Glomus* followed by *Acaulospora* was reported in other studies undertaken in different parts of Ethiopian soils (Muleta *et al*., 2008; Dobo *et al*.,2016; Birhane *et al*.,2017; Berza *et al*.,2021). Wang et al. (2019) have also reported similar result from different plant species in planted forest in eastern China.

The results from isolation frequency show that, *Glomus* and *Acaulospora* were found under all tree species, so they are classified as a generalist and followed by *Racocetra.* The genus *Gigaspora* was recorded as the least isolated genus (Table 4). Similarly, Auliana and kaonongbua (2018) have isolated

Glomus and *Acaulospora* from all soil samples from oil palm plantations in Thailand. Out of total spores identified in our study 45.1% belonged to *Glomus* followed by *Acaulospora* (30.5%) and *Pacispora* (11%) (Table 4). Thus, *Glomus* and *Acaulospora* are widely distributed worldwide across a variety of natural environments that could be due to their great adaptability and high infectivity of their propagules (Wang *et al.*, 2020). The wide distribution of *Glomus* and *Acaulospora* in our study makes them potential candidates of AMF genera for mass multiplication to use as inoculant for recruitment of tree seedlings in the revegetation of degraded lands using these indigenous tree species.

In this study,*Glomus rubiforme* was the dominant species isolated from the rhizosphere of all tree species (IF = 100%) followed by *Acaulospora myriocarpa* (IF = 85.7%), *Glomus magnicaule* (IF = 71.4%) and *Acaulospora mellea* (IF = 71.4%) (Table 5).More than half (51.3%) of identified morphotypes were isolated from one soil sample which was obtained from the rhizosphere of different indigenous tree species. As result, they were considered as rare genus/species according to important value (IV) index. In addition, the highest relative abundance (RA) was recorded from *Glomus rubiforme* (18.9%) followed by *Acaulospora myriocarpa* (7.9%) and *Pacispora dominikii* (7.3%) per spores of all indigenous trees (Table 5).

3.5. AMF species richness and diversity among indigenous tree species

AMF genus richness per 100 g dry soil under indigenous tree species was varied from 3 to 6 (Table 6). *Croton macrostachyus* was associated with the highest species richness (6 genera) followed by *Millettia ferruginea* (5 genera). *Albizia shimperiana, Polycias fulva,Celtis africana* and *Pouteria adolfifriedericii* each associated with the same number of four genera. The lowest number of genera (3) was recorded from *Podocarpus falcatus* (Table 6). The results from relative abundance of genus within tree species showed that *Glomus* was dominantly distributed across four indigenous trees (*Albizia shimperiana, Polycias fulva, Celtis africana* and*Podocarpu sfalcatus*); whereas the Genera*Pacispora* and *Acaulospora* were dominant in *Croton macrostachyus*and *Millettia ferruginea*, respectively. *Glomus* and *Acaulospora* displayed the same number of spores (RA = 44.8%) from soil samples collected from *Pouteria adolfifriedericii* (Table 6).

Table 6
AMF genus richness, number and relative abundance (RA) each genus per tree species

Tree species	Genus richness	Genus name	Number of each genus	RA (%)
Albizia shimperiana	4	*Funneliformis*	2	20
		Pacispora	2	20
		Acaulospora	2	20
		Glomus	4	40
Polycias fulva	4	*Glomus*	9	60
		Racocetra	2	13.3
		Acaulospora	3	20
		Gigaspora	1	6.6
Celtis africana	4	*Glomus*	18	58.1
		Pacispora	5	16.1
		Acaulospora	7	22.6
		Racocetra	1	3.2
Croton macrostachyus	6	*Acaulospora*	5	19.2
		Pacispora	8	30.8
		Glomus	6	23.1
		Paraglomus	3	11.5
		Funneliformis	3	11.5
		Racocetra	1	3.8
Millettia ferrugenea	5	*Acaulospora*	13	39.4
		Glomus	12	36.4
		Racocetra	4	12.1
		Paraglomus	1	3
		Pacispora	3	9.1
Pauteria adolfi	4	*Acaulospora*	13	44.8
		Glomus	13	44.8
		Funneliformis	2	6.9
		Racocetra	1	3.4

Tree species	Genus richness	Genus name	Number of each genus	RA (%)
Podocarpus falcatus	3	*Acaulospora*	8	38.1
		Glomus	12	57.1
		Funneliformis	1	4.8

Unlike AMF genus richness, *Millettia ferruginea* was associated with the highest AMF species richness (18 species) followed by *Croton macrostachyus* and *Pouteria adolfifriedericii* each harbored 14 species. We recorded the lowest number of AMF species (7) from *Albizia shimperiana* (Table 7). Type of vegetation is the main driver of AMF community (Rodriguez *et al.*,2017). AMF mean spore density and AMF species richness showed positive correlation ($r = 0.8$; $p < 0.05$).Similar to AMF species richness, Shannon diversity index also varied 1.83 to 2.78 in *Albizia shimperiana* and*Millettia ferruginea*, respectively. *Millettia ferruginea* showed the highest Simpson dominance index value (14.3); whereas *Polycias fulva* displayed the lowest value (3.3) (Table 7).

Table 7
AMF Species richness (SR), Shannon diversity (H) and Simpson dominance (D) indices associated to different tree species at Munessa forest

Tree species	SR	H'	D
Albizia shimperiana	7	1.83c	5.6bc
Polycias fulva	8	2.02c	3.3c
Celtis africana	13	2.2bc	6.7b
Croton macrostachyus	14	2.5ab	9.1ab
Millettia ferruginea	18	2.78a	14.3a
Pouteria aldolfifriedericii	14	2.5ab	11.1ab
Podocarpus falcatus	12	2.39bc	9.1ab
Similar letters denote not significant difference at $p < 0.05$.			

The differences in AMF species or genus richness and diversity among indigenous trees could be due to AMF host plant preferences (Hazard *et al.*, 2013) and microenvironments host plants offer to AMF (Jinping *et al.*,2019). Other studies also indicated that soil pH affects AMF germination, diversity, richness where more spores are found in neutral than acidic soil (Xu et al., 2016; Jamiolkowska et al., 2018).

4. Conclusion

All tree species were showed AMF colonization in which hyphal colonization was the most observed structure and arbuscular colonization was the least. Thirty nine (39) AMF morphotypes belonging to 7

genera were identified of which genus *Glomus* the most dominant genus was followed by *Acaulospora*. The high of number spores and percentage of root colonization found in this study indicate great diversity of AMF associated with indigenous trees. The mean AMF spore densities per 100 g dry soil under indigenous tree species were significantly ($p \leq 0.05$) varied among tree species. All the tree species were associated with *Glomus* and *Acaulospora*. Among indigenous tree species *Millettia feruginea* associated with the highest AMF species number followed by *Croton macrostachyus.* Studying the diversity of indigenous AMF species associated with indigenous trees could be important for production of AMF inoculums used as bio-fertilizers in seedlings of these trees and rehabilitation of drylands. Further studies should be carried out with broad range of sampling trees in different areas including molecular identification.

Declarations

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jemal Yimer. Fassil Assefa, Belay Berza and Marcela Pagano supervised the work. Marcela Pagano identified AMF species. The first draft of the manuscript was written by Jemal Yimer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data and materials availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics approval and Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. AmogneAsfaw (2014) Forest resource management systems in Ethiopia: Historical perspective. Int J Biod and Cons 6(2):121–131
2. AnnissaMuhammed and Eyasu Elias (2020) Tree species composition, structure and regeneration status in Munessa natural forest. Southeast Ethiopia Eur J For Sci 8(1):35–53
3. Auliana, Kaonongbua W (2018) Preliminary study on biodiversity of arbuscular mycorrhizal fungi in oil palm (*Elaeisguineensis*Jacq.) plantations in Thailand.Earth and Environ. Sci.144 (1)
4. Belay Berza MC, Pagano VR, Prabavathy Z, Belay, Fassil Assefa (2021) Arbuscular mycorrhizal status of *Erythrinabrucei* in different land use types in Ethiopia. Appl Soil Ecol 165:104018
5. BeyeneDobo, FassilAssefa and ZebeneAsfaw (2016) Diversity and abundance of arbuscular mycorrhizal fungi under different plant and soil properties in Sidama, Southern Ethiopia. Adv Biosci Bioengi 4(3):16–24
6. Buckland ST, Borchers DL, Johnston A, Henrys PA, Marques TA (2007) Line transect methods for plant surveys. Biometrics 63(4):989–998
7. Ceulemans T, Van GM, Jacquemyn H, Boeraeve M, Plue J, Saar L et al (2019) Arbuscular mycorrhizal fungi in European grasslands under nutrient pollution. Global Ecol Biogeogr 28:1796–1805
8. Chen K (2012) Diversity of arbuscular mycorrhizal fungi in continuous cropping soils used for pepper production. Afr J Microbiol Res 6(10):2469–2474
9. Dawit Terefe Z, Belay FasilAssefa, KibebewKibret and NigussieDechassa (2021) Abundance and Diversity of Arbuscular Mycorrhizal Fungi (AMF) in Soils under Different Rangeland Use Types in the Middle Awash Basin, Ethiopia.East Afri. J. Sci. 15 (1):25–40
10. Diriba Muleta F, Assefa, Nemomissa S et al (2008) Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biol Fertil Soil 44:653–659
11. Diriba Muleta F, Assefa, Nemomissa S, 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 Manag 241:145–154
12. EmiruBirhane KideGebremedihin, TewodrosTadesse H, Mengesha and, Solomon N (2017) Exclosures restored the density and root colonization of arbuscular mycorrhizal fungi in Tigray, Northern Ethiopia. Ecol Processes 6(1):1–12
13. EmiruBirhane, Kuyper TW, Sterck FJ, Bongers F (2010) Arbuscular mycorrhizal associations in *Boswelliapapyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. For Ecol Manage 260(12):2160–2169

14. EmiruBirhane TeklemariamGebregergs, MengisteabHailemariam L, Norgrove and ErmiasAynekulu (2021) Root Colonization and Spore Abundance of Arbuscular Mycorrhizal Fungi along Altitudinal Gradients in Fragmented Church Natural Forest Remnants in Northern Ethiopia. Microbial Ecol
15. FAO (2022) The State of the World's Forests (2022) Forest pathways for green recovery and building inclusive, resilient and sustainable economies.Rome. FAO
16. Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *endogone* species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244
17. Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New phytologist, pp 489–500
18. Haileab Zegeye, TeketayDemel and EnsermuKelbessa (2011) Diversity and regeneration status of woody species in Tara Gedam and Abebaye forests, northwestern Ethiopia. J For Res 22:315–328
19. Heijden MGA, van der, Boller T, Wiemken A (1998) Different arbuscular mycorrhizal fungi species are potential determinants of plant community structure. *Ecology*79:2082– 2091
20. Hinds AA, Lowe LE (1980) Application of the berthelot reaction to the determination of ammonium-n in soil extracts and soil digests. Commun Soil Sci Plant Anal 11(5):469–475
21. Huange W, Pohjonen V, Johansson S, Nashanda M, Katigula MIL, Luukkanen O (2003) Species diversity, forest structure and species composition in Tanzanian tropical forests. For Ecol Manag 173:11–24
22. Husband R, Herre EA, Young JPW (2002) Temporal variation in the arbuscular mycorrhizal communities colonizing seedlings in a tropical forest. Microbiol Ecol 42:131–136
23. Jakobsen I, Rosendahl LJ (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol s115:77–83
24. Jamiolkowska A, Ksiezniak A, Galazka A, Hetman B, Kopacki M, SkwaryloBednarz B (2018) Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: a Review. Int Agrophys 32:133–140
25. Jinping W, Shilin MG, Geof W, Linhao X, Zhiyuan Fu, Juan S, Zhang J (2019) Arbuscular mycorrhizal fungi communities associated with wild plants in a coastal ecosystem. J For Res 32:683–695
26. Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001) Promotion of AM utilization through reduced P fertilization. Plan tand Soil 231(1):65–79
27. Kedir Nino, MamoYosef, MengeshaGirma, and KefyalewSahle (2017) GIS based ecotourism potential assessment in MunessaShashemene Concession Forest and its surrounding area, Ethiopia. App Geogr 82:48–58
28. Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141
29. Lambers H, Teste PF (2013) Plant Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? Cell and Environment 36:1911–1915

30. Oehl F, Sieverding E, Ineichen K, Mäder P, Wiemken A et al (2009) Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. Agr Ecosyst Environ 134:257–268
31. Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL (ed) *Methods of Soil Analysis*, Part 2: Chemical and Microbiological Properties. ASA Monograph 9: 403–430
32. Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbiosis. Nat Rev Microbiol 6:763–775
33. Picone C (2000) Diversity and abundance of arbuscular mycorrhizal fungus spores in tropical forest and pasture.*Biotropica*. 32:734–7504
34. Rodríguez-Echeverría S, Teixeira H, Correia M, Timóteo S, Heleno R, Öpik M, Moora M (2017) Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. New Phytol 213:380–390
35. Schmitt CB, Denich M, Demissew S, Friis I, Boehmer HJ (2010) Floristic diversity in fragmented Afromontane rainforests: altitudinal variation and conservation importance. Appl Veg Sci 13:291–304
36. Shannon CE (1948) The mathematical theory of communication. Bell Syst Tech J 27(3):379–423
37. Smith S, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic press, Elsevier, Cambridge
38. Tadessse Chanie and Fassil Assefa (2013) Arbuscular mycorrhizal fungi associated with shade trees and *Coffeaarabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afri.Focus.*26 (3):111–131
39. Teketay Demel, Mulugeta D, Bekele L, Yemshaw T, Feleke Y, Tadesse SW, Nigussie D (2010) Forest resources and challenges of sustainable forest management and conservation in Ethiopia.Degraded forests in Eastern Africa: management and restoration.*Earth scan*.Pp:19–63
40. TesfayeWubet, Kottke I, TeketayDemel, Oberwinkler F (2003) Mycorrhizal status of indigenous trees in dry Afromontane forests of Ethiopia. For Ecol Manag 179:387–399
41. Torti S, Coley P, Janos D (1997) Vesicular-arbuscular mycorrhizae in two tropical monodominant trees. J Trop Ecol 13(4):623–629. 10.1017/S0266467400010762
42. Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Appl Environ Microbiol 64(12):5004–5007
43. Walkley A, Black IA (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
44. Wang, Johanna WH, Lutz, Adrian, Natera S, Wang M, Ng V, Grigoriev I, Martin F, Roessner U, Anderson IC, Plett, Jonathan M (2020) The Influence of Contrasting Microbial Lifestyles on the Pre-symbiotic Metabolite Responses of *Eucalyptus grandis* Roots. *Front. Ecol. and Evol.*7:*10.*
45. Wang J, Wang GG, Zhang B, Yuan Z, Fu Z, Yuan Y, …, Zhang J (2019) Arbuscular mycorrhizal fungi associated with tree species in a planted forest of Eastern China. Forests 10(5):424

46. Wang W, Shi W, Xie J, Jiang Q, Yu Y, N and, Wang E (2017) Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Mol Plant 10:1147–1158
47. Geoff Wang WJ, Yuan GZhangB, Zhu ZFuZYuanY, Ma L. S. and, Zhang J (2019) Arbuscular Mycorrhizal Fungi Associated with Tree Species in a Planted Forest of Eastern China. *Forests*10: 424
48. Winagraski E, Kaschuk G, Monteiro PHR, Auer CG, Higa AR (2019) Diversity of arbuscular mycorrhizal fungi in forest ecosystems of Brazil: A review. Cerne 25:25–35
49. Xu TL, Veresoglou SD, Chen YL, Rillig MC, Xiang D, Ondrej D (2016) Plant community, geographic distance and abiotic factors play different roles in predicting AMF biogeography at the regional scale in northern China. Environ Microbiol Rep 8:1048–1057
50. Yonas, Yohannes and FassilAssefa (2007) Phenotypic characteristics of root nodule Bacteria and Arbuscular Mycorrhizal Fungi infecting Acacia polyacantha growing in Gibe wooded grasslands. Ethio J Natu Sci 9(1):123–139
51. Zerihun Belay, Negash M, Kaseva J, Vestberg M, Kahiluoto H (2020) Native forests but not agroforestry systems preserve arbuscular mycorrhizal fungal species richness in southern Ethiopia, vol 30. Mycorrhiza, pp 749–759. 6
52. Zerihun Belay,Vestberg M, FassilAssefa (2013) Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. Afri J Microbiol Res 7(48):5503–5515
53. Zerihun Belay,Vestberg M, FassilAssefa (2015) Diversity and abundance of Arbuscular Mycorrhizal Fungi across different land use types in a Humid Low Land Area of Ethiopia. Trop and Subtrop Agro 8(1):47–69
54. Zhao ZW, Wang GH, Yang L (2003) Biodiversity of arbuscular mycorrhizal fungi in a tropical rainforest of Xishuangbanna, southwest China. Fungal Divers 13:233–242
55. Shi. Z ZM, Yang. M, Cao LuS. L and, Wang X (2021) Molecular Diversity and Distribution of Arbuscular Mycorrhizal Fungi at Different Elevations in Mountain Tibia of Qinling Mountain. Front Microbiol 12:609386
56. Zhang Y, Guo LD, Liu RJ (2004) Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. Plant Soil 261(1–2):257–263
57. Ziadin N, Tran TS (2007) Lime requirement. In: Carter, Martin R (eds) Soil Sampling and Methods of Analysis. Canadian Soil Science Society.Lewis Publishers, Boca Raton, Florida, pp 129–134

Figures

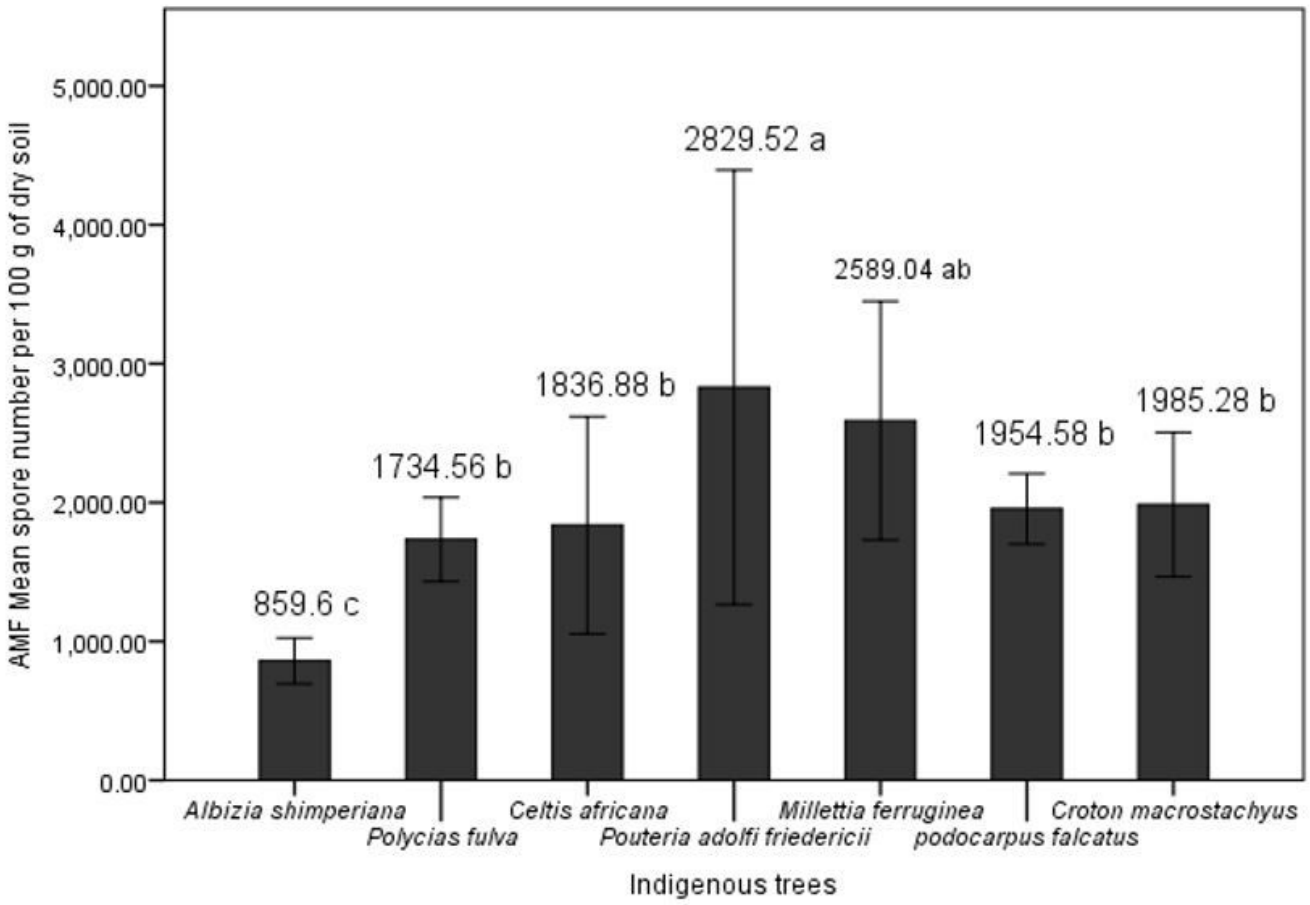

Figure 1

Mean spore density of AMF extracted from indigenous trees of Munessa forest

Means followed by the same letter do not differ significantly by LSD ($p < 0.05$.)

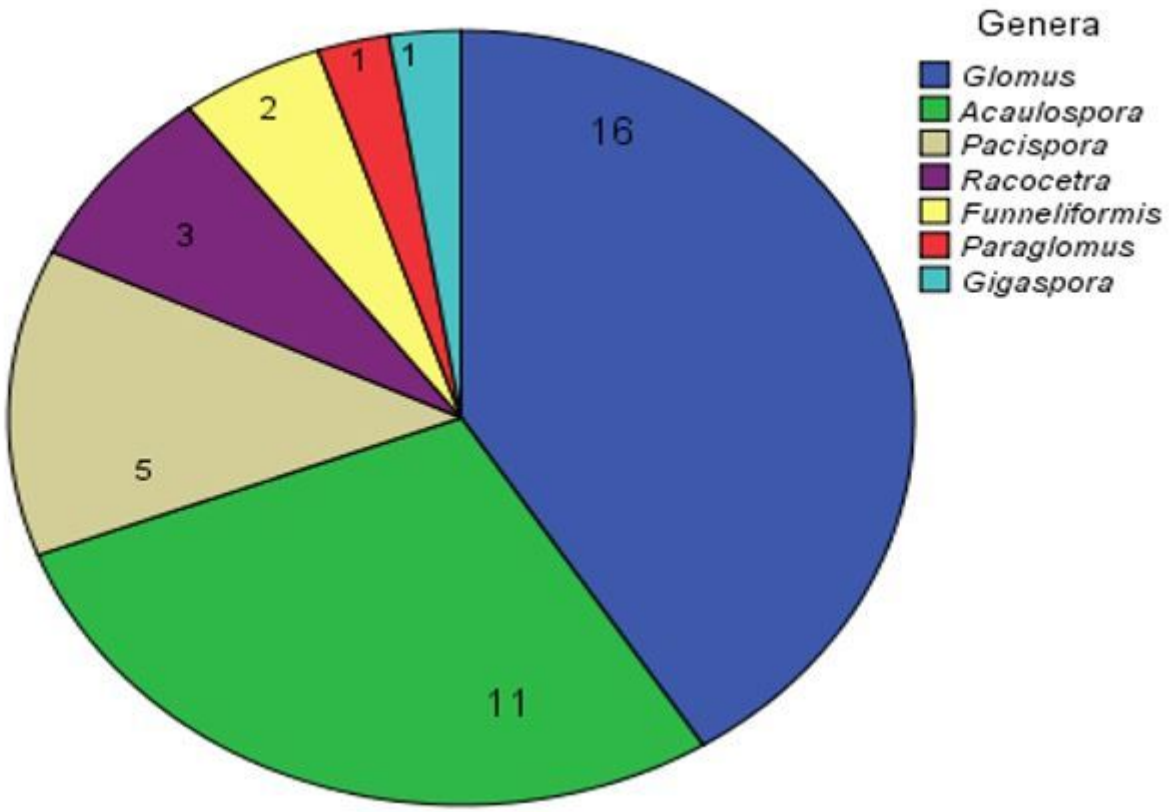

Figure 2

The number of identified morphotypes in a genus level