

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

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Abstract

In terrestrial ecosystems, arbuscular mycorrhizal fungi (AMF) helps land plants in nutrient uptake and protect them against abiotic and abiotic stresses. It is important to study the AMF-plant relationship to fully realize the beneficial potential to support establishment, growth and maintenance of mycorrhizal plants in different ecosystems. 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 soil samples and fine roots were collected from seven dominant indigenous trees within 10 m x10 m plots in the forest. AMF spores were extracted for taxonomic identification and root colonization with AMF structures was determined for each trees. A total of 39 AMF species (morphotypes) belonging to 10 genera were recovered. *Glomus* was the most dominant genus followed by *Acaulospora*. The highest genus richness was recorded from *Croton macrostachyus* (9). Mean AMF spore density was significantly different ($p < 0.05$) among indigenous trees, ranging from 860 spores/100 g of dry soil under *Albizia shimperiana* to 2830 spores/100 g of dry soil under *Pouteria adolfiifriedericii*. The highest root hyphal colonization was observed in *Millettia ferruginea* (71%) and *Podocarpus falcatus* (64%), respectively. *Albizia shimperiana* was the least colonized tree (44%). AMF spore density was positively correlated with soil available phosphorus ($p < 0.05$). Extensive studies are required to select AMF inocula for plant growth promotion and recruitment of tree seedlings for plantation and, restoration of forest vegetation and rehabilitation of degraded and dry 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 disasters and anthropogenic activities. The Ethiopian highlands constitute 60% of the Afromontane tropical forest ecosystems of eastern Africa that are rich in plants and animals and endemic plants and animals species (Schmitt et al., 2010). The woody plant species are the important components of forest ecosystems that 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 timber, 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 arbuscular 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 the uptake of poorly mobile soil nutrients such as P, K and Mg to plants (Yadeva et al, 2017), 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 tree species (Muthukumar et al., 2022). AMF also increase the volume of soil to be exploited and thereby improve phosphorous (P) use efficiency and P access to plants and its 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 communities in a natural ecosystems could potentially affect the way plant species coexist, and determine plant community structure (Heijden et al., 1998). Moreover, knowledge about AMF diversity may help forest scientists to understand relationships of species selection, AMF production and inoculation.

In Ethiopia, 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 south west Ethiopia (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 ecosystems and agro-forestry systems in central Shewa (Belay et al., 2013; Belay et al., 2015), species diversity and population density of AMF in Sidama agro-forestry systems (Dobo et al., 2016), AMF status of *Erythrina brucei* in different land use types in Ethiopia (Berza et al., 2021) and species 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 across the globe. 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 still dearth of sufficient information on species and population density of arbuscular mycorrhizal fungi in dense and protected forests in Ethiopia. Therefore, the objective of this study was to evaluate the AMF species diversity, abundance, population density and root colonization in indigenous trees in Munessa Forest.

2. Materials and Methods

2.1. Study area

The study was conducted in Munesa 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. Munesa forest encompasses 48% of Arsi forest enterprise which include Munesa, 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 names (in Amharic and in Afan Oromo language) of trees were recorded (Table 1). 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 collected indigenous trees species in Munesa forest, their scientific and their Amharic and Afanoromo names.

Scientific name	Family	Amharic	Afanoromo
<i>Podocarpus falcatus</i>	<i>Podocarpaceae</i>	Zigba	Birbisa
<i>Croton macrostachyus</i>	<i>Euphorbaceae</i>	Bisanna	Bakkanisa
<i>Albizia shimperiana</i>	<i>Fabaceae</i>	Sassa	Mukarba (Karchafe)
<i>Millettia ferruginea</i>	<i>Fabaceae</i>	Birbira	Dhadhattu
<i>Celtis africana</i>	<i>Ulmaceae</i>	Kawoot	Amallaqqaa
<i>Pouteria adolfriedericii</i>	<i>Sapotaceae</i>	Keraro	Suduba, (keraro)
<i>Polycias fulva</i>	<i>Araliaceae</i>	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 °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 Gerdemann 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. The spores 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 2000 rpm 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 °C for 24 h. 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 Munesa 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). Mean AMF spore densities and log transformed root colonization data were subjected to One way ANOVA to test the significant difference among mean AMF spore densities in relation to indigenous tree species. The 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 Discussion

3.1. Soil physico-chemical characteristics

The soil physicochemical properties varied among different indigenous tree rhizospheres. The mean value of soil pH, organic carbon (%), organic matter (%), total nitrogen (%) and available phosphorus were 7.45, 11.5, 19.8 (%), 1.25(%) and 4.37 ppm, respectively in the Munesa forest. The highest pH (7.96) was observed under *Celtis africana* and lowest (6.78) was observed under *Albizia schimperiana*. Thus, in this particular forest, pH was varied between neutral and alkaline. The rhizosphere soil of *Pouteria adolfifriedericii* was exhibited the highest in organic carbon (14.9%) and the lowest (8.2%) was recorded under *Podocarpus falcatus*. Similarly, total nitrogen was the highest (1.55%) under *Pouteria adolfifriedericii* rhizosphere and the lowest (0.96%) under *Podocarpus falcatus* rhizosphere. Available phosphorus was also varied between (1.11 ppm) under *Podocarpus falcatus* and (8.46 ppm) in the rhizosphere of *Millettia ferruginea* (Table 2). This implies that the Munesa forest was poor in available phosphorous. Similarly, Bajigo (2017) has also recorded poor available phosphorous ranging between 1.6 and 10.9 ppm.

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

Tree species	Soil chemical parameters				
	PH	O.C	O.M	TN	AV.P
	(H ₂ O)	(%)	(%)	(%)	(ppm)
<i>Podocarpus falcatus</i>	7.27	8.2	14.1	0.96	1.11
<i>Croton macrostachyus</i>	7.42	11.7	20.1	1.27	4.55
<i>Albizia schimperiana</i>	6.78	9.7	16.6	1.09	1.22
<i>Millettia ferruginea</i>	7.72	14.2	24.4	1.48	8.46
<i>Celtis africana</i>	7.96	12.6	21.7	1.35	6.19
<i>Pouteria adolfifriedericii</i>	7.36	14.9	25.8	1.55	7.67
<i>Polyscias fulva</i>	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* was least colonized (44%) (Table 3). However, with regard to the vesicular colonization, *Celtis africana* (36.37%) and *Polycias fulva* (33.36%) showed significantly higher vesicular colonization compared to the other trees. In this study, indigenous trees exhibited variations in arbuscular mycorrhization ranging from 10.18% (*Albizia shimperiana*) to 13.92% (*Croton macrostachyus*). This higher arbuscular colonization could be associated to poor available phosphorous in the particular forest and might be highly engaged in P exchange between AMF and trees. Another study by Dobo *et al.* (2016) has reported higher hyphal colonization in *Croton macrostachyus* (64.11%) and similar arbuscular colonization in *Millettia ferruginea* (11.67%) in Sidama agro forestry systems in southern Ethiopia. In addition, Channie and Assefa (2013) have also found higher hyphal colonization in *Millettia ferruginea* (69%) and in *Polycias fulva* (56%). However, they had 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 physico-chemical properties, agroforestry practices and climatic conditions that can influence both plants and the AMF. Moreover, the reduced root length colonization could also be associated to the 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 $\pm$ SE).

Tree species	Root colonization by AMF structures		
	AC	VC	HC
<i>Podocarpus falcatus</i>	11.24 $\pm$ 4.26a	26.57 $\pm$ 8.48ab	64.02 $\pm$ 8.76a
<i>Croton macrostachyus</i>	13.92 $\pm$ 4.47a	17.75 $\pm$ 4.61b	59.60 $\pm$ 6.11a
<i>Albizia shimperiana</i>	10.18 $\pm$ 4.11a	15 $\pm$ 5.65b	44.44 $\pm$ 5.50b
<i>Millettia ferruginea</i>	11.68 $\pm$ 3.44a	25.69 $\pm$ 7.60ab	71.00 $\pm$ 8.19a
<i>Celtis africana</i>	10.92 $\pm$ 3.16a	36.37 $\pm$ 5.00a	52.78 $\pm$ 3.77ab
<i>Pouteria adolfifriedericii</i>	11.85 $\pm$ 3.84a	15 $\pm$ 5.65b	52.78 $\pm$ 3.77ab
<i>Polycias fulva</i>	12.89 $\pm$ 3.36a	33.36 $\pm$ 3.81a	59.48 $\pm$ 6.41ab
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 to a study by Birhane *et al.* (2021) who have reported AMF colonization of church forests in northern Ethiopia which was in the range of 31–82%. Our results are much higher than hyphal colonization (18.6%-34%) reported from grassland ecosystems in the middle Awash basin in Ethiopia (Terefe *et al.*, 2021) that could be due to the differences in plant species, soil physicochemical properties and 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) who have studies AMF status *Erythrina brucei* in different land use types in Ethiopia and Birhane *et al.* (2021) who reported more hyphal colonization compared to the other AMF structures in church forests in northern Ethiopia. Parniske (2008) has reported that colonization by arbuscules is lower than others, which are partly due to the quick degradation of arbuscules 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 systems. Plants having less extensive root systems are more dependent on mycorrhizae compared to 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 ($p < 0.05$). The mean AMF spore density extracted and collected under indigenous trees soil varied from 860 to 2830 with average of 1970 per 100 g of dry soil. The highest mean AMF spore density was recorded from the rhizosphere soil of *Pouteria adolfifriedericii* (2830 per 100 g dry soil) followed by *Millettia ferruginea* (2589 per 100 g dry soil) and the lowest spore number recorded from *Albizia shimperiana* (860 per 100 g dry soil) (Fig. 1).

The spore density recorded in this study was almost twice higher than those recorded by Channie and Assefa (2013) from *Croton macrostachyus* (1098 per 100 g of dry soil), four times higher than the record on *Millettia ferruginea* (670 per 100 g of dry soil), *Pouteria adolfifriedericii* (787 per 100 g of dry soil), and three times higher than reported from *Polycias fulva* (638 spores per 100 g dry soil) in Bonga forest south western Ethiopia (Channie and Assefa, 2013). It is also twice higher than the spore density (927 spores per 100g of dry soil) recorded from *Podocarpus falcatus*, and four times higher (617 spores per 100 g of dry soil) from *Millettia ferruginea* (Asmelash *et al.*, 2021). However, Birhane *et al.* (2017) have reported much higher AMF spore density from *Croton macrostachyus* (5729 per 100 g of dry 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 g of dry soil) from different plant species in planted forest in eastern China.

This particular study showed similar patterns of spore densities compared to those reported from Central American tropical forest (110–2600 spores per 100 g of 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 ours is much higher than those 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 dry soil) (Terefe *et al.*,2021). The mean AMF spore density can be affected by seasons 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) have also reported positive correlation of AMF spore densities 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 (= species) belonging to 10 genera of Glomeromycota were identified (Table 5; online resource 1). All morphotypes were identified to genus level and 19 to the species level. The results showed that the Genus *Glomus* comprised of the largest number of morphotypes (14), followed by *Acaulospora* (10). The other genera: *Pacispora* (5), *Racocetra* (3), *Funneliformis* (2), *Paraglomus* (1),*Sclerocystis* (1), *Redeckera* (1) ,*Archaeospora* (1) and *Gigaspora* (1) (Fig. 2).

Thus, *Glomus* and *Acaulospora* were categorized as dominant in Munesa forest; whereas the genera *Pacispora*, *Racocetra*, *Funneliformis* and *Paraglomus* were considered as common and *Gigaspora*, *Sclerocystis*, *Redeckera* and *Archaeospora* were rare (Table 4).

Table 4 Isolation frequency, relative abundance and importance value of AMF genus isolated from indigenous trees of Munessa forest.				
Genus	IF (%)	RA (%)	IV (%)	Status
<i>Glomus</i>	100	45.1	72.6	Dominant
<i>Acaulospora</i>	100	30.5	65.3	Dominant
<i>Pacispora</i>	57.1	11	34.1	Common
<i>Racocetra</i>	71.4	5.5	38.5	Common
<i>Funneliformis</i>	57.1	4.9	31	Common
<i>Paraglomus</i>	28.6	2.4	15.5	Common
<i>Gigaspora</i>	14.3	0.6	7.5	Rare
<i>Sclerocystis</i>	14.3	0.6	7.5	Rare
<i>Redeckera</i>	14.3	0.6	7.5	Rare
<i>Archaeospora</i>	14.3	0.6	7.5	Rare
IV ≥ 50%: dominant, 10% < IV < 50%: common, IV ≤ 10%: rare (Chen <i>et al.</i> , 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	<i>Funnelformis geosporus</i>	57.1	3	30.1	common
2	<i>Pacispora dominikii</i>	57.1	7.3	32.2	common
3	<i>Acaulospora rehmi</i>	14.3	0.6	7.5	rare
4	<i>Sclerocystis rubiformis</i>	14.3	0.6	7.5	rare
5	<i>Archaeospora myriocarpa</i>	14.3	0.6	7.5	rare
6	<i>Glomus magnicaule</i>	71.4	4.9	38.2	common
7	<i>Racocetra castanea</i>	57.1	3	30.1	common
8	<i>Acaulospora lacunose</i>	57.1	8.5	32.8	common
9	<i>Acaulospora mellea</i>	71.4	3	37.2	common
10	<i>Paragomus bolivianum</i>	28.6	2.4	15.5	common
11	<i>Pacispora franciscana</i>	14.3	0.6	7.6	rare
12	<i>Pacispora corralloidea</i>	42.9	1.8	22.4	common
13	<i>Acaulospora delicate</i>	14.3	1.2	7.8	rare
14	<i>Racocetra gregaria</i>	14.3	1.8	8.1	rare
15	<i>Acaulospora spinosa</i>	28.6	2.4	15.5	common
16	<i>Glomus multicaule</i>	14.3	1.2	7.8	rare
17	<i>Glomus melanosporum</i>	14.3	1.8	8.1	rare
18	<i>Redeckera fulvum</i>	14.3	0.6	7.5	rare
19	<i>Funnelformis mosseae</i>	28.6	1.8	15.2	common
20	<i>Glomus sp1.</i>	28.6	1.8	15.2	common
21	<i>Glomus sp2.</i>	14.3	1.8	8.1	rare
22	<i>Glomus sp3.</i>	14.3	1.2	7.8	rare
23	<i>Glomus sp4</i>	28.6	1.8	15.2	common
24	<i>Glomus sp5</i>	28.6	1.8	15.2	common
25	<i>Glomus sp6</i>	28.6	1.8	15.2	common
26	<i>Glomus sp7</i>	42.9	1.8	22.4	common
27	<i>Glomus sp8</i>	14.3	1.2	7.8	rare
28	<i>Glomus sp9</i>	14.3	1.2	7.8	rare
29	<i>Glomus sp10</i>	14.3	1.2	7.8	rare
30	<i>Glomus sp11</i>	42.9	2.4	22.7	common
31	<i>Acaulospora sp1</i>	42.9	3	23	common
32	<i>Acaulospora sp2</i>	14.3	1.2	7.8	rare
33	<i>Acaulospora sp3</i>	14.3	1.2	7.8	rare
34	<i>Acaulospora sp4</i>	14.3	0.6	7.5	rare
35	<i>Acaulospora sp5</i>	14.3	0.6	7.5	rare
36	<i>Pacispora sp1</i>	14.3	0.6	7.5	rare
37	<i>Pacispora sp2</i>	14.3	0.6	7.5	rare
38	<i>Racocetra sp</i>	14.3	0.6	7.5	rare
39	<i>Gigaspora sp</i>	14.3	0.6	7.5	rare
IV ≥ 50%: dominant, 10% < IV < 50%: common, IV ≤ 10%: rare (Chen <i>et al.</i> , 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). In addition, the number of morphotypes were comparable to the *Erythrina brucei* in different land use type (33 AMF species) (Berza *et al.*, 2021) and the number of genera (10) recorded in this study were also comparable to 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. With regard to genera, our results are in line with Terefe *et al.* (2021). The isolation frequency showed that *Glomus* and *Acaulospora* were dominant. The 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) are in line with our results. 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* was recovered under all tree species, whereas, *Acaulospora* was isolated from 71.4% of trees, 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 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, 43.5% of the AMF species were common species isolated from the rhizosphere of majority of tree species, *Glomus magnicaule* (IV = 38.2%) followed by *Acaulospora mellea* (IV = 37.2%), *Pacispora dominikii* (IV = 32.2%) and *Acaulospora lacunose* (IV = 32.2%) (Table 5). The majority of the morphotypes (56.5%) were recovered and identified from single soil sample obtained from the rhizosphere of different indigenous tree species. As result, they were considered as rare species according to important value (IV) index (Table 5). In addition, the highest relative abundance (RA) was recorded from *Acaulospora lacunose* (8.5%) followed by *Pacispora dominikii* (7.3%) and *Glomus magnicaule* (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 9 (Table 6). *Croton macrostachyus* was associated with the highest genera richness (9 genera) followed by *Millettia ferruginea* (5 genera), *Albizia shimperiana* (5 genera) and *Pouteria adolfifriedericii* (5 genera). The lowest number of genera (3 genera) was recorded from *Podocarpus falcatus* (Table 6). The results from frequency of occurrence of AMF genera among tree species showed that *Glomus* and *Acaulospora* were dominantly distributed across all the indigenous trees; whereas the genera *Racocetra* (5 tree species), *Pacispora* (4 tree species) and *Funneliformis* (4 tree species) (Table 7).

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 (%)
<i>Podocarpus falcatus</i>	3	<i>Acaulospora</i>	8	38.1
		<i>Glomus</i>	12	57.1
		<i>Funneliformis</i>	1	4.8
<i>Croton macrostachyus</i>	9	<i>Acaulospora</i>	5	19.2
		<i>Pacispora</i>	8	30.8
		<i>Glomus</i>	6	23.1
		<i>Paraglomus</i>	3	11.5
		<i>Funneliformis</i>	3	11.5
		<i>Racocetra</i>	1	3.8
		<i>Sclerocystis</i>	6	23.1
		<i>Redeckera</i>	6	23.1
<i>Albizia shimperiana</i>	5	<i>Funneliformis</i>	2	20
		<i>Pacispora</i>	2	20
		<i>Acaulospora</i>	2	20
		<i>Glomus</i>	4	40
<i>Millettia ferruginea</i>	5	<i>Acaulospora</i>	13	39.4
		<i>Glomus</i>	12	36.4
		<i>Racocetra</i>	4	12.1
		<i>Paraglomus</i>	1	3
		<i>Pacispora</i>	3	9.1
<i>Celtis africana</i>	4	<i>Glomus</i>	18	58.1
		<i>Pacispora</i>	5	16.1
		<i>Acaulospora</i>	7	22.6
		<i>Racocetra</i>	1	3.2
<i>Pouteria adolfiifriedericii</i>	5	<i>Archaeospora</i>	13	39.4
		<i>Acaulospora</i>	13	44.8
		<i>Glomus</i>	13	44.8
		<i>Funneliformis</i>	2	6.9
		<i>Racocetra</i>	1	3.4
<i>Polycias fulva</i>	4	<i>Glomus</i>	9	60
		<i>Racocetra</i>	2	13.3
		<i>Acaulospora</i>	3	20
		<i>Gigaspora</i>	1	6.6

Table 7
Frequency of occurrence of AMF genera under the rhizosphere of indigenous trees in Munesa forest

Tree species	AMF genera									
	<i>Glomus</i>	<i>Acaulospora</i>	<i>Pacispora</i>	<i>Racocetra</i>	<i>Funnelliformis</i>	<i>Paraglomus</i>	<i>Gigaspora</i>	<i>Sclerocystis</i>	<i>Redeckera</i>	<i>Archae</i>
<i>Podocarpus falcatus</i>	X	X	-	-	X	-	-	-	-	-
<i>Croton macrostachyus</i>	X	X	X	X	X	X	X	X	X	-
<i>Albizia shimperiana</i>	X	X	X	-	X	-	-	-	-	-
<i>Millettia ferruginea</i>	X	X	X	X	-	X	-	-	-	-
<i>Celtis africana</i>	X	X	X	X	-	-	-	-	-	-
<i>Pouteria adolfifriedericii</i>	X	X	-	X	X	-	-	-	-	X
<i>Polycias fulva</i>	X	X	-	X	-	-	X	-	-	-

X = recovered from a particular tree rhizosphere; - = not recovered from a particular tree rhizosphere.

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 (Table 8). The lowest number of AMF species (7) was recovered from *Albizia shimperiana* rhizosphere (Table 8). 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 between 1.83 (in *Albizia shimperiana*) and 2.78 (in *Millettia ferruginea*). Moreover, *Millettia ferruginea* showed the highest Simpson dominance index value (14.3); whereas *Polycias fulva* exhibited the lowest value (3.3) (Table 8).

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

The differences in AMF species and/or genus richness and/or 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 arbuscular mycorrhizal and colonized with vesicles, arbuscules and hyphae. Thirty nine (39) AMF morphotypes (= species) belonging to 10 genera were identified in this study. Genus *Glomus* the most dominant followed by *Acaulospora*. The high of number spores and percentage of root colonization found in this study indicated great diversity of AMF associated with indigenous trees. The mean AMF spore densities per 100 g dry soil under indigenous tree species were significantly ($p \leq 0.05$) different among tree species. All the tree species were associated with *Glomus* and *Acaulospora*. Among indigenous tree species *Millettia feruginea* was associated with the highest number of AMF species followed by *Croton macrostachyus*. Studying the diversity of indigenous AMF species associated with indigenous trees could be important for the production of AMF inocula that can be used as bio-fertilizers in the establishment of seedlings of these trees and rehabilitation of degraded and dry lands. Further studies should be carried out with a broad range of sampling trees in different areas including molecular AMF 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. Annissa Muhammed and Eyasu Elias (2020) Tree species composition, structure and regeneration status in Munessa natural forest, Southeastern Ethiopia. *Eur. J. Forest Sci.* **8** (1):35-53.
3. Asmelash, F., Bekele, T., Belay, Z., & Kebede, F. (2021) Soil physicochemical property and arbuscular mycorrhizal fungi resilience to degradation and deforestation of a dry evergreen Afromontane forest in central Ethiopia. *Land Degradation & Development*, 32(11), 3338-3350.
4. Auliana and 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)
5. Bajigo, A. (2017) The Effect of Land Use Change on Soil Physicochemical Properties over Time in Ethiopia. *Journal of Developing Country Studies*, 7(3), 60-71.
6. Belay Berza, Marcela Claudia Pagano, V.R. Prabavathy, Zerihun Belay and Fassil Assefa (2021) Arbuscular mycorrhizal status of *Erythrinabrucei* in different land use types in Ethiopia. *Appl. Soil Ecol.* **165**:104018.
7. Beyene Dobo, FassilAssefa and ZebeneAsfaw (2016) Diversity and abundance of arbuscular mycorrhizal fungi under different plant and soil properties in Sidama, Southern Ethiopia.*Advances in Biosci and Bioengi.* **4** (3):16-24.
8. Buckland, S. T., Borchers, D. L., Johnston, A., Henrys, P. A. and Marques, T. A (2007) Line transect methods for plant surveys. *Biometrics* **63**(4):989–998.
9. Ceulemans, T., Van, G. M., 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.
10. Chen, K (2012) Diversity of arbuscular mycorrhizal fungi in continuous cropping soils used for pepper production.*Afr. J. Microbiol. Res.* **6** (10):2469–2474.
11. Dawit Terefe, Zerihun 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.
12. Diriba Muleta, Fassil Assefa and 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.
13. Diriba Muleta, Fassil Assefa, Nemomissa, S. and Granhall, U (2007) Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. *Forest Ecol. Manag.* **241**:145–154.
14. Emiru Birhane, KideGebremedihin, TewodrosTadesse, Hailemariam, Mengesha and Negasi Solomon (2017) Exlosures restored the density and root colonization of arbuscular mycorrhizal fungi in Tigray, Northern Ethiopia. *Ecological Processes*, **6** (1): 1–12.
15. Emiru Birhane, Kuyper, T.W., Sterck, F.J. and Bongers, F (2010) Arbuscular mycorrhizal associations in *Boswelliapapyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *Forest Ecol. and Management*, **260** (12): 2160–2169.
16. Emiru Birhane, TeklemariamGebregergs, MengisteabHailemariam, Lindsey 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.*

17. FAO 2022. The State of the World's Forests (2022) Forest pathways for green recovery and building inclusive, resilient and sustainable economies. Rome, FAO.
18. Gerdemann, J. W. and Nicolson, T. H (1963) Spores of mycorrhizal *endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**: 235-244.
19. Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New phytologist*, 489-500.
20. Haileab Zegeye, Teketay Demel and Ensermu Kelbessa (2011) Diversity and regeneration status of woody species in Tara Gedam and Abebaye forests, northwestern Ethiopia. *J. Forest. Res.* **22**: 315–328.
21. Hazard, C., Gosling, P., Van Der Gast, C. J., Mitchell, D. T., Doohan, F. M., & Bending, G. D. (2013) The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. *The ISME journal*, 7(3), 498-508.
22. Heijden M.G.A van der, Boller, T. and Wiemken, A (1998) Different arbuscular mycorrhizal fungi species are potential determinants of plant community structure. *Ecology* **79**: 2082– 2091.
23. Hinds, A.A. and Lowe, L.E (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.
24. Husband, R., Herre, E.A., and Young, J.P.W (2002) Temporal variation in the arbuscular mycorrhizal communities colonizing seedlings in a tropical forest. *Microbiol. Ecol.* **42**: 131-136.
25. Jamiolkowska, A., Ksiezniak, A., Galazka, A., Hetman, B., Kopacki, M., and Skwarylo Bednars, 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.
26. Jinping, W., Shilin, M. G., Geof, W., Linhao, X. Zhiyuan, Fu., Juan, S. and Zhang, J (2019) Arbuscular mycorrhizal fungi communities associated with wild plants in a coastal ecosystem. *J. For. Res.* **32**: 683–695.
27. Kahiluoto, H., Ketoja, E., Vestberg, M. and Saarela, I (2001) Promotion of AM utilization through reduced P fertilization. *Plan tand Soil*, **231** (1): 65–79
28. Kedir Nino, Mamo Yosef, Mengesha Girma, and Kefyalew Sahle (2017) GIS based ecotourism potential assessment in Munessa Shashemene Concession Forest and its surrounding area, Ethiopia. *App. Geography* **82**: 48–58.
29. Muthukumar, T., Sumathi, C. S., Rajeshkannan, V., & Bagyaraj, D. J. (2022) Mycorrhizosphere Revisited: Multitrophic Interactions. In *Re-visiting the Rhizosphere Eco-system for Agricultural Sustainability* (pp. 9-35). Singapore: Springer Nature Singapore.
30. Oehl, F., Sieverding, E., Ineichen, K., Mäder, P. and 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, S.R. and Sommers, L.E (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** (4): 734–750.
34. Rodríguez-Echeverría, S., Teixeira, H., Correia, M., Timóteo, S., Heleno, R., Öpik, M. and Moora, M (2017) Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. *New Phytol.* **213**: 380-390
35. Schmitt, C.B., Denich, M., Demissew, S., Friis, I. and Boehmer, H.J (2010) Floristic diversity in fragmented Afromontane rainforests: altitudinal variation and conservation importance. *Appl. Veg. Sci.* **13**: 291–304.
36. Siddiqui, Z. A., and Pichtel, J. (2008) "Mycorrhizae: an overview" in *Mycorrhizae: Sustainable agriculture and forestry*. eds. Z. A. Siddiqui, M. S. Akhtar and K. Futai (Dordrecht: Springer), 1–35.
37. Smith, S. and Read, D (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic press, Elsevier, Cambridge
38. Tadesse Chanie and Fassil Assefa (2013) Arbuscular mycorrhizal fungi associated with shade trees and *Coffea arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afri. Focus*. **26** (3): 111-131.
39. Tesfaye Wubet, Kottke, I., Teketay Demel and Oberwinkler, F (2003) Mycorrhizal status of indigenous trees in dry Afromontane forests of Ethiopia. *Forest Ecol. Manag.* **179**: 387- 399.
40. Torti, S., Coley, P., & Janos, D. (1997). Vesicular-arbuscular mycorrhizae in two tropical monodominant trees. *Journal of Tropical Ecology*, 13(4), 623-629. doi:10.1017/S0266467400010762
41. Vierheilig, H., Coughlan, A. P., Wyss, U. and Piche, Y (1998) Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* **64** (12): 5004–5007.
42. Walkley, A. and Black, I. A (1934) An examination of the Degtareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**: 29–38.
43. Wang, Johanna, W. H., Lutz, Adrian, Natera, Siria; Wang, Mei; Ng, Vivian; Grigoriev, Igor; Martin, Francis; Roessner, Ute; Anderson, Ian C.; Plett and 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.
44. Wang, J., Wang, G. G., 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.
45. Winagraski, E., Kaschuk, G., Monteiro, P. H. R., Auer, C. G., & Higa, A. R (2019) Diversity of arbuscular mycorrhizal fungi in forest ecosystems of Brazil: A review. *Cerne*, 25, 25-35.

46. Xu, T. L., Veresoglou, S. D., Chen, Y. L., Rillig, M. C., Xiang, D. and 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
47. Yadav, R. S., Mahatma, M. K., Thirumalaisamy, P. P., Meena, H. N., Bhaduri, D., Arora, S., & Panwar, J. (2017) Arbuscular mycorrhizal fungi (AMF) for sustainable soil and plant health in salt-affected soils. *Bioremediation of salt affected soils: an Indian perspective*, 133-156.
48. 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.
49. Zerihun Belay, Negash, M., Kaseva, J., Vestberg, M. and Kahiluoto, H (2020) Native forests but not agroforestry systems preserve arbuscular mycorrhizal fungal species richness in southern Ethiopia. *Mycorrhiza* 30 (6): 749–759.
50. Zerihun Belay, Vestberg, M. and 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.
51. Zerihun Belay, Vestberg, M. and 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.
52. Zhao, Z. W., Wang, G. H., & Yang, L (2003) Biodiversity of arbuscular mycorrhizal fungi in a tropical rainforest of Xishuangbanna, southwest China. *Fungal Diversity*, 13, 233-242.
53. Zhang, Y., Guo, L. D., & Liu, R. J (2003) Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Duijiangyan, southwest China. *Plant and Soil*, 261(1-2), 257-263.
54. Ziadin, N. and Tran T.S (2007) Lime requirement. Pp. 129-134. In: Carter and Martin, R. (eds.). *Soil Sampling and Methods of Analysis*. Canadian Soil Science Society. Lewis Publishers, Boca Raton, Florida.

Figures

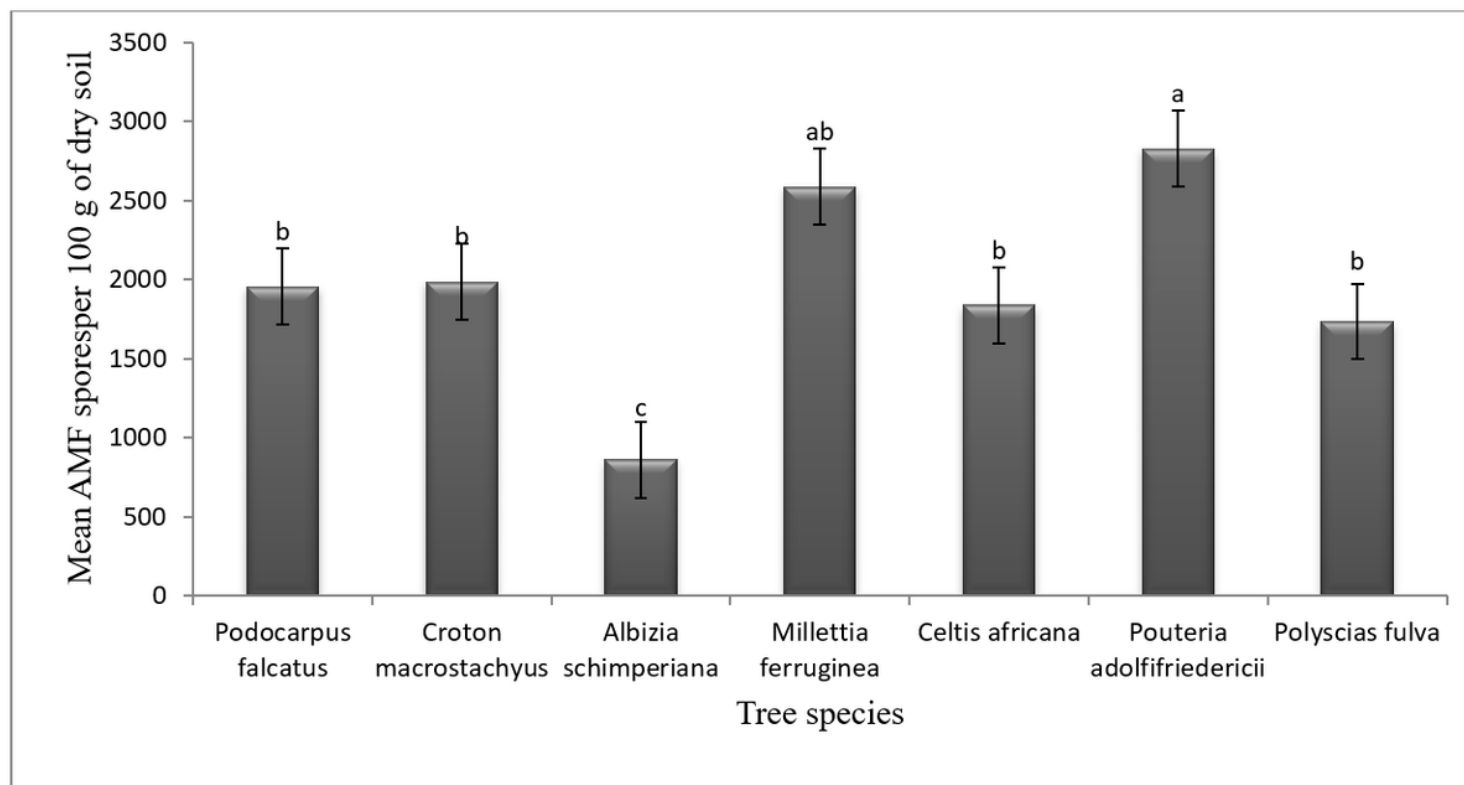


Figure 1

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

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

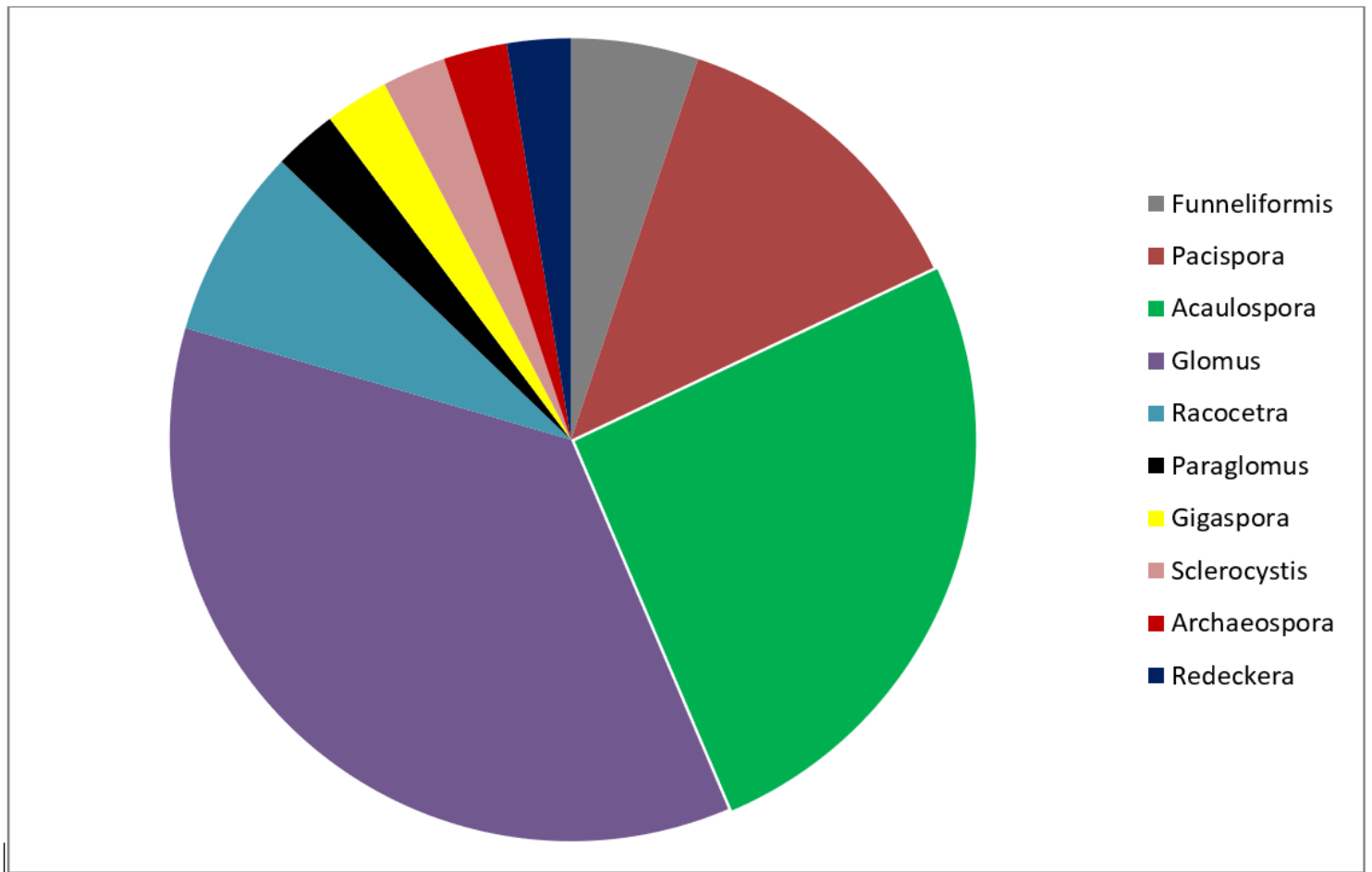


Figure 2

the number of identified morphotypes in a genus level