Science of the Total Environment 942 (2024) 173718

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Untangling the effect that replacing Ethiopia's natural forests with exotic tree plantations has on arbuscular mycorrhizal fungi

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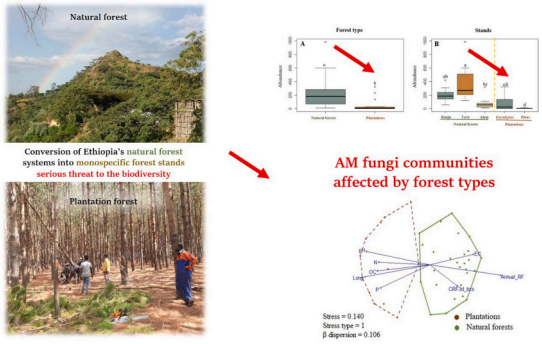
HIGHLIGHTS

- Changes in forest types resulted in the divergence of AM fungi communities.
- Soil pH, organic carbon, and annual rainfall, significantly influenced AMF diversity.
- *Septoglomus fuscum*, *Diversispora insculpta*, and *Funneliformis mosseae* emerge as dominant AMF species.
- Glomeraceae is found as a dominant contributor to AMF community.
- Conserving native forests is promising to enhance diverse AMF communities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Manuel Esteban Lucas-Borja

Keywords:
Arbuscular mycorrhizal fungi
Natural forest conservation
Ethiopian forest
Fungal composition
Soil fungi
Non-wood forest products

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have a broad distribution and establish symbiotic relationships with vascular plants in tropical regions. They play a crucial role in enhancing plant nutrient absorption, mitigating pathogenic infections, and boosting the resilience of host plants to abiotic stresses, including drought under specific conditions. Many natural forests in Ethiopia are being replaced by monospecific plantations. However, the impact of these actions on AMF is unknown and, despite their ecological functions, AMF communities in various forest systems have not been thoroughly investigated. In this study, we assessed soil AMF communities in natural and plantation forests by DNA metabarcoding of the ITS2 rDNA region and assessed the influence of climate and environmental variables on the AMF community. In total, 193 AMF operational taxonomic units (OTUs), comprising nine families and 15 genera, were recorded. Glomerales was the dominant order (67.9 % of AMF OTUs) and *Septoglomus fuscum*, *Diversispora insculpta*, and *Funneliformis mosseae* were the dominant species. AMF were more abundant in natural forests than in plantation forests and the composition of AMF communities differed significantly from those of plantation forest. In plantation forests, soil pH, organic carbon, total nitrogen, and available phosphorus significantly influenced the composition of AMF communities, whereas in natural forest, electrical conductivity, annual rainfall, and cumulative rainfall before sample collection were significantly correlated with AMF. SIMPER analysis identified the AMF responsible for composition variances among different forest types, with the Glomeraceae family being the most significant contributor, accounting for nearly 60 % of

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https://doi.org/10.1016/j.scitotenv.2024.173718
Received 20 March 2024; Received in revised form 24 May 2024; Accepted 31 May 2024
Available online 6 June 2024

the dissimilarity. Our findings further our understanding of the ecological niche function and the role of AMF in Ethiopia's natural forest systems and highlight the importance of prioritizing the sustainable development of degraded natural forests rather than plantations to ensure the preservation of habitats conducive to maintaining various AMF communities when devising conservation and management strategies.

1. Introduction

In tropical regions, biodiversity faces significant threats due to habitat loss and fragmentation (Haddad et al., 2015). These threats are generated mainly from human activities such as deforestation (Del Mar Alguacil et al., 2016; Torrecillas et al., 2013). Ethiopia, like many other developing countries, is experiencing forest land degradation due to various factors, such as a high population density, increased forest product needs, and continuous cropping practices (Lemenih and Teketay, 2005; Wassie, 2020). Consequently, physical and biological changes have occurred within natural forests, resulting in their fragmentation into small patches of natural forest. This fragmentation is causing the isolation of species populations and disrupting ecological processes (Jones et al., 2012). Moreover, to alleviate pressure on natural forests, the planting of exotic tree species has become a prevalent forestry practice (Bekele, 2011). This practice could directly contribute to the loss of biodiversity within the forest ecosystem (Bekele, 2011; Friis et al., 2010), including the diversity of microbial organisms in forest soils (Fierer and Jackson, 2006). As key components of the belowground ecosystem, alterations in the composition of these microorganisms might have effects on nutrient cycling, soil structure, and overall ecosystem health (Smith and Read, 2008). Hence, the degradation and subsequent transition of natural forests to plantations may aggravate challenges to biodiversity, especially within the belowground ecosystem. However, to date, belowground ecosystems have been given less attention than other systems with regard to research and conservation efforts relating to Ethiopia's forest systems.

Arbuscular mycorrhizal fungi (AMF) are important components of most soil microbial communities, forming mutualistic relationships with most vascular plant species (72 %), mainly in tropical forest systems, by supplying nutrients to the host plant in exchange for carbon (Smith and Read, 2008). This symbiotic relationship may enhance host plant productivity (Nakmee et al., 2016), resistance to drought (Hodge and Storer, 2014; Masebo et al., 2023), and tolerance to heavy metals (Hildebrandt et al., 2007), and can also influence soil development (Masebo et al., 2023; Oehl et al., 2010). Moreover, AMFs can protect their hosts against abiotic (Hodge and Storer, 2014; Smith and Read, 2008) and biotic factors (Felton et al., 2021; Vos et al., 2012). Soils with a high diversity of AMF are associated with increased aboveground plant biomass productivity (Wagg et al., 2011). Different species of AMF vary in their function across different ecosystems and how they benefit plants with diverse life-history strategies (Felton et al., 2021). These differences can potentially enhance overall productivity of the ecosystem, particularly in terms of aboveground biomass production.

Thus, AMF diversity influences the overall productivity of an ecosystem, particularly in terms of plant biomass production (Wagg et al., 2011). As such, understanding relationships between AMF diversity and tree communities, and their influence on ecosystem functioning is essential for effective ecosystem management and conservation efforts.

Different factors play a role in influencing the diversity and community composition of AMF. The type of host plant species exerts a significant and robust impact on AMF diversity and distribution (Vos et al., 2012). Edaphic factors, encompassing soil type and properties, are pivotal in determining AMF richness. Soil type exerts a profound influence on AMF composition because different soil types harbor distinct microenvironments that favor certain AMF species over others (Fitzsimons et al., 2008). Moreover, variations in soil properties, such as pH (Dumbrell et al., 2010), organic carbon (OC) content (Bai et al., 2009), nitrogen levels (Fitzsimons et al., 2008), and land-use intensity (Oehl et al., 2010) further contribute to nuances in the composition of AMF communities. In addition, land-use management practices that involve the conversion of forests to a different forest type, and climatic variables have emerged as crucial factors shaping AMF composition. Higher levels of AMF richness have been documented in less-disturbed land-use systems (Lacombe et al., 2009), with significantly greater quantities of AMF in diverse tree-based systems than in mono-cropping systems. Climatic variables such as rainfall, relative air humidity, and precipitation exert influences on AMF community composition, sporulation (Sun et al., 2013), spore density, and richness (Melo et al., 2017). Understanding these intricate relationships is crucial for effectively conserving and managing AMF populations in diverse ecosystems.

Ethiopia has diverse forest ecosystems that harbor vast biodiversity; however, research studies exploring fungal communities within these forest systems have been limited (Alem et al., 2020a; Castaño et al., 2019; Dejene et al., 2017a; Kewessa et al., 2022), particularly with regard to AMF. Wubet et al. (2004, 2009) reported mycorrhizas associated with indigenous tree species such as *Olea europaea* subsp. cuspidate and *Prunus africana*, as well as from *Podocarpus falcatus* seedlings. Furthermore, there is a notable gap in understanding the specific impacts of habitats and associated factors on AMF communities across different habitats. Examining the composition of AMF communities in various forest ecosystems is crucial for gaining a comprehensive understanding of ecological processes within these environments (Hazard et al., 2013). In addition, this type of research would help to unravel the complex interactions and functions of AMF in forest ecosystems. Therefore, by studying AMF in both natural and plantation forests, we can gain insights into ecosystem dynamics and inform sustainable forest management as comparing these forest types will reveal the ecological roles of AMF and their responses to different land management strategies. In this study, we hypothesize that Ethiopia's natural forests support a greater diversity and abundance of AMF than plantation forests due to their complex structures and diverse plant communities. In contrast, plantation forests, with their monoculture stands and simplified ecosystems, may have reduced AMF diversity and abundance due to limited host plant diversity and altered soil conditions. Thus, the specific objectives of this study were: (i) to evaluate overall differences in the diversity and composition of AMF between plantation and natural forest systems; (ii) to identify environmental variables responsible for governing the composition of AMF communities in both forest systems; and (iii) to identify the AMF most responsible for compositional differences between the two forest systems.

2. Materials and methods

2.1. Study area

The study involved three natural forests located in the Amhara region and two plantation forests located in the Sidama region. The three natural forests were the Banja Forest in Banja district, the Alemsaga forest in Farta district, and the Taragedam forest in Libokemkem district (Fig. 1). The two plantation forests comprised a *Eucalyptus grandis* forest and a *Pinus patula* forest in the Wondo Genet district (Fig. 1). The three natural forests are remnants of the Dry Afromontane forests that once grew in Northern Ethiopia (Gebeyehu et al., 2019; Masresha et al., 2015; Zegeye et al., 2011). The two plantations were established by replacing native vegetation that had been depleted due to logging and clearance for cultivation (Teshome, 2011). Detailed descriptions of both forest

types are provided in Table 1. The climate of both regions is characterized by a bimodal rainfall pattern: the main rainy season is in the summer (June to August), while the shorter rainy season is in spring (March to May).

2.2. Sampling

Sample plots were established in 2019. Within each of the five forest stands, three transects were established about 500 m apart from each other. Rectangular sampling plots (2 m × 50 m) were established along these transects at least 250 m apart and laid out using systematic random sampling to avoid confounding spatial effects inherent to such a plot-based design (Hiiesalu et al., 2017; Rudolph et al., 2018) and to reduce environmental heterogeneity (O'Hanlon and Harrington, 2012). In total, nine plots were established in each of the five forest stands (i.e., 45 sampling plots in total) (Table S3). Despite the native forest stands, and plantation sites are geographically distinct and may experience different environmental conditions, in selecting the sites, we ensured that both the plantation and natural forests were located in the highland regions of the country, at elevations >1500 m above sea level (Table 1). These areas share relatively similar temperature and rainfall patterns, which helps us to minimize the environmental variability and we assume that this condition could allow us for a more accurate comparison of the AM fungi communities between the plantation and natural forests in our study sites. However, the replication of our results to other areas should be taken in caution.

2.3. Environmental data collection and analysis

Environmental data, including soil variables, climate (temperature and rainfall), and spatial data (elevation, latitude, and longitude), were collected to assess their influence on the composition of AMF communities (Table 1). Soil samples were collected from each of the sample plots using a soil corer (2 cm radius, 20 cm depth, and 250 cm^3). In total, five soil core samples were extracted from each plot: one core was extracted from the center of the plot and one from each of the four corners of the plot. The five soil core samples collected from each plot were pooled to form a composite, relatively homogeneous sample, approximately 500 g of which was then placed in a plastic bag. The litter layer (comprising intact and partially decomposed leaves) was discarded because the composition of fungal communities in litter tends to diverge from that in soil (Voříšková et al., 2014). The soil samples were first dried and then sieved through a 1 mm mesh before being ground with a mortar and pestle to form a fine powder, which was used for physico-chemical analyses and DNA extraction. The soil sample analyses were performed by Water Works Design and Supervision Enterprise, Addis Ababa, Ethiopia. Soil OC was assessed using wet digestion (Walkley and Black, 1934). Total nitrogen (N) was measured using the Kjeldahl method (Kim et al., 2005). Available phosphorus (P) was calculated following the standard procedure described by Tan (2005). A pH meter was used to analyze a soil:water (1,2.5) suspension to determine the pH of the soil samples (van Reeuwijk, 2002).

Climate variables (weather data) were obtained from nearby meteorological stations for each of the five forest locations. Detailed descriptions of the main environmental variables assessed in this study are shown in Table 1.

2.4. Molecular analysis

For molecular analysis, the samples were transported to the

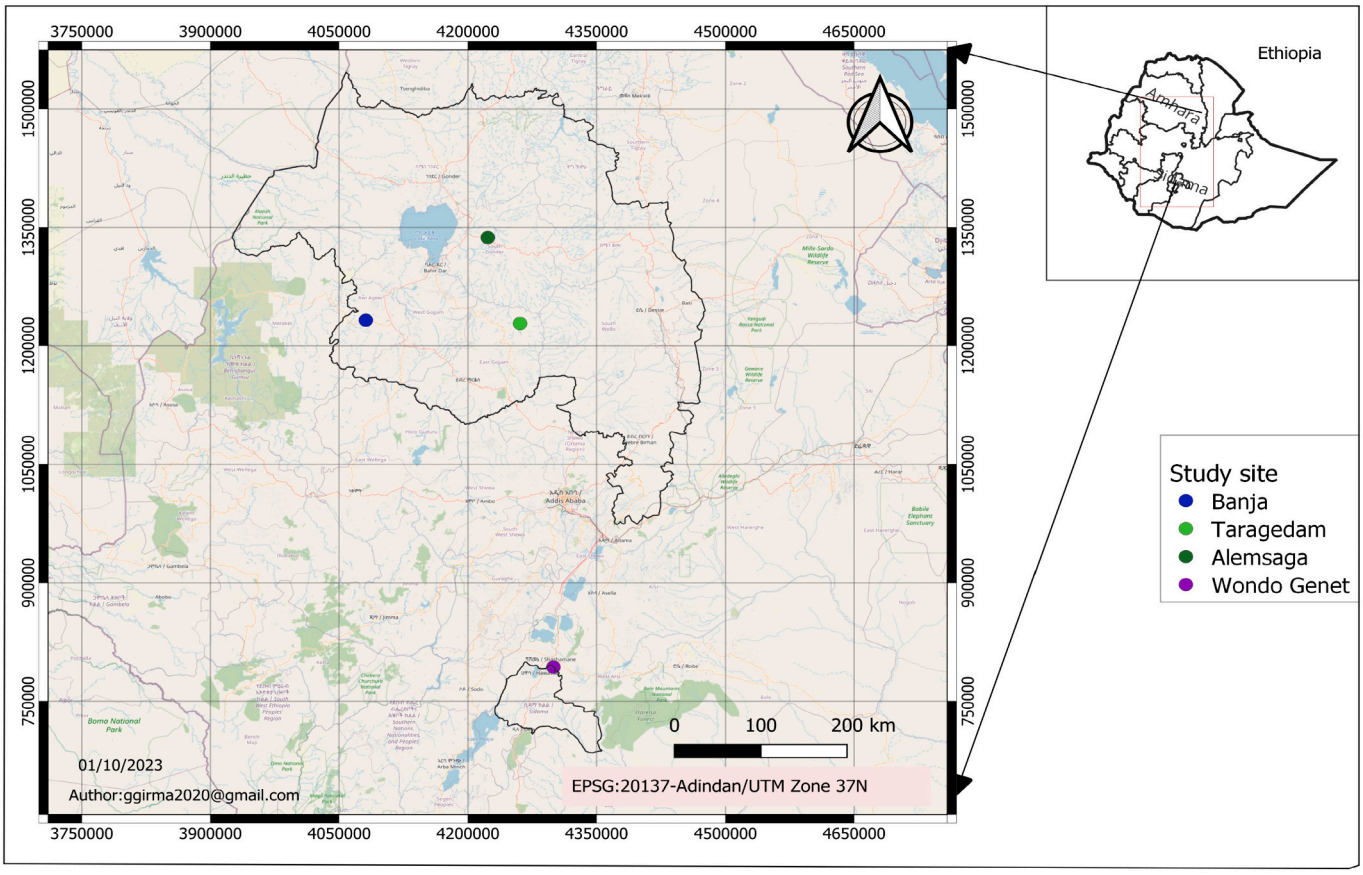

Fig. 1. Map of the Amhara and Sidama regions in Northern and Southern Ethiopia showing the location of the three natural forests and the plantations, respectively, in which the study plots were located.

Table 1
Description of the study sites and selected edaphic properties.

Variables	Description	Plantation forests		Natural forests		
		Eucalyptus grandis	*Pinus patula*	Taragedam	Banja	Alemsaga
Location	Location	7°06′–7°07′ N		12°06′–12°07′ N	10°57′–11° 03′ N	11°54′–11°56′ N
	Altitude range (m asl)	1600–2580		2142–2484	1870–2570	2180–2470
	Annual prec. (mm)	1210		1098	1884	1929
	Annual temp. (°C)	20		19.5	18.7	15.8
Edaphic	pH_H_2O	6.01 ± 0.28	5.65 ± 0.39	5.14 ± 0.18	4.86 ± 0.19	4.79 ± 0.27
	Ca (ppm)	18.17 ± 5.73	17.43 ± 5.93	13.95 ± 1.79	13.55 ± 2.62	9.19 ± 1.57
	Mg (ppm)	6.11 ± 2.11	6.47 ± 1.71	6.16 ± 0.30	5.54 ± 0.60	4.58 ± 0.45
	Na (ppm)	1.19 ± 0.38	0.91 ± 0.29	1.95 ± 0.15	1.82 ± 0.35	2.05 ± 0.30
	K (ppm)	0.63 ± 0.13	0.84 ± 0.41	0.72 ± 0.17	0.78 ± 0.19	0.61 ± 0.12
	EC	0.18 ± 0.20	0.13 ± 0.09	0.43 ± 0.15	0.81 ± 0.43	0.28 ± 0.10
	CEC (%)	39.59 ± 11.68	39.88 ± 9.95	47.21 ± 4.08	44.51 ± 5.87	34.89 ± 2.77
	OC (%)	3.69 ± 1.35	6.04 ± 1.68	2.59 ± 0.35	2.83 ± 0.06	1.94 ± 0.77
	OM (%)	2.32 ± 0.85	3.80 ± 1.06	4.46 ± 0.60	4.87 ± 0.10	3.35 ± 1.34
	N (%)	0.33 ± 0.10	0.63 ± 0.23	0.23 ± 0.04	0.26 ± 0.02	0.17 ± 0.05
	P (ppm)	41.64 ± 21.68	37.08 ± 6.18	17.18 ± 17.16	17.64 ± 18.16	7.80 ± 2.20
Climate	CRF3d	24.33 ± 0.00	24.33 ± 0.00	59.50 ± 0.00	18.60 ± 0.00	77.40 ± 0.00
	CRF10d	77.62 ± 0.00	77.62 ± 0.00	149.70 ± 0.00	99.60 ± 0.00	184.80 ± 0.00
	CRF20d	146.51 ± 0.00	146.51 ± 0.00	211.30 ± 0.00	221.30 ± 0.00	307.30 ± 0.00
	CRF30d	181.05 ± 0.00	181.05 ± 0.00	334.30 ± 0.00	344.60 ± 0.00	452.30 ± 0.00
	CRF_ucs	596.70 ± 0.00	596.70 ± 0.00	1098.40 ± 0.00	1408.70 ± 0.00	1368.30 ± 0.00
	Annual_RF	972.62 ± 0.00	972.62 ± 0.00	1488.50 ± 0.00	1884.30 ± 0.00	1926.10 ± 0.00
	AvDmaxT_CM	21.97 ± 0.00	21.97 ± 0.00	26.10 ± 0.00	21.60 ± 0.00	19.42 ± 0.00
	AvDmiT_CM	12.78 ± 0.00	12.78 ± 0.00	12.50 ± 0.00	8.30 ± 0.00	10.80 ± 0.00
	AvDmaxT_yr	24.66 ± 0.00	24.66 ± 0.00	29.80 ± 0.00	24.30 ± 0.00	22.80 ± 0.00
	AvDminT_yr	11.85 ± 0.00	11.85 ± 0.00	10.70 ± 0.00	7.90 ± 0.00	10.00 ± 0.00
	CRF3d_J	586.75 ± 0.00	586.75 ± 0.00	801.90 ± 0.00	1124.20 ± 0.00	936.60 ± 0.00
	CRF10D_J	563.56 ± 0.00	563.56 ± 0.00	679.70 ± 0.00	1048.20 ± 0.00	804.50 ± 0.00
	CRF20d_J	533.44 ± 0.00	533.44 ± 0.00	617.10 ± 0.00	938.70 ± 0.00	675.20 ± 0.00
	CRF30d_J	508.41 ± 0.00	508.41 ± 0.00	504.10 ± 0.00	802.40 ± 0.00	537.20 ± 0.00
Vegetation	Dominant trees	*Eucalyptus* spp., *Cupressus lusitanica*, *Grevillea robusta*, and *P. patula*		*Olea* sp., *Juniperus procera*	*Albizia gummifera*, *Brucea* sp.	*Acacia abyssinica*, *Acacia nilotica*
References		Alem et al., 2020b Dejene et al., 2017a Teshome, 2011		Gedefaw and Soromessa, 2014 Zegeye et al., 2011 Zerihun et al., 2013	Abere et al., 2017	Birhane et al., 2017 Masresha et al., 2015 Wubet et al., 2004

Note: Values shown are means ± the standard error of the mean. Mean annual precipitation (prec.) and mean annual temperature (temp.) values are based on nearby meteorological station data recorded in each study area during the collection of field data. Abbreviations: Ca, calcium; Mg, magnesium; Na, sodium; K, potassium; N, nitrogen; P, phosphorus; EC, electrical conductivity; CEC, cation exchange capacity; OC, organic carbon; OM, organic matter; CRF-3d, -10d, -20d, -30d, cumulative rainfall 3, 10, 20, or 30 days before soil sampling; CRF_ucs, cumulative rainfall before sampling; Annual_RF, annual rainfall (mm) during collection year; AvDmaxT_CM, average daily maximum temperature (°C) during collection month; AvDmiT_CM, average daily minimum temperature (°C) during collection month; AvDmaxT_yr, average daily maximum temperature (°C) during collection year; AvDminT_yr, average daily minimum temperature (°C) during collection year; CRF-3d_J, -10d_J, -20d_J, -30d_J, cumulative rainfall (mm) from January up to 3, 10, 20, or 30 days before collection; m asl, m above sea level. References relate to the climatic, vegetation, and geographical descriptions of the study areas.

laboratory in sterile plastic bags and stored at 4 °C. The samples were frozen immediately upon return to the laboratory and kept at −20 °C until DNA was extracted. Each sample was subjected to genomic DNA analysis, which was performed using 100 g of soil. We maintained care in fieldwork and lab work to avoid cross-contamination among samples as much as possible, e.g., sampling tools were cleaned with alcohol (96 %) after sampling each plot.

A PowerSoilTM DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) was used to extract DNA from 0.25 g of soil per sample. PCRs of each sample were carried out in triplicate to minimize PCR bias. The three samples were subsequently pooled before sequencing. PCRs were performed in 20 μL reaction volumes containing 11.22 μL of modified quantification (MQ) water, 1.60 μL of DNA template, 2.00 μL of 10 × buffer, 1.40 μL of MgCl2 (50 mM), 1.60 μL of dNTPs (10 mM), 0.50 μL of bovine serum albumin (BSA) (2 %), 0.80 μL of reverse and forward primers (10 μM), and 0.08 μL of Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA). The following PCR conditions were used: initial denaturation at 94 °C for 3 min; 35 cycles at 94 °C for 45 s, 50 °C for 1 min, and 72 °C for 1.5 min; and one cycle at 72 °C for 10 min. To amplify the ITS2 rDNA region (ca. 260 bp), we used the forward primer fITS794 and reverse primer ITS495, which were amplified with Illumina adaptors. To link the sequences to the sample source, a second PCR was performed to append sample-specific tags to the Illumina adaptors. The second PCR was conducted using Phusion HF PCR master mix and llumina indexes with the cycling conditions of 98 °C for 30 s, followed by 6 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s, and a subsequent extension at 72 °C for 5 min and holding at 4 °C. Each set of PCR replicates also included a negative control comprising MQ water instead of DNA that underwent PCR under the same experimental conditions and was shown to be amplicon-free on a gel. The amplicon library was sequenced at BaseClear B.V. (Leiden, Te Netherlands) using a paired-end (2 × 250 bp) Illumina MiSeq platform.

2.5. Bioinformatic analysis

Primers and poor-quality ends in both directions (3′ and 5′) were removed based on a 0.02 error probability limit in Cutadapt v.2.8 with Phyton 3.6.796. Because length differences in OTU clustering algorithms are often counted as terminal gaps that may result in clustering otherwise identical sequences into different OTUs, all sequences were truncated to 200 bp and then filtered with USEARCH v.8.097 to discard sequences with an expected error of >1. The remaining sequences were collapsed into unique sequence types on a per-sample basis using USEARCH v.8.097 while preserving read counts. First, we discarded singleton sequence types before grouping the remaining high-quality sequences into 193 OTUs with USEARCH at a 97 % sequence similarity level while simultaneously excluding sequences representing OTUs with <70 % similarity or < 150 bp pairwise alignment length to a fungal sequence. Sequences were assigned to taxonomic groups based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence database, version v.8.0, released on November 18th, 2018, which contains identified fungal sequences with assignments to species hypothesis (SH) groups (Kõljalg et al., 2013). We recognize the limitations of functional inference based on partial ITS sequences and here use these guilds as hypothetical trophic groups. OTUs with >90 % similarity to a fungal SH with known ecological function were assigned to plant pathogens, animal parasites, ectomycorrhizal (ECM) fungi, arbuscular mycorrhizal fungi, functional saprotrophs, or other groups. Ecological functions at the genus level were assigned using the published classification (Põlme et al., 2020), and finally, arbuscular mycorrhizal fungi were selected for further analysis in this study. A list of AMF OTUs is provided as supplementary data in Table S1.

2.6. Data analysis

AMF diversity indices in different forest types and forest stands were determined using PAST software (ver. 4.03) to calculate species richness (Chao1), Shannon–Wiener index (H′), Simpson's dominance index (D), Evenness (E), and alpha-species richness (Hammer et al., 2001). The Rényi diversity profile (Tothmeresz, 1995), was used to depict the diversity curves of the five stands (Fig. S1). It depends upon a parameter alpha, such that for alpha = 0, this function gives the total species number and alpha = 1 gives an index proportional to the Shannon index. The AMF diversity indices were then subjected to a one-way analysis of variance (ANOVA). Tukey's Honest Significant Difference test (HSD, $p < 0.05$) was used to check significant differences between forest stands when there was a significant difference among the groups. Relationships between AMF communities and soil properties (OC, P, N, and pH) were determined using Pearson's correlation analysis.

A permutation-based nonparametric ANOVA (PERMANOVA) (Anderson, 2001) using Euclidean distance was performed to analyze differences to test whether AMF communities detected in the two forest types and in the five studied forest stands were statistically different. Communities were visualized using non-metric multidimensional scaling (NMDS) based on an abundance species data matrix and soil and climate data (Clarke, 1993). Direct ordination was used to relate variability in the composition of AMF communities to forest types and forest stands, as well as to other environmental factors, such as soil OC, N, P, electrical conductivity (EC), and pH, annual rainfall, cumulative rainfall three days before collection (CRF3d), and longitude. We plotted the composition of AMF communities on NMDS ordinations for forest types and forest stands using the Euclidean distance. Correlations between the two NMDS axes with significant environmental variables were assessed using the *envfit* function in R. One-way ANOSIM (analysis of similarity) was performed on Bray–Curtis resemblance matrices (incorporating 999 permutations) to determine the significance of differences between forest types, forest stands, and environmental variables, as described in previous studies (Anderson, 2001). An analysis of similarity percentages (SIMPER) was also performed to identify which AMF species were most responsible for pattern similarities and to determine the percentage contribution of AMF taxa to significant dissimilarities between the two forest types and among the five forest stands (Parravicini et al., 2010). Statistical analyses were performed using R software (version 4.2.0) (R Core Team, 2020) and PAST (Version 4.03) (Hammer et al., 2001).

3. Results

3.1. Taxonomic identification of AMF

In this study, 193 operational taxonomic units (OTUs) of AMF belonging to the phylum Glomeromycota were identified (Table S1). These OTUs comprised three classes, six orders, nine families, and 15 genera. Most of the OTUs belonged to the class Glomeromycetes (n = 158), which was also the most abundant class (81.9 %), followed by Paraglomeromycetes (n = 12, 6.2 %), and Archaeosporomycetes (n = 5, 2.6 %). We were unable to identify 18 OTUs. Most of the OTUs belonged to the order Glomerales (n = 131, 68 %), followed by Diversisporales (n = 21, 11 %) and Paraglomerales (n = 10, 5 %) (Fig. 2). At the family level, most of the OTUs belonged to the Glomeraceae (n = 119, 61.7 %), followed by Diversisporaceae (n = 12, 6.2 %), and Claroideoglomeraceae (n = 10, 5.2 %) (Table S1). The most frequently detected AMF species in the sampled plots were *Septoglomus fuscum*, *Diversispora insculpta*, and *Funneliformis mosseae* (Table 2).

3.2. AMF species abundance and richness

The abundance of AMF was significantly higher in natural forests than in plantations (F = 39.93; p = 0.000; Fig. 3A). Furthermore, the abundance of AMF differed significantly between stands (F = 15.73; p =

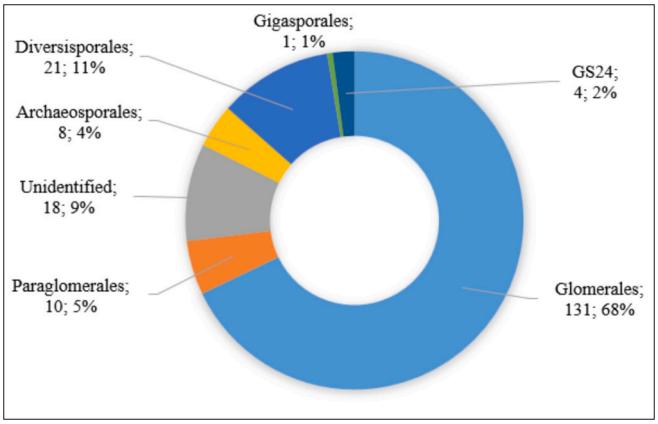

Fig. 2. Operational taxonomic units of arbuscular mycorrhizal fungi identified in five different forest systems in Ethiopia grouped by order.

0.000; Fig. 3B), with the highest level of AMF abundance detected in Taragedam natural forest soil and the lowest level detected in *Pinus* stand soil (Fig. 3B).

AMF species richness was significantly higher in Taragedam forest (106 AMF species, $p < 0.05$; Table 3) and significantly lower in the *Pinus* plantation (13 species) and Alemsaga forest (23 species) than in other forest stands. The total AMF species richness for each forest type and forest stand is shown in Table 3.

3.3. Diversity of AMF in different forest types and forest stands

AMF taxa richness (Chao 1) was estimated to be significantly higher in natural forests than in plantations ($F = 8.808$; $p = 0.005$). The Shannon–Wiener diversity index indicated that AMF taxa richness differed significantly between forest types ($F = 9.389$; $p = 0.004$, Table 3). A comparison of AMF species evenness in natural and plantation forests revealed no significant differences ($F = 1.393$; $p = 0.245$). However, AMF species dominance ($F = 5.367$; $p = 0.026$) and Simpson ($F = 5.287$; $p = 0.027$) indices varied significantly between the two forest types.

3.4. AMF community composition

Differences were observed in AMF community composition. NMDS analyses indicated that the total AMF community composition differed significantly according to forest type ($F = 7.969$, $R^2 = 0.156$, $p = 0.001$) (Fig. 4A) and forest stand ($F = 2.999$, $R^2 = 0.231$, $p = 0.001$) (Fig. 4B).

To elucidate the drivers of the gradient in arbuscular mycorrhizal fungal dissimilarities between forest type and forest stands, we fitted 31 environmental variables (Fig. S2). Of these, soil pH, EC, soil OC, N, P, annual rainfall, CRF3d, and aspects such as longitude were significantly correlated with the AMF communities detected in the five different stands (Table 4).

ANOSIM analyses confirmed that AMF community composition differed significantly between forest types ($F = 8.439$, $R = 0.690$, $p = 0.000$) and between forest stands ($F = 3.250$, $R = 0.327$, $p = 0.000$). The SIMPER analysis identified the cumulative contribution of the most influential OTUs to the dissimilarity between forest types and among forest stands is also shown in Table S2. At the family level, most OTUs contributing to the dissimilarity belonged to the Glomeraceae (almost 60 % of the dissimilarity contribution) (Table 5). Other secondary contributors were OTUs belonging to the Ambisporaceae and Claroideoglomeraceae. Only two OTUs identified at the species level, *Funneliformis mosseae* and *Ambispora fennica*, contributed significantly to the dissimilarity, with an individual contribution to the dissimilarity of 2.6 and 1.7, respectively.

4. Discussion

Despite the abundant biodiversity and ecological significance of Ethiopian forests, only a limited number of studies have characterized the diversity and structure of AMF communities within these ecosystems. In this study, we detected a high level of AMF diversity (193 OTU) in the forests we examined. This high level of diversity could be attributed to factors such as plant diversity (Janos, 1996; Laurindo et al.,

Table 2
Selection of the Arbuscular Mycorrhizal OTUs identified at species level according to forest type and forest stand in which they were detected.

Species	Family	Forest type	Forest stand
Septoglomus constrictum	Glomeraceae	Plantation	*Eucalyptus*
Septoglomus constrictum	Glomeraceae	Plantation	*Eucalyptus*
Funneliformis mosseae	Glomeraceae	Plantation	*Eucalyptus* and *Pinus*
Glomus indicum	Glomeraceae	Plantation	*Eucalyptus*
Funneliformis mosseae	Glomeraceae	Plantation	*Eucalyptus* and *Pinus*
Septoglomus fuscum	Glomeraceae	Natural forest	Tara and Banja
Dominikia disticha	Glomeraceae	Natural forest	Banja
Dominikia disticha	Glomeraceae	Natural forest	Tara
Dominikia bernensis	Glomeraceae	Natural forest	Tara
Septoglomus fuscum	Glomeraceae	Natural forest	Alem
Rhizophagus intraradices	Glomeraceae	Natural forest	Tara
Septoglomus fuscum	Glomeraceae	Natural forest	Tara
Septoglomus jasnowskae	Glomeraceae	Natural forest	Tara
Septoglomus fuscum	Glomeraceae	Natural forest	Tara
Diversispora insculpta	Diversisporaceae	Plantation	*Eucalyptus*
Diversispora insculpta	Diversisporaceae	Natural forest	Tara and Banja
Diversispora eburnea	Diversisporaceae	Natural forest	Banja
Redeckera megalocarpum	Diversisporaceae	Natural forest	Tara and Banja
Corymbiglomus tortuosum	Diversisporaceae	Natural forest	Tara
Claroideoglomus etunicatum	Claroideoglomeraceae	Plantation	*Eucalyptus*
Paraglomus brasilianum	Paraglomeraceae	Plantation	*Pinus*
Paraglomus occultum	Paraglomeraceae	Natural forest	Tara
Ambispora gerdemannii	Ambisporaceae	Plantation	*Eucalyptus*
Ambispora fennica	Ambisporaceae	Plantation	*Eucalyptus*
Ambispora fennica	Ambisporaceae	Natural forest	Tara
Archaeospora trappei	Archaeosporaceae	Plantation	*Pinus*
Pacispora scintillans	Pacisporaceae	Natural forest	Tara

Note: Tara, Taragedam; Alem, Alemsaga.

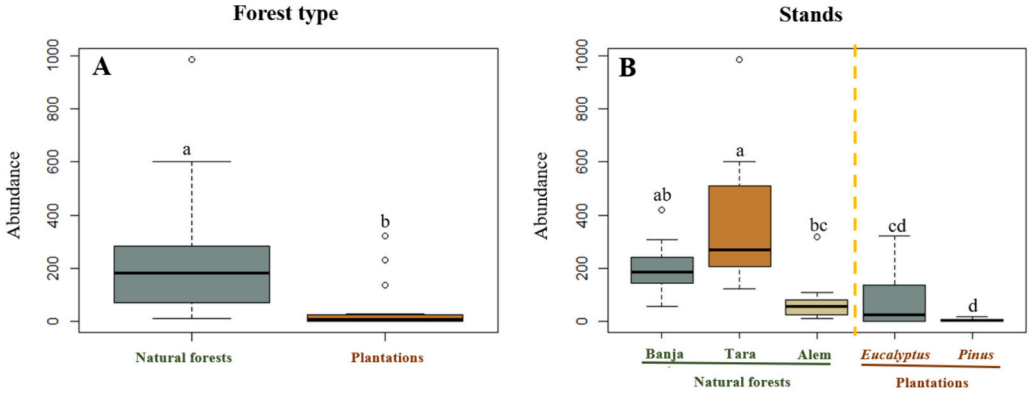

Fig. 3. Abundance of arbuscular mycorrhizal fungal operational taxonomic units in two forest types (A), and at five forest stand locations (B) in Ethiopia. Box-and-whisker plots indicate the range of the data; the horizontal lines, the median; and the dots, outliers. Generalized linear mixed effect models were fitted, and post hoc (Tukey's honest significant difference) tests revealed significant differences at $p \leq 0.05$. Significant differences are indicated by different lowercase letters ($p \leq 0.05$). Tara, Taragedam; Alem, Alemsaga.

Table 3
Comparison of arbuscular mycorrhizal fungal species diversity indices calculated for two forest types and five forest stands in Ethiopia.

Diversity indices	Forest type		Forest stand				
	Plantation	NF	*Eucalyptus* plantation	*Pinus* plantation	Taragedam NF	Alemsaga NF	Banja NF
Chao 1	42b	151a	34b	13b	106b	23b	90a
Shannon (H′)	2.595b	3.565a	2.481bc	2.294bc	3.489a	2.281c	3.428a
Evenness (J)	0.319a	0.234a	0.352bc	0.763a	0.309c	0.426ab	0.342bc
Dominance	0.129a	0.079b	0.137a	0.129ab	0.078b	0.194a	0.068b
Simpson (1-D)	0.871b	0.920a	0.863b	0.870ab	0.922a	0.806b	0.932a
Fisher-alpha	9.424b	28.180a	7.337b	5.374b	20.78a	4.533b	19.78a

Different lowercase letters within the same row for forest type or forest stand indicate a significant difference in the species diversity index between forest types or between forest stands at $p < 0.05$ using Tukey's honest significant difference test. NF, natural forest.

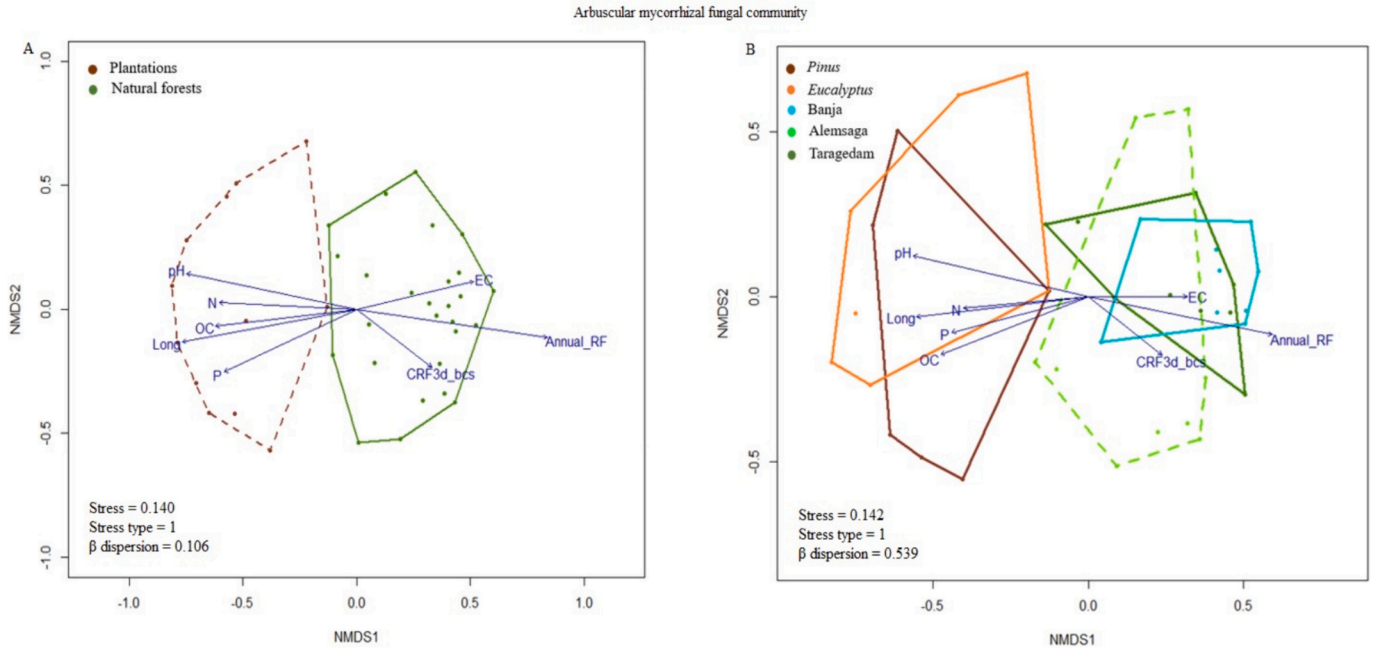

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination graph with fitted explanatory variables based on dissimilarities calculated using the Euclidean distance of fungal operational taxonomic unit compositions based on forest type (A) and forest stand (B) in Ethiopia. Arrows represent environmental variables that were most significantly ($p < 0.05$) related to ordination. Explanatory variables are shown in blue: N, nitrogen; OC, organic carbon; EC, electrical conductivity; P, available phosphorus; Annual RF, annual rainfall; CRF3d, cumulative rainfall three days before collection; Long, longitude.

Table 4
Influence of forest type and forest stand soil physicochemical properties on the total arbuscular mycorrhizal fungal community composition at $p < 0.05$ using the 'benefit' function in R.

Environmental variables	Forest type		Forest stand	
	R^2	p	R^2	p
pH	0.5076	0.001	0.5479	0.001
EC	0.2433	0.001	0.1662	0.021
Organic carbon	0.3396	0.001	0.4196	0.002
Nitrogen	0.3189	0.001	0.2665	0.003
Phosphorus	0.3529	0.001	0.3359	0.003
Annual rainfall	0.6272	0.001	0.5968	0.001
CRF3d	0.1426	0.048	0.1439	0.035
Longitude	0.5299	0.001	0.5069	0.001

CRF3d, cumulative rainfall three days before collection; EC, electrical conductivity.

Table 5
Arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs) responsible for differentiating AMF communities in natural forests from those in plantations (SIMPER analysis).

OTUs	Individual contribution to the dissimilarity (%)	Cumulative contribution to the dissimilarity (%)
Glomeraceae	18.63	18.63
Glomeraceae	6.68	25.31
Glomeraceae	5.70	31.01
Glomeraceae	5.20	36.21
Glomeraceae	4.55	40.76
Glomeraceae	3.32	44.07
Glomus	3.28	47.35
Glomeraceae	2.73	50.08
Funneliformis mosseae	2.61	52.68
GS24	2.28	54.96
Glomeraceae	2.13	57.09
Glomeraceae	1.70	58.79
Ambispora fennica	1.68	60.47
Archaeosporales	1.49	61.95
Claroideoglomus	1.23	63.17
Glomeraceae	1.08	64.25
Glomus	1.01	65.25
Rhizophagus	0.98	66.22
Glomeraceae	0.95	67.17
Glomus	0.92	68.09
Glomeraceae	0.90	68.99
Claroideoglomus	0.88	69.86
Glomeromycota	0.83	70.69
Glomeraceae	0.82	71.51
Glomus	0.78	72.29
Glomeraceae	0.66	72.95
Glomeromycota	0.62	73.57
Glomeraceae	0.57	74.14
Glomeraceae	0.56	74.70

2021) and favorable environmental conditions (Hanson et al., 2012; Hazard et al., 2013; Melo et al., 2017), which promote diversity across the country (Wardle et al., 2004). Thus, the observed AMF richness in this study suggests the necessity of obtaining comparable descriptive data on soil fungal communities through more comprehensive sampling designs across diverse ecosystems (Tchabi et al., 2008) in Ethiopia.

As expected, AMF communities detected in plantation and natural forests were distinctly different from each other in terms of diversity and structure. The presence of distinctly different communities in plantation and natural forests can be attributed to several interrelated factors specific to each forest type. For example, natural forests in Ethiopia are characterized by their relatively undisturbed ecological processes, which provide a diverse range of habitats and niches that support a rich variety of plant species (Chen et al., 2022; Gong et al., 2012; He et al., 2017; Melo et al., 2017; Vieira et al., 2019). The wide diversity of plant species provides a wide range of resources and ecological niches for AMF colonization and proliferation, leading to a complex and diverse AMF community structure (Alemu, 2013; Dejene et al., 2017b). By contrast, plantation forests, which are typically managed for commercial purposes and are often composed of monocultures or limited species compositions, have a simpler ecological environment than natural forest, with reduced plant diversity and altered soil conditions (He et al., 2017; Melo et al., 2017). Such ecosystems may favor specific AMF species that are better adapted to conditions prevailing in plantation forests, resulting in distinct AMF communities. In addition, management practices such as soil disturbance, fertilization, and pesticide use in plantation forests can further influence AMF communities by altering soil properties and disrupting natural ecological processes (Gong et al., 2012; Kazenel et al., 2019; Kivlin et al., 2011; Tian et al., 2018; Torrecillas et al., 2013). These considerations, highlight the importance of considering ecological context when studying fungal biodiversity and ecosystem dynamics (Chen et al., 2022; Gong et al., 2012; He et al., 2017; Melo et al., 2017; Vieira et al., 2019).

The Shannon–Wiener diversity index of AMF species varied across different forest types and stands. Specifically, higher AMF diversity was detected in natural forests than in plantation forests. The abundance, richness, and number of AMF species were also significantly higher in natural forest environments than in plantations. However, a significant number of AMF species were identified in *Eucalyptus* stands, indicating a potential influence of forest management practices on the taxonomic composition of AMF communities within plantation forests. Plant diversity plays a crucial role in shaping the resources available to fungi for survival and growth, as highlighted in previous studies (Tedersoo et al., 2016; Wardle et al., 2004). Analyses of AMF richness in the five different stands revealed that AMF richness and diversity values were greatest in the Taragedam and Banja forests. This finding might be associated with the greater availability of a broader host range (Alem et al., 2020a; Roy et al., 2008) in these forests than in Alemsaga forest or in the plantations. This suggests that stands with several plant species have a positive effect on AMF diversity (Oehl et al., 2010). A previous study (Ayana, 2021) reported that the richness of ectomycorrhizal fungal species was highest in Taragedam forests. Among the three natural forests, the lowest abundance, diversity indices, and species richness levels were detected in soil samples from Alemsaga forest, which could be attributed to the land-use history of the forest. Alemsaga forest was converted to agricultural land in 1990, which could have had a negative impact on fungal resources in this area (Ayana, 2021; Tervonen et al., 2019). A decline in vegetation and the constant removal of dead wood from forests results in the loss of associated fungi (Berg et al., 2002; Jiang et al., 2018). The lower levels of AMF diversity and species richness in the Alemsaga forest could also be due to the lower soil fertility level of Alemsaga forest compared with that of Taragedam or Banja forests (Garo et al., 2022; Oehl et al., 2010; Soudzilovskaia et al., 2015), and perhaps due to an absence of other forests in the vicinity that could serve as a source of fungal propagules (Redondo et al., 2020). In addition, the host could influence AMF diversity (Melo et al., 2017; Tedersoo et al., 2016) through its impact on the quantity and quality of carbon resources (Genevieve et al., 2019).

Many other environmental factors can also influence AMF communities, such as soil nutrients, temperature, light availability, rainfall, and possible interactions with these factors (Chaudhary et al., 2014; Gong et al., 2012; Vieira et al., 2019). In our study, AMF community composition was significantly affected by soil pH, EC, N, OC, P, annual rainfall, CRF3d, and longitude. Soil pH, EC, and N had a positive influence on AMF communities, whereas OC and P had a negative influence on AMF communities. Similar observations have also been reported by previous studies (Lakshmipathy et al., 2012). The available P in soil plays an important role in the composition of the AMF community by influencing mycorrhizal colonization and spore production. Several studies (Gong et al., 2012; Tervonen et al., 2019) have indicated that high available P content could reduce mycorrhizal colonization and spore production. The high level of available P in the plantation soil

samples seems to have had a significant negative influence on AMF communities. Previous studies have also reported that high P availability modifies the composition and diversity of AMF communities as well as spore and mycelium densities in temperate and tropical systems (Lakshmipathy et al., 2012; Melo et al., 2017; Wang et al., 2015). Our analyses indicate that forest type and forest stand significantly influence the composition of AMF communities, which suggests that the conversion of native forests to exotic plantations impacts the diversity of AMF communities. This conclusion aligns with the conclusions drawn by other studies (Kivlin et al., 2011; Melo et al., 2017; Öpik et al., 2010).

5. Conclusions

Our analyses of the AMF present in plantation and native forest soils indicate that forest type has a huge impact on the diversity and abundance of AMF, as well as on the composition of these fungal communities. Forest type is therefore of enormous importance due to the fundamental role that AMF play in ecosystem functioning. Based on our findings, we conclude that the conversion of Ethiopia's natural forest systems into agricultural crops or monospecific forest stands poses a serious threat to the biodiversity of its ecosystems. Specifically, the impact on microorganisms could be irreversible with enormous ecological consequences. Although this study could be considered to be a case study, our findings indicate the importance of prioritizing the sustainable development of degraded natural forests over promoting plantations to ensure the preservation of habitats conducive to maintaining various AMF communities, which is vital when devising conservation and management strategies.

Further research is needed to disentangle the effects of environmental variations from the transition from natural forests to plantations. We suggest future studies that involve controlled experiments or long-term monitoring to better understand these dynamics. We also propose conducting parallel studies on AMF and ECM fungi across different forest types to provide a more comprehensive understanding of how mycorrhizal associations across different forest types.

Funding

This research was supported by the projects SUSTFUNGI_ET I: 2017/ACDE/002094; MYCOPROED_ET: 2019/ACDE/000921, and SUSTFUNGI_ET III: 2022/ACDE/000201 funded by the Spanish Agency for International Development and Cooperation. Gonfa Kewessa also received funds through the call for predoctoral contracts of the UVa 2021, co-financed by Banco Santander.

CRediT authorship contribution statement

Gonfa Kewessa: Writing – original draft, Methodology, Investigation, Formal analysis. **Tatek Dejene:** Writing – review & editing, Supervision. **Pablo Martín-Pinto:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to express our gratitude to the people who, in one way or another, contributed to the success of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173718.

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