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Root Colonization and Spore Abundance of Arbuscular Mycorrhizal Fungi Along Altitudinal Gradients in Fragmented Church Natural Forest Remnants in Northern Ethiopia

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Abstract

Arbuscular mycorrhizal fungi (AMF) spore density and root colonization are considered sensitive to host species and abiotic factors such as climate and soil. However, there is a knowledge gap about how fragmented native forest remnants might contribute to AMF conservation, what is the AMF spore density and root colonization, and to what extent climate change, particularly warming, might impact AMF. The aim of the study was to quantify the AMF spore density and root colonization along altitudinal gradients in three agro-ecological zones of nine church forests in northern Ethiopia. Data were collected from 45 plots. All the surveyed church forest species were colonized by AMF. However, we found a significant ($p < 0.05$) decrease in root colonization and AMF abundance in forests at high elevation. The topsoil had significantly ($p < 0.05$) higher root colonization and AMF abundance than subsurface soil. We found strong negative correlations between altitude and both spore density and root colonization and soil fertility. While we cannot separate whether spore density was temperature or soil limited, we can demonstrate the importance of conserving certain tree species, particularly *Ficus* species, which harbor high spore densities, in both lowland and midland church forests. In the highland, no *Ficus* species were found. However, *Hagenia abyssinica*, another Rosales, had the highest spore density in the highland ecoregion.

Keywords Arbuscular mycorrhizal fungi · Spore density · Root colonization · soil properties · Altitudinal gradient · Church forest

Introduction

Traditionally, soil biological diversity conservation efforts focus on large and relatively undisturbed forest habitats because large areas are assumed to conserve relatively more species than small forest patches [1]. However, small and moderately disturbed forests can accommodate high diversity. In Ethiopia, forest fragments are retained near churches and considered sacred, and these can harbor high degrees of species richness and biodiversity compared to their surroundings [2]. Church forests are Ethiopian Afromontane sacred groves associated within the central and northern Ethiopian Orthodox Tewahedo Churches (EOTC) and monasteries [2–4]. The sacred forests have spiritual, cultural, and religious values and an intrinsic function to biodiversity conservation [2]. These forests are sources of germplasm to biodiversity and superior examples in conserving exceptionally high plant diversity [5]. Church forests are in situ and ex situ conservation sites acting as a refuge and buffer for red list endemic and endangered plant species [6]. These forests play a pivotal role in supplying quality germplasm for the restoration of degraded landscapes in Ethiopia [2]. Church forests serve as important in situ conservation habitats to indigenous and threatened species in Ethiopia [7]. This is though mycorrhizal symbiotic association between plant root and microbial fungi in the soil [8]. Arbuscular mycorrhizae is among the most important fungi obligate symbionts that have a symbiosis with more than 80% of terrestrial plant species [9]. Arbuscular mycorrhizal fungi

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(AMF) spore and colonization play a central role in many microbiological decomposition and ecological processes [10]. As deforestation continues, church forests have an increasing importance as a source of propagules/germplasm [5]. Thus, they have widespread significant future conservation and sustainable forest management of biodiversity and forest-based livelihoods [2]. However, the existing systems of research and information gathering regarding the knowledge of AMF in church forests have not been adequately documented and exploited [11].

Inoculation of tree seedlings with AMF fungi has huge potential for the rehabilitation of degraded land [12, 13]. However, AMF inoculum due to obligate symbiotic behavior cannot be cultivated in vitro pure cultures away from their host plants [14]. The source of the propagule spore bank abundance, diversity, and infectivity is not identified [14]. Lack of cheap and robust AMF inoculum production limits the source of inoculum [12]. The best and mandatory solutions for the identified problem are to set up AMF inoculum sources as reference collections and identify to what extent the host plant is colonized by AMF [14].

Some studies have evaluated the conservation value of church forests [2, 3, 11]. However, there was a knowledge gap on the status and potential patterns of AMF spore density and root colonization of host tree species dynamics in relation to variant agro-ecologies at the species level in remnant church forests [10]. Therefore, the overall objective of this paper was to quantify AMF spore density and root colonization along an altitudinal gradient in church forests in northern Ethiopia. Specifically, the aims of this paper were (i) to assess trends in physiochemical properties along elevational gradient; (ii) to assess variation in AMF spore density and root colonization between different tree species in church forests and whether this is a "species effect" or due to dendrometric parameters; and (iii) to establish whether AMF spore density and root colonization vary along an altitudinal gradient.

Methods

Study Area

The study was conducted in old (100–600 years old) church forests in northern Ethiopia (Fig. 1). The church forests were located in three ecoregions, differentiated by altitude: lowland (500–1500 m a.s.l); midland (1500–2300 m a.s.l); and highland (2300–3200 m a.s.l) (Table 1) [after 15]. The study area has a diverse geological formation soil variability. The dominant soil types are Humic Cambisols in the Tsegede highlands, Oxisols and Ultisols in the wet and humid midland of Welkait, and Vertisols in the valley bottoms [16, 17].

The study sites were located in a semi-arid agro-ecological zone where the climate is influenced by topography and exposure to rain-bearing winds [18]. The mean annual temperature, mean minimum, and mean maximum, respectively, were 28 °C, 15 °C, and 40 °C for Kolla Welkait-Maygaba; 20 °C, 12 °C, and 30 °C for Weina Dega Welkait-Adiremets; and 14 °C, 6 °C, and 23 °C for Dega Tsegede-Ketemanigus; it was 28 °C with a mean minimum and maximum of 15 °C and 40 °C, respectively [19].

Mean annual precipitation for Kolla Welkait-Maygaba, Weina Dega Welkait-Adiremets, and Dega Tsegede-Ketemanigus was 911, 1381, and 2526 mm, respectively [19].

The church forests in the study area are categorized as dry evergreen Afromontane forest and *Combretum-Terminalia* woodland ecosystems [20]. The *Combretum-Terminalia* woodland ecosystems are characterized by drought-resistant, moderately sized trees with large, deciduous leaves occurring between 500 and 1900 m a.s.l. [20]. This ecosystem has high species diversity with high litter foliage biomass [20]. Dry evergreen Afromontane forests have canopies usually dominated by *Juniperus procera* as a dominant tree species occurring between altitudinal ranges of 1900 and 3300 m [20]. Most of the species found in the study area of church forest along the altitudinal gradient of the agro-ecologies were dominated by indigenous, native, and endangered species. The lowland church forest canopies were dominated by *Ficus vasta*, followed by *F. sycomorus*, *Diospyros mespiliformis*, *Balanites aegyptiaca*, *Tamarindus indica*, and *Terminalia brownii.* In the midlands, church forests were also dominated by *Ficus vasta* followed by *F. sur*, *Teclea nobilis*, *Olea europaea*, *Schefflera abyssinica*, and *Mimusopis kummel*. *Juniperus procera*, *Eucalyptus globules*, *Hagenia abyssinica*, and *Podocarpus falcatus* were among the dominant species in the highland church forests.

Sampling Design

In August 2017, a reconnaissance survey was carried out with key informants and stakeholders including the Orthodox Tewahedo Church (OTC) representatives, community elders, district forestry experts, and administrative bodies using discussion and interviews. This information was used for site selection. The selected sites represented church forests which ranged from 100 to 600 years old.

Line transect method and systematic sampling of plots was conducted [21]. A transect line is a line along which samples are taken deliberately across areas where there are rapid changes in vegetation and marked along environmental gradients [22]. Placing plots along the transect line on the field may become difficult in the dense and mountainous forest; hence, using a perfect grid of plot on the geographic map is recommended [22]. This can increase the accuracy of the global positioning system (GPS) points and can cover the total area for being a representative [22]. Points of sample plots were systematically marked at the intersection line of the

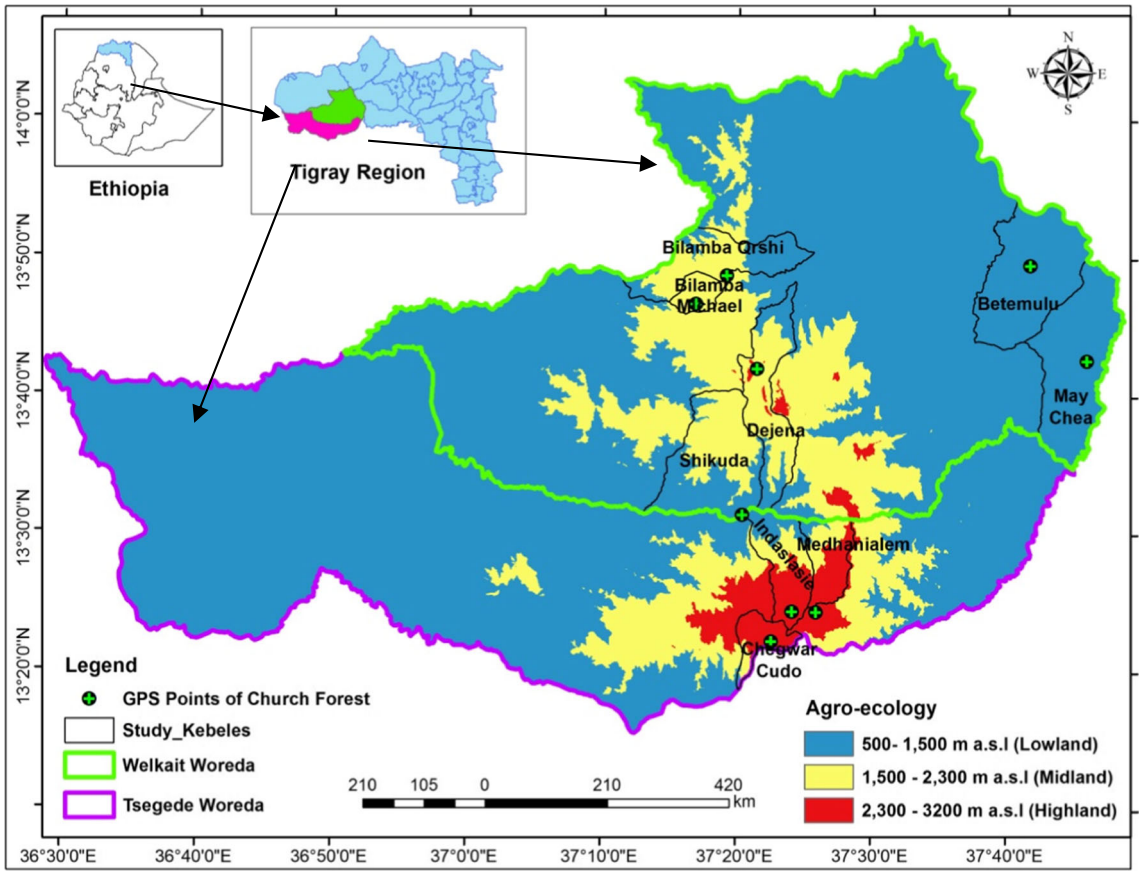

Fig. 1 Location map of study sites and elevation strata along agro-ecologies

topographic map of the study area, with 50-m interval distance between consecutive parallel grid lines and 50-m distance between plots, and edge effects were reduced by leaving a buffer of 25 m at the edge of the forest. After employing a transect line on the map, 45 quadrants of 20 m × 20 m were identified for data collection. The sampling strategy used across the studies considered accessibility, permission, and local knowledge of forest and was used to document the pattern of AMF spore density and root colonization along an altitudinal gradient.

Data Collection

Diameter at breast height (dbh) of all woody plant species > 2 m height was measured using a caliper [23]. Thirty-tree species from all churches, representing 20 families, were identified from 45 study plots along an altitudinal gradient in the three agro-ecologies. Species taxonomic names were identified using published volumes of Flora of Ethiopia and Eritrea [24] which is summarized and shortened in the Natural Database for Africa (NDA) [25].

Table 1 Geographical location and altitudinal ranges of the church forests

Church names	Latitude	Longitude	Altitude (m a.s.l)	Agro-ecologies	Age (year)	Area (ha)	Number of plots
Goworgis Waldba	13° 42′ 39″ N	37° 45′ 55″ E	878	Lowland	550–600	4.50	5
Kaza Eyesus	13° 31′ 23″ N	37° 20′ 26″ E	1079	Lowland	500–550	5.45	5
Mariam Debregenet	13° 49′ 33″ N	37° 41′ 41″ E	930	Lowland	100–150	4.25	5
Michael	13° 46′ 41″ N	37° 16′ 54″ E	1946	Midland	450–500	4.75	5
Cherkos Dejena	13° 42′ 01″ N	37° 21′ 28″ E	2265	Midland	550–600	6.125	6
Medhanialem Debregenet	13° 48′ 46″ N	37° 19′ 11″ E	2004	Midland	300–350	4.50	4
Mamariam Cheguar Cudo	13° 22′ 13″ N	37° 22′ 37″ E	2912	Highland	450–500	4.50	5
Mariam	13° 24′ 21″ N	37° 25′ 55″ E	2878	Highland	550–600	4.25	5
Rufaael	13° 24′ 23″ N	37° 24′ 09″ E	2765	Highland	300–350	5.60	5

The rhizosphere soil samples were collected during the rainy season. Soil samples were collected from four corners of the tree using a rectangular plot "×" pattern. Soil samples were collected from upper depth (0 to 30 cm) and bottom soil depths (30 to 60 cm). Soil samples were taken at four points of 3–5-m radius from the crown edge of each selected tree [26]. Rhizosphere soil samples were collected from all the woody species and replicated three times for the dominant woody species in each plot. A total of 342 soil samples were collected from the two depth ranges.

To account for variability between sites, soil was also sampled with an auger at 0–30 and 30–60 cm depth from four corners of the tree using a rectangular plot "×" pattern. Assessments of fine roots for AMF root colonization were done from 30 tree species. Samples of all plants were collected by excavating soil starting from the plant's trunk base in four directions of the plant and working out towards to get live fine roots within 3–5-m radius [25]. For the dominant tree species, sampling was replicated three times [27]. A total of 171 tree root samples were collected. We collected a 10-g fine root (diameter < 2 mm) composite sample from all four sampling points for laboratory analysis. The fine root samples were tap fresh water to remove any soil particles [27]. We randomly selected 30 fine root samples, put them into tightly sealed plastic jar, filled it with 97% ethanol to preserve the roots, and stored them at 4 °C room temperature until they were ready for further laboratory analysis [27].

Laboratory Analysis

Soil mycorrhizal spores were isolated using the wet-sieving and decanting method [26]. The rhizosphere soils were air-dried and sieved through a 2-mm sieve to remove unwanted material. A 10-g soil sample of the dried soil was then weighed out on a balance to 0.01 g resolution. This sample was mixed with 100 mL of tap water in a plastic bottle and shaken for 30 min, and then left to settle before separating the supernatant from the sediment. The supernatant was passed successively through 300-μm, 100-μm, and 50-μm sieves, placing one below the other in descending order of mesh sizes. An 850-μm sieve was placed on the top to remove rock fragments, woods, and other unwanted materials, which might have remained after the initial sieving. The spores in each of the different sieves were carefully poured into plastic jars and water was added, covered tightly, and centrifuged at 2000 rpm for 5 min. The samples were again washed with tap water and sieved using the smallest 50-μm sieve. They were poured into the plastic vials and centrifuged again using 50% sucrose at 2000 rpm for 3 min. After that, they were transferred to a 50-μm sieve and washed well to remove the sucrose. Finally, each sample was poured onto a filter paper and kept in a petri dish divided into compartments with a glass marker for easy counting of spores. The petri dishes were observed under a stereo-binocular dissecting microscope, with 100 magnification power. AMF propagules existing as spores and spore carps were counted by scanning each filter paper with the 300-μm, 100-μm, and 50-μm sieves. Total AMF spore density was calculated per 100 g of moisture-free soil.

The preserved roots were stained, cleared, and bleached using the procedure in [26]. The grid line intersection method was used to observe the presence or absence of arbuscules, vesicles, and hyphae of AMF [28]. The roots were randomly selected and mounted lengthwise on slides in replicates of nine from every individual plant sample. The samples were observed under a compound microscope with 400 times magnifying power for arbuscule, hypha, and vesicle identification. At each intersection, there were six possible mutually exclusive outcomes [29].

The 90 soil samples were analyzed for pH, total nitrogen (TN), available phosphorus (Av.P), organic carbon (OC), electrical conductivity (EC), cation exchange capacity (CEC), and exchangeable potassium (K). pH and EC were determined using a suspension of 1:2.5 soil:water ratio. Soil texture was determined using the hydrometer method [30]. Organic carbon (OC) was determined using the Walkley-Black method [31]. Av.P content was determined using the Bray II method for the pH of the acidic soil < 5.5 of highland Tsegede conducted using ascorbic acid as a reductant in the presence of potassium antimony and read in a spectrophotometer [32]. The Olsen method was used for soils with pH > 5.5 [33]. Total nitrogen (TN) was determined using the Kjeldahl method [34]. Exchangeable potassium (Av.K) was determined using the flame spectrophotometer method [35]. Cation exchange capacity (CEC) was determined using the ammonium acetate (pH 7) method using the percolation tube procedure [35].

Statistical Analysis

Normality test was carried out and variables that were not normally distributed were $\log_{10}(x)$ transformed to achieve normality. The variation of AMF spore density (for each soil depth) and root colonization structures between the three landscape positions and between tree species in the given landscape position were tested using a one-way ANOVA. The variation of AMF spore density in each soil depth and root colonization structures across the three agro-ecologies were tested using a one-way ANOVA. Differences in soil properties between the three landscape positions and between tree species in the given landscape position were tested using a one-way ANOVA. Tukey's honestly significant difference (HSD) post hoc test was used for pair-wise multiple mean comparisons tests between the three landscape positions and between tree species in the given landscape position. The relationships between AMF spore density and root colonization with soil physicochemical properties, AMF spore density, and

root colonization with tree size was determined using Pearson's correlation analysis. All the tests of statistical significance were decided at $p < 0.05$. The data were analyzed in R statistical software [36].

Results

Variation in Soil Properties Along an Altitudinal Gradient

The soil properties had shown a significant difference between the ecoregions ($p < 0.001$). Most soil properties showed a decreasing trend with increasing altitude and soil depth (Table 2). For example, the lowland soils had about 30% higher total nitrogen content compared to the highland.

The AMF spore density in both soil depths and root colonization structures, mainly HC, had shown a significant relationship with tree dendrometric structures (DBH, height, and crown diameter) in the lowland and midland ecoregions, yet not in the highlands (Table 3).

AMF Spore Density and Root Colonization of Different Tree Species

The average number of AMF spore density enumerated in the church forests ranged from 58 to 5408 (Table 4). The maximum number of spores was found in the lowland church forests followed by midland and highland. In lowland church forests, AMF spore density was different ($p < 0.05$) between soil depths . The average spore densities were 5408 per 100 g of dry soil for the topsoil and 744 for the subsoil depths. The highest spore densities were found associated with *Ficus vasta*, *F. sycomorus*, and *Diospyros mespiliformis* trees in the lowlands. *Anogeissus leiocarpus* species had the lowest AMF spore density of sampled trees (Table 4).

In midland church forests, AMF spore density was also different ($p < 0.05$) between soil depths (Table 5). The average spore densities were 3547 per 100 g of dry soil for the topsoil and 375 for the subsoil. Highest spore densities were found associated with *Ficus vasta*, *F. sur*, and *Teclea nobilis* trees species in the midland ecoregion. *Euphorbia abyssinica* species had the lowest AMF spore density.

In highland church forests, AMF was also different ($p < 0.05$) between soil depths (Table 6). The average spore

Table 2 The mean soil properties in the three ecoregions

Physicochemical Soil property	Soil depth (cm)	Lowland 0–30 cm	Midland 0–30 cm	Highland 0–30 cm	*p* value
TN (%)	0–30	0.38 (± 0.02) b	0.31 (± 0.01) a	0.26 (± 0.02) ab	< 0.001
	30–60	0.34 (± 0.03) b	0.29 (± 0.01) b	0.24 (± 0.01) b	< 0.001
CEC (cmol (+)/kg)	0–30	35.38 (± 1.00) b	32.34 (± 1.10) b	28.38 (± 0.57) a	< 0.001
	30–60	34.65 (± 0.19) c	31.58 (± 0.33) b	27.61 (± 0.56) a	< 0.001
Av.k (mg/kg)	0–30	335.96 (± 11) b	280.63 (± 13) a	230.67 (± 20) a	< 0.001
	30–60	327.07 (± 1.4) c	270.56 (± 12.0) b	190.93 (± 3.6) a	< 0.001
pH (H_2O)	0–30	6.66 (± 0.06) c	6.23 (± 0.08) b	5.35 (± 0.03) a	< 0.001
	30–60	6.54 (± 0.08) b	6.12 (± 0.15) b	5.21 (± 0.13) a	< 0.001
EC (dS/m)	0–30	1.74 (± 0.28) b	0.84 (± 0.18) a	0.36 (± 0.02) a	< 0.001
	30–60	1.03 (± 0.09) c	0.39 (± 0.01) b	0.20 (± 0.01) a	< 0.001
Av.P (mg/kg)	0–30	30.41 (± 0.53) b	28.66 (± 0.58) b	24.47 (± 0.66) a	< 0.001
	30–60	29.94 (± 0.33) c	28.03 (± 0.43) b	23.83 (± 0.68) c	< 0.001
OC (%)	0–30	4.33 (± 0.2) a	5.18 (± 0.10) b	5.95 (± 0.17) b	< 0.001
	30–60	4.20 (± 0.05) a	4.84 (± 0.12) b	5.32 (± 0.16) c	< 0.001
Sand (%)	0–30	39.7 (± 0.3) c	30.1 (± 0.5) b	25.6 (± 0.7) a	< 0.001
	30–60	36.0 (± 0.8) c	32.2 (± 0.5) b	24.3 (± 0.4) a	< 0.001
Silt (%)	0–30	34.8 (± 0.2) b	36.7 (± 0.4) c	33.4 (± 0.4) a	< 0.001
	30–60	34.4 (± 0.6) a	30.6 (± 0.3) b	34.2 (± 0.4) c	< 0.001
Clay (%)	0–30	25.6 (± 0.5) a	33.2 (± 0.3) b	41.0 (± 0.4) c	< 0.001
	30–60	29.6 (± 0.5) a	37.2 (± 0.5) b	41.4 (± 0.5) c	< 0.001

TN total nitrogen, *CEC* cation exchange capacity, *Av.K* available potassium, *pH* power of hydrogen, *Av.P* available phosphorus, *EC* electrical conductivity, *OC* organic carbon. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

Table 3 Pearson's correlation coefficient between AMF spore density along two soil depths and root colonization structures with dendrometric structures along with the agro-ecologies

Altitude (m a.s.l)	Tree size	Spore density		Root colonization structures			
		0–30 cm	30–60 cm	HC	MHC	AC	VC
Lowland 500–1500	Diameter (cm)	0.575**	0.579**	0.506**	0.258*	0.547**	0.383**
	Height (m)	0.296*	0.339**	0.288*	0.244	0.341**	0.257*
	Crown diameter (m)	0.513**	0.536**	0.449**	0.299*	0.506**	0.325*
Midland 1500–2300	Diameter (cm)	0.432**	0.433**	0.356**	0.308*	0.233 ns	0.189 ns
	Height (m)	0.221*	0.286*	0.315*	0.294*	0.268*	0.258 ns
	Crown diameter (m)	0.343**	0.349**	0.328*	0.294*	0.233*	0.205*
Highland 2300–3200	Diameter (cm)	-0.009ns	0.002ns	-0.085ns	-0.086ns	-0.105ns	-0.118ns
	Height (m)	0.097ns	0.079ns	-0.175ns	-0.163ns	-0.149ns	-0.126ns
	Crown diameter (m)	0.068ns	0.052ns	0.110ns	0.123ns	0.108ns	0.107ns

Correlation is significant at level 0.05 (*), 0.01 (**), and ns (nonsignificant) (2-tailed). *HC* hyphal root colonization, *MHC* mycorrhizal hyphal colonization, *AC* arbuscular colonization, *VC* vesicular colonization

densities were 2080 per 100 g of dry soil for the topsoil and 58 for the subsoil. The highest spore densities were found in *Hagenia abyssinica*, *Acacia abyssinica*, and *Dombeya torrida* roots. *Eucalyptus globules* species had the lowest AMF spore density.

All species in church forests were colonized by AMF. The percentage of AMF root colonization significantly differs between tree species in the lowlands ($p < 0.05$) (Table 7). Average colonization ranged between 64 and 90%. The highest colonization was found in *Ficus vasta* species. The lowest colonization was in *Anogeissus leiocarpus* species. HC was the highest root colonization structure followed by MHC, AC, and VC with a mean percentage of 90, 86, 80 and 74%, respectively.

The root colonization structures of AMF were different ($p < 0.05$) between tree species in the midland ecoregion (Table 8). The average colonization ranged from 31 to 82%. The highest colonization was found in *Ficus vasta* species. The lowest colonization was found in *Euphorbia abyssinica* species. Hyphal root colonization (HC) was the most numerous structure followed by mycorrhizal hyphal colonization (MHC), arbuscular colonization (AC), and vesicular colonization (VC) with mean percentages of 82, 80, 72, and 64%, respectively.

In highland church forests, root colonization structures of AMF varied ($p < 0.05$) between tree species (Table 9). The

Table 4 Mean spore density (mean ± SE) of AMF 100 g^{-1} dry soil in each soil depth between different tree species in lowland church forests

Species	Soil depth (cm)	
	0–30	30–60
Acacia polyacantha	1934 (± 50) a	1474 (± 130) c
Anogeissus leiocarpus	1460 (± 46) a	744 (± 110) a
Balanites aegyptiaca	1815 (± 210) a	1323 (± 36) bc
Diospyros mespiliformis	3014 (± 160) b	2042 (± 67) d
Ficus sycomorus	3853 (± 58) c	2990 (± 21) e
Ficus vasta	5408 (± 190) d	4028 (± 54) f
Tamarindus indica	1507 (± 100) a	900 (± 17) ab
Terminalia brownii	1714 (± 110) a	1106(± 57) abc

Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

Table 5 Spore density (mean ± SE) of AMF 100 g^{-1} dry soil in each soil depth between different tree species in midland church forests

Species	Soil depth (cm)	
	0–30	30–60
Acokanthera schimperi	1074 (± 47) abc	810 (± 10) bcd
Albizia amara	1272 (± 150) abc	1065 (±68) cdef
Croton macrostachyus	903 (± 110) abc	650 (± 220) abc
Dracena steudneri	850 (±91) abc	527 (± 58) ab
Euphorbia abyssinica	713 (± 150) a	375 (± 76) a
Euphorbia tirucalli	827 (± 220) ab	450 (± 23) ab
Ficus sur	1905 (± 480) c	1600 (± 100) g
Ficus vasta	3547 (± 290) d	2240 (± 200) h
Mimusops kummel	1241 (± 76) abc	852 (± 27)bcd
Olea europaea	1406 (± 75) abc	1272 (± 30) efg
Rhus natalensis	977 (± 61) abc	710 (± 120) abcd
Schefflera abyssinica	1343 (± 120) abc	1153 (±120) defg
Syzygium guineense	1250 (± 61) abc	880 (± 40) bcde
Teclea nobilis	1606 (± 260) bc	1362 (± 50) fg

Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

Table 6 Spore density (mean ± SE) of AMF 100 g^{-1} dry soil in each soil depth between different tree species in highland church forests

Species	Soil depth (cm)	
	0–30	30–60
Acacia abyssinica	1127 (± 86) c	375 (±35) d
Dombeya torrida	837 (± 29) b	266 (±23) cd
Eucalyptus globules	252 (± 7) a	58 (±6.4) a
Hagenia abyssinica	2080 (± 160) d	697 (±100) e
Juniperus procera	284 (± 20) a	98 (±4.1) ab
Maytenus undata	352 (± 58) a	110 (±11) ab
Maytenus arbutifolia	344 (± 21) a	102 (±3.7) ab
Podocarpus falcatus	463 (± 50)a	180 (±10) bc
Rhus vulgaris	407 (± 32) a	160 (±17) abc

Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

mean percentage of colonization ranged from 9 to 67%. The highest colonization was found in *Hagenia abyssinica* trees and the lowest colonization was in *Eucalyptus globules* trees. HC was the most numerous structure followed by MHC, AC, and VC (Table 9).

AMF Spore Density and Root Colonization Along an Altitudinal Gradient

AMF spore density decreased with increasing altitude with significant differences between ecoregions ($p < 0.05$) and this pattern was consistent for both the topsoil and the subsoil (Fig. 2).

Similarly, % AMF root colonization showed a decreasing trend with increasing altitude with significant differences between the ecoregions of different altitudes ($p < 0.05$) (Fig. 3). The mean percentage colonization of AMF along the agro-ecologies ranged from 81% in the lowlands to 23% in the uplands. HC was the highest % root colonization followed by MHC, AC, and VC with mean percentages of 81, 78, 73, and 67%, respectively.

Discussion

AMF Spore Density and Root Colonization Within Each Ecoregion

All the 30 species surveyed in the study ecoregions were colonized in general agreement with previous studies in northern Ethiopia [37]. The present study also showed comparable spore densities to those found in Central American tropical forest (110 to 2600 spores 100 g^{-1} soil) [38] and tropical savanna in SW China (5 to 6400 spores 100 g^{-1} soil) [39].

Roots have different sources of organic compounds derived from photosynthesis making the rhizosphere a hot spot for microbial activities, particularly fungi [40]. *Ficus vasta* species has harbored the greater spore and colonization, followed by *F. sycomorus* and *F. sur*. *Ficus vasta* species have unique biological, physiological, morphological, and structural attributes with dense lateral and deep taproot root systems and big canopy acting as efficient "nutrient pump" with huge diversity and high litterfall that is easily decomposable, and a host of organisms [41].

Mycorrhizal dependency is related in part to the morphology of the plant root system. Plants having extensive fibrous roots are often less dependent on mycorrhiza than with less extensive root systems [42]. These factors may provide the widespread presence of spore bank and root colonization in each species in our study area. This has an implication for AMF spore bank multiplication for better seedling inoculation and ability to remain active and maintain a mycorrhizal network during periods of extended drought, which is very important for better land restoration establishment [12].

HC was the most numerous root colonization structure followed by MHC, AC, and VC along agro-ecologies. The

Table 7 AMF root colonization structure (%) for tree species in lowland church forests (mean ± SE)

Species	AMF root colonization structures (%)			
	HC (%)	MHC (%)	AC (%)	VC (%)
Acacia polyacantha	82.5 (± 0.5) bcd	79.2 (± 0.8) bcd	74.8 (± 1.7) bcd	67.7 (± 2.4) ab
Anogeissus leiocarpus	76.5 (± 1.2) a	73.2 (± 1.6) a	68.4 (± 1.5) a	63.9 (± 0.9) a
Balanites aegyptiaca	80.1(± 1.2) abcd	77.4 (± 0.3) abcd	72.4 (± 1.1) abd	66.6 (± 0.9) b
Diospyros mespiliformis	85.2 (± 1.2) cde	81.0 (± 1.5) cde	75.4 (± 1.5) cd	69.2 (± 0.8) ab
Ficus sycomorus	87.6 (± 1.2) de	82.6 (± 0.5) de	78.2 (± 0.7) de	71.4 (± 1.1) ab
Ficus vasta	90.1 (± 0.7) e	85.7 (± 2.2) e	80.4 (± 0.6) e	74.3 (± 4.4) b
Tamarindus indica	78.3 (± 1.2) ab	75.3 (± 0.7) ab	70.6 (± 0.4) ab	65.0 (± 1.1) a
Terminalia brownii	79.2 (± 0.6) abc	76.9 (± 0.3) abc	71.6 (± 0.4) abc	65.7 (± 1.7) a

HC hyphal root colonization, *MHC* mycorrhizal hyphal colonization, *AC* arbuscular colonization, *VC* vesicular colonization. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

Table 8 AMF root colonization structure in (%) for tree species in midland church forests (mean ± SE)

Species	AMF root colonization structures (%)			
	HC (%)	MHC (%)	AC (%)	VC (%)
Acokanthera schimperi	57.1 (± 0.5) e	55.6 (± 0.7) de	53.0 (± 0.6) d	52.1 (± 0.2) f
Albizia amara	61.1 (± 0.6) f	57.7 (± 0.7) e	53.8 (± 1.1) d	50.9 (± 0.5) ef
Croton macrostachyus	52.7 (± 0.6) d	50.3 (± 0.8) c	47.9 (± 0.7) c	44.8 (± 0.5) d
Dracena steudneri	47.5 (± 0.5) c	43.4 (± 0.6) b	40.5 (± 0.6) b	39.2 (± 0.6) c
Euphorbia abyssinica	37.8 (± 0.6) a	34.4 (± 0.4) a	31.8 (± 0.5) a	30.8 (± 0.3) a
Euphorbia tirucalli	43.4 (± 0.49) b	40.6 (± 0.8) b	37.5 (± 0.6) b	34.6 (± 0.8) b
Ficus sur	73.1 (± 0.7) h	68.1 (± 0.6) h	63.3 (± 1.2) f	59.7 (± 0.7) gh
Ficus vasta	82.4 (± 1.6) i	79.7 (± 0.5) i	71.8 (± 0.9) g	64.0 (± 1.2) i
Mimusopis kummel	61.3 (± 0.4) f	60.5 (± 0.4) f	59.9 (± 0.5) e	58.6 (± 0.3) g
Olea europaea	65.2 (± 0.5) g	64.2 (± 0.3) g	62.8 (± 0.3) f	62.1 (± 0.3) ij
Rhus natalensis	55.9 (± 0.5) de	53.9 (± 0.8) d	52.5 (± 0.6) d	48.8 (± 0.4) e
Schefflera abyssinica	64.9 (± 0.5) g	63.7 (± 0.6) g	62.7 (± 0.5) ef	61.7 (± 0.4) hij
Syzygium guineense	58.9 (± 0.3) ef	56.5 (± 0.4) de	54.8 (± 0.6) d	52.7 (± 0.2) f
Teclea nobilis	67.2 (± 0.3) g	64.3 (± 0.2) g	63.0 (± 0.3) f	60.4 (± 0.3) ghi

HC hyphal root colonization, *MHC* mycorrhizal hyphal colonization, *AC* arbuscular colonization, *VC* vesicular colonization. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

results were in line with the finding of Belay et al. [43] who found higher hyphae than in other structures. Hyphae are the primary structures of AMF existing for months or years [44]. AMF hyphae are much thinner that exclusively colonize the root cortex and form highly branched structures inside the cells, i.e., arbuscules, which are considered the functional site of nutrient exchange [14]. Hyphae are a bridge to the soil-root gap created during drought through binding and maintain a liquid flow that avoids the loss of hydraulic conductivity caused by air gaps because hyphae increase levels of glomalin that improves soil aggregate and water retention [45]. The overall implication of AMF to soil carbon storage could depend significantly on the hyphae produced.

AMF Pore Density and Root Colonization Along an Altitudinal Gradient and Correlations with Soil Properties and Tree Dendritic Characteristics

We found a decreasing trend in AMF spore bank and colonization with increasing elevation. This finding was similar to [46] from the Andes and the Rocky Mountains, and Lugo et al. [47] from the Puma grasslands showed similar trends with significant decreasing pattern in AMF with increasing elevation. Different edaphic factors such as soil type, soil pH, and decomposition rate of OM might also be responsible for variations of AMF [48]. Furthermore, spore density and root colonization were

Table 9 AMF root colonization structure (%) for tree species in highland church forests (mean ± SE)

Species	AMF root colonization structures (%)			
	HC (%)	MHC (%)	AC (%)	VC (%)
Acacia abyssinica	59.3 (± 0.6) g	54.2 (± 0.3) g	52.6 (± 0.4) g	49.4 (± 0.4) g
Dombeya torrid	50.0 (± 0.9) f	46.4 (± 0.6) f	45.3 (± 0.6) f	41.6 (± 0.7) f
Eucalyptus globules	12.7 (± 0.4) a	11.2 (± 0.2) a	10.5 (± 0.5) a	8.5 (± 0.3) a
Hagenia abyssinica	67.0 (± 0.8) h	61.4 (± 0.6) h	59.5 (± 0.8) h	56.3 (± 0.7) h
Juniperus procera	19.7 (± 0.3) b	16.8 (± 0.3) b	14.2 (± 0.3) b	12.5 (± 0.3) b
Maytenus undata	30.6 (± 0.4) cd	29.4 (± 0.4) d	29.0 (± 0.4) d	26.3 (± 0.4) d
Maytenus arbutifolia	28.4 (± 0.4) c	24.5 (± 0.3) c	22.9 (± 0.2) c	17.2 (± 0.3) c
Podocarpus falcatus	39.5 (± 0.6) e	36.2 (± 0.6) e	35.5 (± 0.5) e	31.6 (± 0.5) e
Rhus vulgaris	32.3 (± 0.7) d	30.5 (± 0.6) d	29.3 (± 0.9) d	26.3 (± 0.6) d

HC hyphal root colonization, *MHC* mycorrhizal hyphal colonization, *AC* arbuscular colonization, *VC* vesicular colonization. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

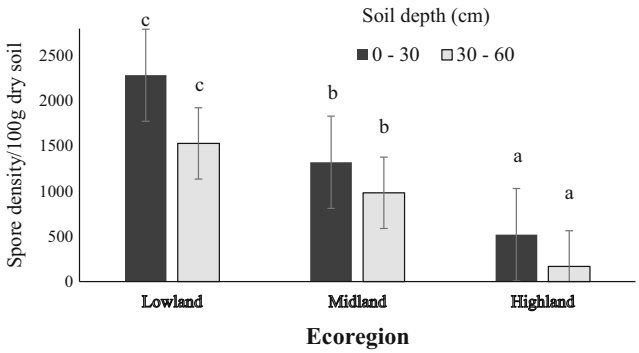

Fig. 2 Variation of AMF spore density between ecoregions and soil depth. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

positively correlated with tree size. In general, species with the largest contribution in the basal area can be considered the most important for a large proportion of biomass and carbon, which could bring a variation of AMF [49]. These results support that the sporulation and colonization are positively correlated with the growth and size of mycorrhizal [28].

Our results demonstrated strong negative correlations between altitude and both spore density and root colonization. The low spore density and root colonization in the highland ecoregion could be due to low temperature and poor soil fertility compared to the lowland and midland ecoregions. We found that species like *Ficus* and *Hagenia abyssinica* host high spore density that need conservation priority. Dense cover also produces higher litterfall and greater amounts of root biomass for maintaining a diverse AMF community [50]. Therefore, the unequal contribution of species to AMF in church forest could be due to the dendrometric structure difference, density, and host ability responsiveness among species.

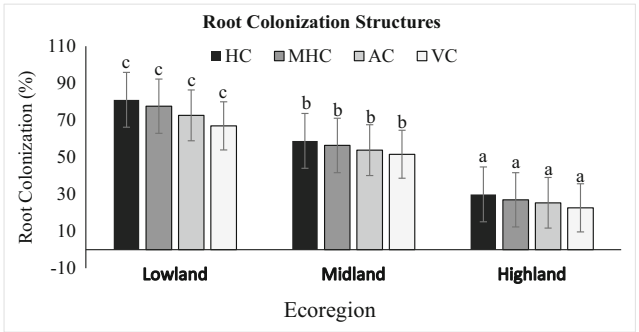

Fig. 3 Variation of AMF spore density between ecoregions and soil depth. Units within a column followed by the same letter/s are not significantly different at $p < 0.05$

Conclusions

The lowland and midland church forests' dendrometric structural composition of species had a positive significant correlation with spore density and with almost all root colonization structures mainly hyphal colonization. Generally, there was a decreasing trend in arbuscular mycorrhizal fungi with increasing altitude. This variation might be due to structural vegetation biomass, host specificity, root structure, physicochemical soil properties, and variations on climatic conditions. The difference in the presence of dense litter foliage in the top surface among the forest species leads to the variation of spore bank and colonization. Generally, conserving remnant church forests is a central point to the source of arbuscular mycorrhizal fungi spore bank inoculums as biofertilizers to better seedling inoculation and rehabilitation efforts of dryland area which could potentially decrease the cost used for agrochemicals.

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