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Effects of Forest Composition and Disturbance on Arbuscular Mycorrhizae Spore Density, Arbuscular Mycorrhizae Root Colonization and Soil Carbon Stocks in a Dry Afromontane Forest in Northern Ethiopia

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Abstract: We investigated arbuscular mycorrhizal fungi (AMF) spore density and root colonization in three distinct dry Afromontane forest plant communities, representing differing levels of disturbance and soil properties. Soil and root samples were collected from sixty-five 50 × 50-m plots from four plant communities. We collected data for AMF spore density, AMF root colonization and soil organic carbon stocks in 0–25 and 25–50 cm soil depth ranges. AMF spore density, and root colonization differed significantly among plant communities. The least disturbed *Juniperus procera–Maytenus senegalensis* (*Jupr-Mase*) plant community, which contained high tree and shrub density, had the highest AMF spore density, root colonization and soil carbon stocks. The most disturbed *Cadia purpurea–Opuntia ficus-indica* (*Capu-Opfi*) community which contained the lowest tree and shrub density supported the lowest AMF spore density, root colonization and soil carbon stocks. There was no significant difference in spore density between the two soil depths, but AMF root colonization was significantly higher in the upper soil than in the subsoil ($p < 0.001$). The difference in soil properties was not uniform between plant communities. Conserving remnant dry Afromontane forests and restoring the degraded forests are critical to improve and maintain forest ecosystem functioning and sustain ecosystem services.

Keywords: arbuscular mycorrhizal fungi; biodiversity; forest disturbance; plant community; soil biology; soil properties

1. Introduction

Tropical dry forests have some potential to help mitigate climate change, reduce desertification and land degradation, and provide livelihood products and services for millions of people living in dryland regions. Yet, unsustainable use of forest products, combined with increased drought frequency, has reduced forest cover, and thus the ecosystem services they provide [1,2]. Disturbance increases tree die back, changes population structure, limits natural regeneration, reduces the soil

seedbank and causes species loss [3–6]. Such changes may have cascading effects on soil microorganisms, including arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi are an ancient group of micro fauna, dating back to when plants first colonized the land [7,8]. AMF possess fungal structures in the form of arbuscules, vesicle and hyphae, which are found in roots, and external hyphae and spores in the soil [8]. The relationship between these fungal symbionts and their hosts is generally mutualistic, with the host plant benefitting primarily through the enhanced acquisition of soil nutrients, particularly phosphorus [7]. Other benefits include improved plant hormone production and induction [9]; heightened resistance to root pathogens [10]; improved water uptake [11] and soil structure [12,13]. In return, all obligated AMF depend on the host plant for photosynthetically-fixed carbon sources [7,8]. Nevertheless, they are occasionally parasitic, depending on the host-plant species and the species of AMF [14].

The contribution of AMF to supporting plant growth and promoting ecological stability is more important in marginal soils in semi-arid regions where nutrients and water are limiting factors [7]. The productivity of plant communities depends on the symbiotic effectiveness of the colonizing fungi and perhaps also the diversity of the fungal assemblage [2]. In turn, the distribution and abundance of AMF is strongly affected, both by habitat conditions and the diversity and abundance of host species [15]. At high plant densities, nutrient extraction rates are higher, stimulating AMF reproduction [16]. Densities of AMF spores in soils can also differ within the same region, depending on soil physical and chemical properties, particularly soil organic matter content, and soil microbial activity [8]. These are modified by land use practices [17,18]; season, successional stage of the vegetation [4,19] and natural and anthropogenic disturbances [20,21], including land use change [22–24]. In semi-arid environments, soil temperature and moisture content [25] and elevation [26,27] also affect AMF spore density and colonization.

The factors influencing the composition and density of AMF in restored forests (e.g., forests protected by exclosures) and remnant forest patches, such as church forests, are poorly known. Yet, it is essential to understand the determinants of spore density and root colonization by indigenous AMF in these ecosystems and how they affect carbon sequestration. Some studies on AMF in the dry Afromontane forests of Ethiopia have used classical techniques based on characterizing spore morphology and levels of root colonization [28,29]. In the Hugumburda dry Afromontane forest in the northern Ethiopian highlands, Aynekulu [30] identified three distinct plant communities based on their dominant species: *Juniperus procera–Maytenus senegalensis* (hereafter referred to as Jupr-Mase), *Pterolobium stellatum–Celtis africana* (Ptst-Ceaf) and *Cadia purpurea–Opuntia ficus indica* (Capu-Opfi). We hypothesized that these distinct communities varied in the amount of AMF root colonization and spore density, with effects on plant productivity and furthermore soil carbon stocks, so we assessed these differences in an observational, false-chronosequence study.

2. Materials and Methods

2.1. Study Area

The study was conducted in Hugumburda forest, a 1687 ha, isolated (surrounded by agricultural landscape and separated from the rest of dry afromontane forests), natural forest remnant, located between 12°36′ and 12°40′ N, and 39°31′ and 39°34′ E in northern Ethiopia (Figure 1A). The terrain is undulating to steep (Figure 1), and frequently incised by streams. The underlying geology is tertiary basalt, alkali-alluvial basalt and tuff. The dominant soils are Leptosols, which are shallow and lie on the slopes, and Regosols, thick deposits of which lie in the valley bottoms.
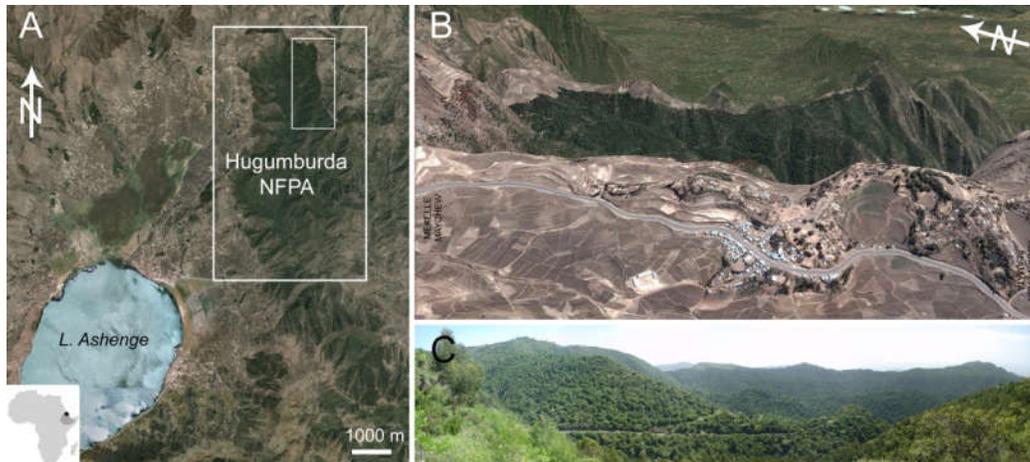
Figure 1. Location of the study area, Hugumburda Forest, in the highlands of northern Ethiopia: **A**—overhead and, **B**—oblique satellite imagery over a global Digital Terrain Model (DTM) in Google Earth, with vertical exaggeration factor 3 and **C**—corresponding photograph (panorama of five images) by Raf Aerts. The boundary of the forest in **A** is approximate. Scale in **B** varies—the boundary of the forest along the upper ridge is 5.7 km. Satellite imagery © 2020 GeoEye, Cnes/Spot Image and DigitalGlobe, by way of Google Earth.

The study area is in a semi-arid agro-ecological zone where the climate is influenced by topography and exposure to rain-bearing winds. The wet season is between June and September, whereas the remaining months are more or less dry. The nearest meteorological station to Hugumburda dry Afromontane forest is at Korem, 14 km south of Hugumburda. Mean annual rainfall (10-year average from 2007–2016) in the study area is 991 mm. Yet, there is high interannual variability, with 1480 mm and 480 mm in the study years of 2014 and 2015, respectively. The average annual temperature is 15 °C (Figure 2) with a mean minimum of 5 °C and mean maximum of 24 °C in November and May, respectively.
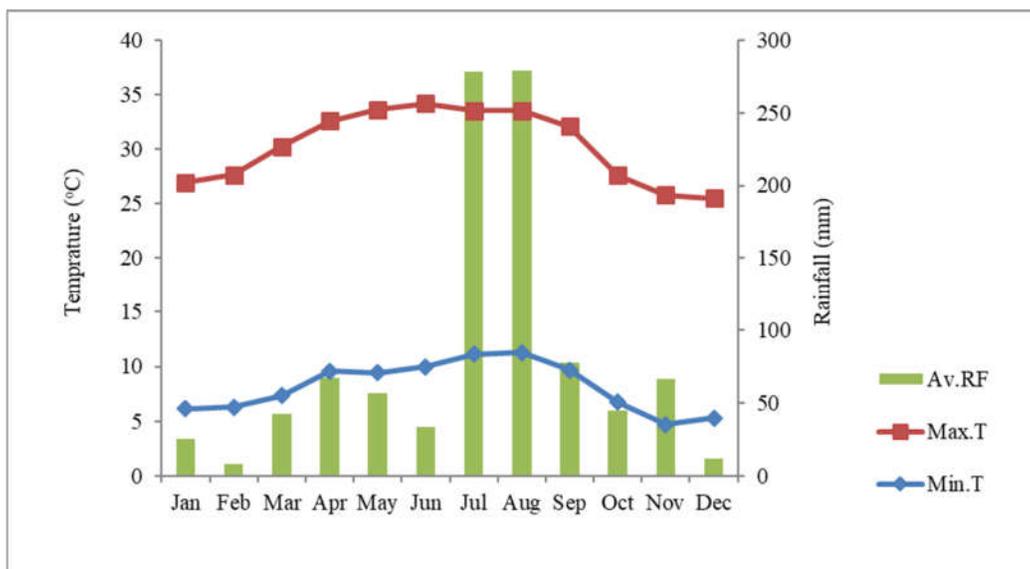

Figure 2. Maximum temperature, minimum temperature (°C) and monthly rainfall depth (mm) of Hugumburda forest recorded at Korem meteorological station from 2007–2016 (Ethiopia National Meteorological Agency 2017)**.**

Hugumburda forest can be broadly categorized as a dry co-dominant Afromontane forest, with *Juniperus procera* and *Olea europaea* subsp. *cuspidata* as the dominant tree species [31]. Tree species constitute 30% of all vascular plant species present, the balance comprising herb and shrub species, which predominate in the understory. It is the only more or less intact large forest containing many locally rare tree species from the pre-disturbance period, which are also rare in other parts of northern Ethiopia, including *Afrocarpus falcatus* [30]. There are several small settlements around the forest, where people cut trees illegally. Exotic tree species, mainly *Cupressus lusitanica*, *Eucalyptus camaldulensis* and *Acacia saligna*, are scattered throughout the forest. Crop cultivation is widely practiced adjacent to the forest. Browsing, grazing and trampling by livestock, especially in and near the forest edge, create disturbances in the forest. Soil depth varies from shallow (21–50 cm deep) to moderately deep (>100 cm deep) [32].

2.2. Experimental Design and Layout

We assessed AMF spore density by sampling 65 plots, laid out the samples systematically along five transects established in the Hugumburda forest by Aynekulu (30). These transects were placed at 1-km intervals, parallel to each other, and extended downslope across an elevational gradient from 2700 to 1860 m a.s.l. Along each transect, at 100 m a.s.l. elevation intervals, a series of 50 m × 50 m plots were laid out horizontally at right angles to the slope (Figure 3).
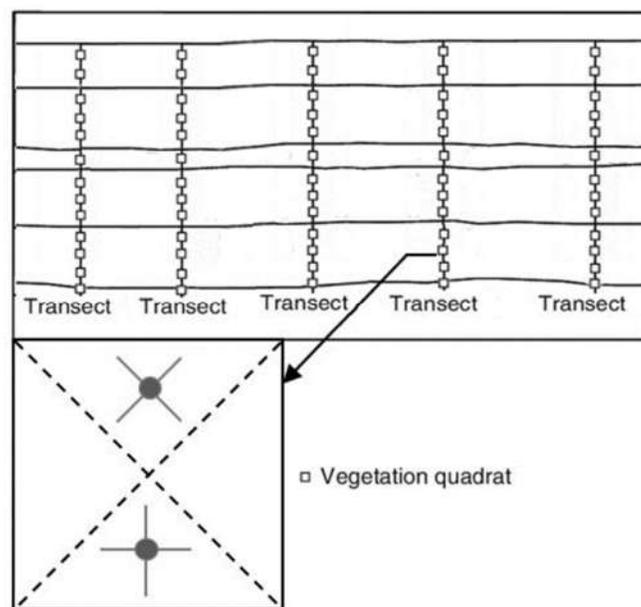
Figure 3. Layout of transects, plots and schematic sampling scheme.

Diversity and grouping of plant species into communities were done in a previous study [30]. The level of disturbance on each plot was assessed subjectively, based on grazing intensity, number of cut stems (stumps), human trails, and categorized as moderate to high (Table 1, [33]).

Table 1. Criteria used for ranking disturbances.

Disturbance levels	Description
No disturbance (0)	No grazing observed, no human trails, no cut stems or droppings
Slightly disturbed (1)	1–2 animal dung patches and the presence of cut stem within the plot
Moderately disturbed (2)	3–5 grazing animals observed; human trails, cut stems and droppings present
Highly disturbed (3)	> 6 grazing animals observed; human trails, cut stems present

2.3. Data Collection

Root and Rhizosphere Soil Sampling

Roots and rhizosphere soil were sampled from all woody species > 1.5 m height and > 2 cm DBH, at the end of the rainy season in September 2015. Three replicates of each sample were taken from the dominant woody species in each plot, resulting in 1920 samples overall. For each selected woody species, root samples were collected by excavating the soil using a hoe to dig outwards from the base of the tree trunk in four directions, until the fine roots were exposed. The fine roots were collected in each quadrant and mixed well to form a composite sample. This was placed in a bottle, to which 97% ethanol was added immediately to preserve the roots, until processed and analyzed for AMF root colonization. Rhizosphere soil samples were collected at two depths (0–25 cm and 26–50 cm) around each tree [34] and mixed to form a composite sample. These were stored in plastic bags and closed tightly. The bags were labeled and taken to the laboratory at Mekelle University, for the enumeration of AMF spore density and root colonization.

2.4. Soil Sampling

Soil samples were collected from the surface (0–25 cm) and subsoil (25–50 cm) layers in each plot, as described by Sewnet and Tuju [34]. Undisturbed samples for bulk density analysis and the subsequent analysis of soil carbon stocks were taken from the center of each plot, using a 5-cm high, 5-cm diameter soil corer. Samples for soil chemical analysis were collected in a diagonal pattern within a plot and mixed to form a single representative composite sample for the plot. These composite samples were dried, crushed and sieved through a 2-mm sieve before determining pH, electrical conductivity (EC), organic carbon (OC), total nitrogen (TN), exchangeable potassium (Exch. K) and available phosphorus (Av. P).

2.5. Laboratory Analysis

2.5.1. Soil Laboratory Analysis for AMF Spore Density

Spores produced by AMF were examined after wet sieving 100 g of dry rhizosphere soil [35], followed by centrifugation in a 50% sucrose solution [36]. 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, 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, placed 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 material, 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.

2.5.2. Arbuscular Mycorrhiza Fungi Root Colonization Analysis

Roots were washed thoroughly with tap water to remove ethanol and soil particles, cut into ~1-cm-long segments and placed in autoclave-resistant bottles containing 10% KOH and heated at 90 °C for 1 hr [37]. The root pieces were then washed with distilled water and placed into 10% H_2O_2 for

15 min for further bleaching. Bleached roots were washed with tap water and acidified in 1% HCl for 1 h and then stained overnight using trypan blue solution (0.05% in a 1:1:1 ratio solution of lactic acid: glycerol: distilled water) [38]. The stained root pieces were washed and placed into 50% glycerol for 1 h. They were then washed and mounted lengthwise on slides in replicates of nine and arbuscules, hyphae and vesicles were identified and enumerated by observation under a compound microscope with 400x magnification. The grid-line intersection method was used to observe fungi structures by inserting a hairline graticule into an eyepiece of the microscope and using the grid lines as the lines of intersection with each root [39]. AMF root colonization was determined by recording separately the number of intersections with roots, overall and with mycorrhizae. Root colonization by AMF was then the number of intersected mycorrhizae, expressed as the percentage of the number of roots intersected overall [40].

2.5.3. Analysis of Soil Chemical Properties

Soil pH, OC, EC, TN, Exch. K and Av. P were determined for each soil sample, using standard laboratory procedures. Soil pH and EC were measured in a 1:5 soil: water suspension. Organic carbon and available P were determined by the Walkley–Black and anion resin extractable phosphorus methods, respectively [41,42]. Total nitrogen content in the soil was measured using the Kjeldahl method [43]. Exchangeable potassium was determined with a flame spectrophotometer. Soil texture was assessed using a hydrometer, as suggested by [43].

2.5.4. Soil Organic Carbon Stock Estimation

The soil organic carbon stock for each horizon was calculated as:

$$\text{SOC } (Mg \text{ C } ha^{-1}) = [\text{WBC (\%)} \times \text{Bd (g cm}^{-3}) \times \text{D}] \times 100, \tag{1}$$

where SOC is total soil organic carbon stock, WBC (%) = Walkley–Black carbon expressed as a decimal fraction, D = soil horizon depth (cm) and Bd = soil bulk density (g cm^{-3}), all for that horizon and site [44]. Soil Bd was determined using core sample method [45]. The soil samples were oven dried at 105 °C for 24 h. The mass of oven dried soil divided by the total volume of core was used to get the Bd.

2.6. Statistical Analysis

Data were analyzed using SPSS Version 20.0. The data sets were tested for normality and variables that were not normally distributed were transformed. Accordingly, spore density and EC values were transformed using log10(x). Pearson's correlation coefficient was calculated to identify the strength of any relationship between AMF spore density and root colonization, and soil parameters such as pH, OC, TN, OM, Exch. K and Av. P. The differences in AMF spore density and total colonization among plant communities were analyzed using a two-way ANCOVA, with plant community at three levels and soil depth at two levels as the factors and disturbance assessment as a covariate. Gabriel's pairwise comparisons test was used for post-hoc multiple pair-wise comparisons of the plant communities. The independent *t*-test was used to determine any differences in spore density and root colonization between the two soil depths. A probability level of $\alpha \leq 0.05$ was used.

3. Results

### 3.1. Assessment of Plant Communities and Levels of Disturbance

Of the 65 plots distributed across the three plant communities, 39 were classified as Jupr-Mase, 13 as Ptst-Ceaf, and 13 as Capu-Opfi, based on their indicator species [30]. Plant diversity differed across these three distinct plant communities. The Ptst-Ceaf community had the highest, while Capu-Opfi had the lowest species diversity. The Jupr-Mase and Ptst-Ceaf communities were classified as moderately disturbed, while Capu-Opfi was more highly disturbed. The Jupr-Mase and

Capu-Opfi plant communities were on shallow soils, whereas the Ptst-Ceaf plant communities occurred on moderately deep soils.

3.2. Differences in AMF Spore Density, Root Colonization and AMF Structures between Plant Communities and at Different Soil Depths

Mean spore density overall was 559 spores 100 g^{-1} dry soil (range 86–1460 spores 100 g^{-1} dry soil (Figure 4a)). Spore density varied significantly between the plant communities ($p < 0.0001$), with the Capu-Opfi community having a significantly lower mean spore density than the Jupr-Mase community ($p < 0.05$). In contrast, spore density in the Ptst-Ceaf community did not differ significantly from either of the other two.

All plant species were colonized by AMF structures. AMF root colonization differed significantly among the three plant communities ($p < 0.05$), with Jupr-Mase having the highest and Capu-Opfi the lowest colonization rate (Figure 4b).
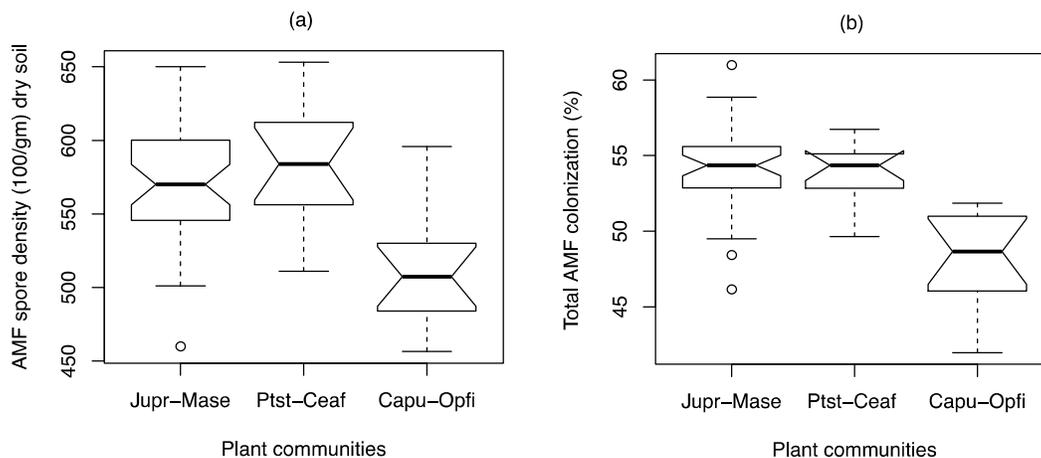
Figure 4. (**a**) Variation of arbuscular mycorrhizal fungi spore density and (**b**) root colonization across plant communities for the 0–25 cm soil depth: Jupr-Mase = *Juniperus procera-Maytenus senegalensis*; Ptst-Ceaf = *Pterolobium stellatum-Celtis africana* and Capu-Opfi = *Cadia purpurea-Opuntia ficus-indica* plant community.

All AMF structures—hyphae, vesicles and arbuscules—were present within the tree roots (Table 2). Hyphal colonization (HC) was highest (mean rate overall 66.5%), followed by mycorrhizal hyphal colonization (MHC, 62.7%), vesicular colonization (VC, 43.2%) and arbuscular colonization (AC, 34.3%). Compared across the communities, HC, MHC and VC were generally significantly lower in the Capu-Opfi community than in either of the other two communities, although this trend was complicated by the high variability in HC colonization in the Ptst-Ceaf community (Table 2). Arbuscular colonization, in contrast, was significantly lower in the Ptst-Ceaf community than in the Jupr-Mase community, but not different from the Capu-Opfi community, although that, in turn, did not differ from the Jupr-Mase community (Table 2). There was no significant difference in spore density between the two soil depths (Table 2), but AMF root colonization was significantly higher in the upper soil than in the subsoil ($p < 0.001$; Table 2).

Table 2. Mean percentage colonization and mean spore density (100 g^{-1}dry soil) (men ± SE) by hyphae (HC), mycorrhizal hyphae (MHC), mycorrhizal vesicles (VC), and arbuscular mycorrhizae (AC), in samples of roots from the *Juniperus procera-Maytenus senegalensis* (Jupr-Mase), *Pterolobium stellatum-Celtis africana* (Ptst-Ceaf) and *Cadia purpurea-Opuntia ficus-indica* (Capu-Opfi) plant communities. Community means for each structure were compared with 1-way ANOVA (α = 0.05, n = 130), followed by post-hoc multiple comparison tests, where these differed significantly. These results are shown by superscripts, with different letters in the rows indicating statistically significant differences between the plant communities concerned ($\alpha \leq$ 0.05).

Structure Colonization	Soil Depth (cm)	Plant Communities			1-way ANOVA	
		Jupr-Mase (*n* = 78)	Ptst-Ceaf (*n* = 26)	Capu-Opfi (*n* = 26)	$F_{2,127}$	*p*
HC	0–25	68.89 ± 1.07[a]	70.04 ± 0.73[a]	64.29 ± 0.93[b]	4.26	< 0.018
	26–50	70.19 ± 0.58[a]	50.05 ± 0.86[b]	63.59 ± 2.03[c]	98.53	< 0.001
MHC	0–25	64.18 ± 1.03[a]	65.73 ± 1.09[a]	54.88 ± 1.78[b]	13.67	< 0.001
	26–50	65.20 ± 0.89[a]	65.10 ± 1.53[a]	53.18 ± 1.94[b]	21.92	< 0.001
VC	0–25	44.81 ±0.79[a]	45.75 ± 0.82[a]	41.07 ± 1.28[b]	4.18	0.020
	26–50	42.59 ± 0.65[a]	43.96 ± 1.04[b]	39.80 ± 1.17[a,b]	3.73	0.029
AC	0–25	38.68 ± 0.51[a]	34.15 ± 0.97[b]	32.07 ± 1.24[b]	20.7	< 0.001
	26–50	33.09 ± 0.82[a]	26.56 ± 1.07[b]	35.51 ± 2.27[a]	9.17	< 0.001
Spore density	0–25	570.45 ±39.43[a]	581.41 ± 45.15[a]	512.14 ± 36.02[b]	12.49	< 0.001
	26–50	559.71 ± 50.15[a]	515.44 ± 47.99[a]	514.83 ±135.48[a]	2.80	0.069
Total root colonization	0–25	54.14 ± 0.48[a]	53.92 ± 0.55[a]	48.08 ± 0.82[b]	23.7	< 0.001
	26–50	52.77 ± 0.42[a]	46.42 ± 0.56[a,b]	48.02 ± 0.58[b]	42.07	< 0.001

3.3. Differences in Soil Properties between Plant Communities and at Different Depths

All soil properties other than pH, EC and available P differed significantly among at least some of the plant communities (Table 3). Total nitrogen concentration was significantly higher in the soil beneath the Jupr-Mase community than beneath either of the other two communities, whereas the concentration of Exch. K was over three times higher in the soils of the Ptst-Ceaf community than in the other two. The soils under the Capu-Opfi community had significantly low organic carbon content (Table 3).

Soil organic carbon stock (SOC) overall was significantly lower in the Capu-Opfi community than either the Jupr-Mase or the Ptst-Ceaf communities (Table 3). As might be expected, across all communities, it was significantly higher in the surface soil than the subsoil (Table 3).

Table 3. Mean values (mean ± SE) of soil properties in the *Juniperus procera-Maytenus senegalensis* (Jupr-Mase), *Pterolobium stellatum-Celtis africana* (Ptst-Ceaf) and *Cadia purpurea-Opuntia ficus-indica* (Capu-Opfi) plant communities. Mean values for each variable were compared across the communities with 1-way ANOVA (α = 0.05, n = 130), followed by post-hoc multiple comparison tests, where these differed significantly. These results are shown by superscripts, with different letters in the rows indicating statistically significant differences between the plant communities concerned ($\alpha \leq$ 0.05).

Soil variable	Unit	Soil Depth (cm)	Plant Communities			1-way ANOVA	
			Jupr-Mase (*n* = 78)	Ptst-Ceaf (*n* = 26)	Capu-Opfi (*n* = 26)	$F_{2,127}$	*p*
pH		0–25	6.75 ± 0.06[a]	7.04 ± 0.51[a]	6.91 ± 0.14[a]	0.67	0.104
		26–50	7.39 ± 0.09[a]	6.94 ± 0.15[b]	7.43 ± 0.12[a,b]	3.78	0.028
Electrical conductivity (EC)	dSm^{-1}	0–25	0.33 ± 0.06[a]	0.37 ± 0.13[a]	0.31 ± 0.08[a]	0.11	0.894

		26–50	0.33 ± 0.05^a	0.23 ± 0.07^a	0.46 ± 0.08^a	2.02	0.141
Available P	ppm	0–25	10.89 ± 0.47^a	10.78 ± 0.76^a	9.46 ± 0.70^a	1.29	0.282
		26–50	6.66 ± 0.70^a	7.66 ± 0.59^a	7.46 ± 0.68^a	0.46	0.631
Exch K	ppm	0–25	28.65 ± 1.53^a	134.76 ± 27.13^b	38.41 ± 4.73^a	28.7	< 0.001
		26–50	19.38 ± 1.36^a	114.80 ± 23.52^b	33.67 ± 3.93^a	30.41	< 0.001
Total N	%	0–25	0.39 ± 0.03^a	0.23 ± 0.03^b	0.16 ± 0.01^b	17.32	< 0.001
		26–50	0.19 ± 0.01^a	$0.17 \pm 0.03^{a,b}$	0.12 ± 0.01^b	5.52	0.006
SOC	%	0–25	4.34 ± 0.30^a	2.78 ± 0.35^a	1.75 ± 0.17^b	14.41	< 0.001
		26–50	1.88 ± 0.12^a	2.20 ± 0.36^a	1.24 ± 0.11^b	4.63	0.013
SOC stocks	Mg ha^{-1}	0–25	126.77 ± 3.01^a	124.72 ± 12.68^a	79.38 ± 5.22^b	16.62	< 0.001
		26–50	89.23 ± 3.78^a	$87.61 \pm 6.60^{a,b}$	71.36 ± 3.78^b	3.29	0.044

3.4. Effect of the Interactions between Plant Communities and Soil Depth on Spore Density and root Colonization of AMF

The interaction between plant communities and soil depth was significant ($p < 0.05$) for root colonization. Spore density differed significantly among plant communities ($p = 0.014$), but there was no significant difference, either with soil depth ($p = 0.093$), or in the interaction between plant community and soil depth ($p = 0.450$). There were significant differences in root colonization among the factors and their interaction (Table 4).

Table 4. Effect of the interactions between plant communities and soil depth on AMF spore density and root colonization (1-way ANOVA, $\alpha = 0.05$, $n = 130$).

		AMF spore density (100 g^{-1} dry soil)		AMF root colonization (%)	
Source	d.f.	F	p	F	p
Plant communities	2, 127	4.387	0.014	46.802	< 0.001
Soil depth	1, 128	2.868	0.093	32.121	< 0.001
Plant community*Soil depth	2, 124	0.803	0.450	16.268	< 0.001

3.5. Correlation between Spore Density and Root Colonization of AMF, with Soil Chemical Property and Soil Carbon Stock

Spore density was positively and significantly correlated with the degree of root colonization, and both in turn were significantly positively correlated with OC, TN and soil carbon stock (SOC) (Table 5).

Table 5. Correlation between spore density and root colonization of AMF with soil chemical properties and soil carbon stocks (SOC).

Variables	AMF Spore Density (100 g^{-1} dry soil)		AMF Root Colonization (%)	
	Pearson r	p	Pearson r	p
Root colonization	0.215	0.014	--	
pH	−0.021	0.810	−0.159	0.071
EC	−0.048	0.584	−0.004	0.965
Avail. P	0.007	0.938	0.156	0.076
Exch. K	−0.053	0.546	−0.085	0.337
SOC content	0.256	0.003	0.404	< 0.001
TN	0.288	0.001	0.403	< 0.001
SOC stocks	0.263	0.003	0.373	< 0.001

4. Discussion

4.1. Differences in AMF Spore Density, Root Colonization and AMF Structures, between Plant Communities and at Different Soil Depths

The original species of the dry Afromontane forests are being overtaken by disturbance-indicator species such as *Opuntia ficus-indica* [46]. Earlier work by Aynekulu et al. [30] found that mean tree densities in the *Jupr-Mase*, *Ptst-Ceaf* and *Capu-Opfi* communities were 731, 519 and 287 stem ha^{-1} respectively, while mean basal areas across the same three communities in turn averaged 11.1, 7.7 and 5.0 m^2 ha^{-1}. We found the lowest mean spore densities in the *Capu-Opfi* community. Disturbance alters plant species composition or the plant community, and these in turn are associated with differing degrees of spore density and root colonization.

Spore density in tropical regions is strongly dependent on vegetation type, as supported by our results. Zhao et al. [47] found that spore density ranged from 50 to 1908 in 100 g^{-1} dry soil across three forest types (i.e., primary, secondary and limestone forest) in the tropical ecoregions of SW China. Similarly, Birhane et al. [4] found up to 69 spores 100 g^{-1} dry soil in dry deciduous wood lands, while Birhane et al. [48] reported up to 2980 spores per 100 g of dry soil in a dry Afromontane forest. Moreover, Dobo et al. [49] found spore densities ranging from 6 to 1010 from different plant species in three land-use types in southern Ethiopia. Low spore density has been recorded in grazing land because of high soil disturbance [50]. Due to the fact that AMF can provide favorable services to almost all terrestrial ecosystems, including agroecosystems [51], it is crucial that land management decisions consider what impact there will be on their density and diversity.

Apart from the impact of disturbance, lower spore density may also be due to losses of host plants. This may be attributed to the loss of pioneer plants, many of which serve as primary host plants [3,52], but also to phenology and seasonality of the plant communities. The higher spore density in *Jupr-Mase* and *Ptst-Ceaf* communities could also reflect higher ground coverage. The function of different AMF species in ecosystems is still unknown and needs further investigation, but it is assumed that a high diversity of AMF species is likely to be more beneficial for a plant community [52].

We found that plant communities, distinguished by differing levels of disturbance, have different AMF root colonization. Communities with higher plant density increase root colonization. Dense plant cover also produces higher litter fall and more root biomass for maintaining a diverse AMF community. Plant density is directly associated with AMF diversity, since rhizosphere of mature tree has high percentage of spores. The density and diversity of AMF increased with increasing tree canopy cover, especially in deciduous forest, because plants in this habitat may more efficiently convert the higher solar inception into photosynthetic products which are directed to roots, providing a carbon source for AMF [52]. Boddington and Dodd [53] reported that AMF availability and activity can be affected by vegetation removal, which significantly decreases spore density and AMF root colonization, so human-induced changes aboveground have cascading implications belowground [54]. Land use change has been previously shown to reduce AMF spore density and root colonization [55], due to the breakdown of AMF hyphae network in the process of cultivation and grazing, which leads to a reduction in mycorrhizal colonization of the root [56].

All AMF structures, such as hyphae, vesicles and arbuscules were present within the roots of plants in our study area. Moreira-Souza et al. [57] obtained similar results for all the AMF structures present in the roots of the species they studied. Hyphal colonization (HC) was the highest followed by mycorrhizal hyphal colonization (MHC), vesicular colonization (VC) and arbuscular colonization (AC). The results were in line with the finding of Belay et al. [58], who observed higher hyphae and vesicular structural colonization than all the other structures. This might be because hyphae are the primary structures of AMF and can exist for months or years. In our study, the relative proportions of arbuscular and vesicular colonization were similar. Yet, in another study, Sarkar et al. [52] recorded a vesicular colonization rate of 26.6–33.0%, this high rate perhaps being because vesicles develop to accumulate storage products in many AMF associations and remain in the roots for months or years, whereas the arbuscules are short-lived [52].

We found no difference in spore density between soil depths, consistent with [4]. AMF species were found to be associated with plants at sites with different soil structures [59]. AMF composition changed with soil depth and the spore density decreased with increasing soil depth, corroborating previous results [59,60]. Low availability of oxygen in deeper soil zones can reduce spore density

[61,62]. Spore density and root colonization by AMF were higher in the topsoil (0–25 cm) than the subsoil, suggesting higher biological activity in this zone of the soil profile. This could be due to its proximity to the litter layer on the soil surface beneath different canopy layers, with differences between the communities reflecting different rates of biomass production and a more amenable micro-climate in the better vegetated communities [63].

Other studies from the highlands of Ethiopia and elsewhere have shown that exclosures can improve ecosystem services such as soil and aboveground carbon sequestration. On the other hand, increased vegetation cover in exclosures would further reduce erosion and increase organic matter input into the soil [64]. Disturbance affects soil quality through the process of biomass removal, limiting organic inputs into the soil [65]. Evidently, the degree of soil fertility affected the aboveground carbon density and caused a significant difference between the two soil depths. The total soil carbon stock recorded in Hugumbrda forest was 202 Mg ha^{-1} (Table 3). Soils that form under forests tend to accumulate high levels of soil organic carbon near the surface and have lower carbon levels in the subsoil [66,67]. Therefore, it is likely that the activity of AMF is an important factor in regulating the cycling of nutrients in ecosystems [68]. Symbiotic AMF depend heavily on living plants for carbon, so will be impacted by any process which alters the belowground carbon allocation [8].

4.2. Correlation of AMF Spore Density and Root Colonization with Soil Property and Soil Carbon Stock

Spore density was positively and significantly correlated with root colonization, as has been reported by others [48,69–71]. However, some researchers indicated no correlation between spore density and root colonization [15,49]. The irregular spatial distribution of AMF spores and the complex structure of the underground root component should be considered as the most important factors affecting AMF spore density that could contribute to variable rates of AMF colonization between plants [16,47]. The relationship of spore density and root colonization with different soil characteristics is a result of the interactions between them and could be specific for each case [70].

According to MOA and ATA [72], almost all soil types in the Tigray region of northern Ethiopia are classed as either neutral or moderately alkaline. Some AMF species are found only in acidic or alkaline soils, but others occur in both [8]. Variations in soil pH may alter the concentration of many nutrients and toxic ions in the soil and thereby affect the development and function of AMF. In the present study, however, both AMF spore density and root colonization were negatively correlated with soil pH, which could hinder spore germination and root colonization [73]. Ghorbani et al. [74] and [75] also observed that EC had a strong negative correlation with the number of spores and percent root colonization. The influence of organic matter on arbuscular mycorrhiza showed varying results, indicating variable response on plants and fungi [76].

Exchangeable K was negatively correlated with spore density and root colonization. Similar results have been shown by [48,77,78], although Gaur and Kaushik [79] found a positive correlation between exchangeable K and spore density. A negative correlation between exchangeable K and root colonization was also reported by Ardestani et al. [80], in their pot experiment to determine the effect of different K concentrations on the root colonization in maize. The inverse relationship between AMF root colonization and exchangeable K could be attributed to a reduction in the ability to develop arbuscles in higher concentrations of K [81]. Soil total nitrogen was positively correlated with both root colonization and spore density in the plant communities. Amal et al. [82] and Don-Rodrigue et al. [83] also found such positive correlations, although others [84,85] found negative correlations between TN and both spore density and root colonization. Generally, high levels of nutrient elements in soil have been reported to decrease mycorrhizal colonization [86]. Specifically, high soil N content has been shown to suppress AMF spore density and root colonization [85], while low soil nitrogen content has been reported to induce root colonization [87].

Soil organic matter concentration was positively correlated with both AMF spore density and root colonization, as has been shown by Boddington and Dodd [53], Khanam et al. [77] and Sivakumar [70]. AMF are thought to add to the soil carbon pool [88] and in long-term may increase carbon storage [89], because AMF colonization allows plants to increase their photosynthetic rate by

up to 30%, thus fixing more C [90]. The overall contribution of AMF to soil carbon storage may depend significantly on the hyphae type produced, the residence time of accumulated hyphal residues, the production of glomalin and the role played by AMF in the stabilization of soil aggregates [91].

5. Conclusions

In our study in a threatened Afromontane forest, all plant communities, regardless of disturbance level, formed associations with AMF. However, the AMF spore density and root colonization were significantly different between communities, probably due to both the plant host specificity of AMF and the different levels of disturbance between the plant communities. Root colonization was significantly different between different soil depths, whereas spore density did not vary with depth. This result showed that the presence of dense fine roots in the upper surface, coupled with lower levels of disturbance, resulted in a higher percentage of root colonization. Likewise, soil organic carbon stocks were higher in the topsoil and in plant communities with lower disturbances. This implies that dense vegetation cover, lower disturbance and the presence of higher biomass of fine roots might facilitate the exchange of carbon with AMF and enhance carbon storage in soil. Conserving remnant dry Afromontane forests is valuable for mitigating climate change, and preserving belowground biodiversity, such as AMF.

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