Soil physicochemical property and arbuscular mycorrhizal fungi resilience to degradation and deforestation of a dry evergreen Afromontane forest in central Ethiopia

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Abstract: We investigated the soil physicochemical property and arbuscular mycorrhizal fungi (AMF) resilience to degradation and deforestation of the Chilimo dry evergreen Afromontane forest. Topsoil (1-10cm) physicochemical property was determined across four land uses, viz. natural forest (NF), shrubland (ShL), cropland (CrL), and grazing land (GrL). AMF spore abundance (SA) and AMF infectivity of these land uses were also determined. One-way ANOVA results indicated that most soil physicochemical variables were significantly affected by land-use change. According to the nonmetric multidiamentiaonal scaling ordination result, soil physicochemical property was found to be resilient to degradation (NF-ShL conversation) but not deforestation (NF-CrL or NF-GrL conversions) of Chilimo forest. Whereas SA was found to be resilient to both the degradation and deforestation, infectivity was resilient only to NF-CrL conversion. Although our results did not show a similar pattern in soil property, SA and AMF infectivity resilience due to Chilimo forest degradation and deforestation, both the soil physicochemical property and AMF infectivity were found to be not resilient to NF conversion to GrL. Hence, based on our results, it can be concluded that AMF inoculation could be more beneficial to NF restoration if the planting sites are in GrL. However, in the future, the AMF community composition of these four land uses should be determined morphologically and molecularly from field soil and trap culture so that AMF resilience to DAF deforestation and degradation is better understood.

Keywords: arbuscular mycorrhizal fungi (AMF), Chilimo forest, deforestation, dry evergreen Afromontane forests, landuse change, resilience

1. Introduction

The term resilience was first applied in ecology by Holling (1973) to refer to the ability of an ecosystem and/or its components to resist change due to disturbance. Its curent ecological usage include resistance to or recoverery from disturbance or both (Hodgson et al., 2015). Forest degradation and deforestation could significantly modify soil physichochemical and biological properties affecting planted seedlings survival, establishment, and forest restoration (Suding et al., 2004). Hence, knowledge on soil physichochemical property and arbuscular mycorrhizal fungi (AMF) resilience is important for planning forest restoration projects.

Dry evergreen Afromontane forests (DAF) are forests of the African highlands that are characterized by the tree specis such as *Juniperus procera*, *Olea europaea*, *Podocarpus falcatus* and *Acacia abyssinica* (Friis et al., 2010). These forests are predominantly found in Ethiopia and comprise the largest proportion of the world's dry tropical and subtropical forests (Lemeniha and Itanna, 2004). Most of the DAF have been deforested or have severely degraded and hence, their restoration is identified to be a national ecosystem restoration priority in Ethiopia (MEFCC, 2018). The recent article published on Nature journal has also recognized DAF restoration to be a global ecosystem restoration priority (Strassburg et al., 2020).

Available data indicate that DAF regeneration from soil seed bank and see rain is hardly possible (Teketay 1995, 2005; Teketay and Anders, 1995). Therefore, DAF restoration greatly depends on the planting of DAF characterstic tree/shrub seedlings (Aerts et al., 2006). Therefore, owing to their active restoration need, detrmining the soil physichochemical property and AMF resilience to the degradation and/or deforestation of DAF is particularly crucial.

Arbuscular mycorrhizal fungi of the subphylum Glomeromycotina (Spatafora et al., 2016) form a symbiotic relationship with most terrestrial (Brundrett, 2009) and possibly, aquatic

(Moora et al., 2016) plants. AM fungi colonize the root cortex and develop extraradical mycelia (ERM) which are very extensive; as long as 4.2 km per gram of soil (Leake et al., 2004). The ERM permeate into microsites of mineral soil (Finlay, 2008; Barea et al. 2011) and can extend to the litter layer (Gui et al., 2017) to significantly increase root access to essential nutrients (Went and Stark, 1968; Simard and Austin, 2010; Smith et al., 2011; Soka and Ritchie, 2014) and moisture (Gianinazzi et al., 2010). Due to these and other ecological functions, AMF are considered to be important actors in forest restoration process (Asmelash et al., 2016; Neuenkamp et al., 2018).

Arbuscular mycorrhizal fungi communities composition could be greatly affected by forest clearing (Xu M et al., 2017; Sepp et al., 2018), degradation (Gavito et al., 2008), or land-use intensification (Oehl et al., 2003; Moora et al., 2014). However, AMF are ubiquitous, found almost in every soil (Abbott and Robson, 1991; Brundrett and Abbott, 2002; Smith and Read, 2008) including mine soils (Wang, 2017). AMF abundance (Zangaro et al., 2013; Stürmer and Siqueira, 2011; Birhane et al, 2018), species richness (Picone, 2000; Zhang et al., 2004; Stürmer and Siqueira, 2011), and species composition (Johnson and Wedin, 1997; Picone, 2000; Zhang et al., 2004; Violi et al., 2008; Carrillo-Saucedo et al., 2018) have also been found be to resilient despite forest degraration and deforestation. Hence, AMF inoculation could be merited for ecosyetems that exibit low resilience to degradation and deforestation.

Previously there have been a few (Delelegn et al. 2017; Birhane et al., 2018, 2020) studies carried out regarding AMF resilience to DAF degradation and deforestation. However, there has not been any comprehensive study to evaluate soil physichochemical and AMF resilience to the degradation and deforestation of the the Chilimo dry evergreen Afromontane forest. Therefore, in this study, we detrmined soil physichochemical property and AMF spore

abundance and AMF infectivity along the Chilimo forest degradation gradient and evaluated resilince. Our hypotheses for this study are;

H1*: soil physichocemical property is not resilient to the degradation and deforestation of the Chilimo dry evergreen Afromontane forest*

H2*: AMF spore abundance is not resilient to the degradation and deforestation of the Chilimo dry evergreen Afromontane forest*

H3*: AMF infectivity is not resilient to the degradation and deforestation of the Chilimo dry evergreen Afromontane forest*

2. Methodology

2.1. Study area

The Chilimo-Gaji forest, commonly called the Chilimo forest, is one of the few DAF in central Ethiopia. It is located 97 km west of the capital Addis Ababa, 7 km north of Ginchi town, close to the main Addis Ababa-Ambo road. Situated within the Dandi and Ejersa lafo districts of the Oromia administrative region, Chilimo forest is geographically located within 38.09° to 38.2°E longitude and 9.04° to 9.095°N latitude (Fig. 1). The forest is currently found within 2340-2960 m elevation and it comprises of 12 patches that are managed through the participatory forest management (PFM) scheme. The mean annual temperature ranges between 15 and 20 °C while the mean annual precipitation is 1264 mm (Tesfaye et al., 2016). Based on the available literature, the soil types of Chilimo are mainly Vertisols, Luvisols, and Cambisols (Soromessa and Kelbessa, 2014).

Figure 1: A map showing land use features of Chilimo and location of the sample plots

The name Chilimo, to mean darkness, reflects the denseness of the forest in the past (Mohammed and Inoue, 2012). However, in only 42 years, around 54% of the forest has either been deforested and become settlement (1%), cultivated land (26%) or bare land (9.8%) or has been degraded to be changed to shrubland (17.3%) (Tolessa et al., 2017). Currently, the Chilimo forest lays on 1952 hectare of land (Tolessa et al., 2017) of which, around 400 ha comprises of *Cupressus lustanica*, *Eucalyptus saligna*, *E. camaldulensis,* and *Pinus patula* plantation established 30 years ago (Mohammed and Inoue, 2012; Tesfaye et al., 2016).

2.2. Soil sampling

In mid-February 2019, a reconnaissance survey was carried and sampling design determined. In this study, since the four land uses were not adjoined and GrL were present in dispersed patches, stratified systematic sampling (Kershaw Jr. et al, 2017) was employed. Hence, NF and CrL were stratified in to low (2400-2430m), mid (2600-2630m), and high (2800-2830m) elevations while the ShL was stratified in to low (2400-2430m), mid (2500-2530m), and high (2600-2630m) elevations. Moreover, the GrL was stratified in to three where, GrL1a is the patch found at 2800-2830 m elevation and with *Pennisetum clandestinum* the dominant cover, GrL2a is the patch found at 2400-2430 m elevation and with *Pennisetum clandestinum* the dominant cover, and GrL3b is the patch at 2400-2430 m elevation but with *Pennisetum sphacelatum* being the dominant cover (Table1; Fig. 2; S3).

Data collection took place from late-February to early-March 2019, during the dry season. From each stratum or patch of the four land-use gradients, composite soil samples were collected systematically from five plots (1m x 1m) spaced 50 m apart and along the contour (guided by the google map). Hence, the total number of composite soil samples collected were

60 (15 per land use). Topsoil samples (0-10cm), in which AMF are mainly found (Oehl et al, 2005), were collected from the four corners of each plot using a soil auger. Then soil samples were sieved using 2.5 mm sieve at the field until 1kg sieved soil samples were obtained. The soil samples were taken to Addis Ababa University and air-dried for a few days. Air-dried soil samples were kept at room temperature (18-21°C) until AMF abundance and other physicochemical properties were determined. Meanwhile, 50g soil subsamples were used for AMF abundance while 500g subsamples were used for the greenhouse bioassay and the remaining 425g subsamples for soil physicochemical property determination.

Table 1: The characteristics of the four land-use gradients studied.

Land use	**Description**	**Land management**
NF	land with >80% canopy cover and dominated by the trees; *Juniperus procera*, *Podocarpus falcatus*, *Olea europaea* subsp. *cuspidata*, *Allopyllus abyssinicus,* and *Prunus africana*	Selective cutting
ShL	land that is >50% covered by shrubs or shrub/trees mainly *Carissa spinarum, Dovyalis abyssinica, Maytenus gracilipes*, *Olinia rochetiana*, *Osyris quadripartita, Rhus glutinosa*, *Scolopia theifolia* and with non-canopy forming (<5 m tall trees) interspersed	Selective cutting, grazing
CrL	land cultivated with mainly wheat but also *Teff* (*Eragrostis tef*), common bean, field pea, lathyrus	Plowing, maybe chemical fertilizer application
GrL	abandoned farmlands or grasslands open for grazing that are dominantly covered with *Pennisetum clandestinum* (a) or *Pennisetum sphacelatum* (b)	Overgrazing

NF=Natural forest, ShL=Shrubland, CrL=Cropland, and GrL=Grazing land.

Figure 2: Land-use gradients studied. NF=Natural forest, ShL=Shrubland, CrL=Cropland, GrL=Grazing land covered with *Pennisetum clandestinum* (a) or *Pennisetum sphacelatum* (b)

2.3. Soil bulk density, texture, pH and EC

Bulk density, soil texture, pH, and EC were determined at ecology and plant ecophysiology laboratory at the Addis Ababa University, Ethiopia. Bulk density was determined using a modified clod method (Blake, 1965). Soil texture was determined by using ASTM 151H soil hydrometer (g/ml scale) and following Day (1965), Bouyoucos, (1962), and Anderson and Ingram (1993) for soil dispersion, hydrometer reading, and percent texture computation respectively. pH and EC were determined by mixing 20g of sieved soil subsamples with 50 ml distilled water (1:2.5 (w:v)) following Cottenie (1980). The soil mixture was initially shacked for about 5 minutes on a shaker and allowed to settle overnight (20 hours) then just before pH measurement, the samples were very gently shacked by hand and pH of the soil suspension measured using a digital pH meter with a glass electrode (Hi9024, microcomputer pH meter). Then, soil samples were allowed to further settle for 30 minutes and EC was determined on a carefully decanted supernatant using a digital EC meter (Sx713 cond/TDs/Sal/Res meter).

2.4. Total nitrogen, available phosphorus, organic matter, cation exchange capacity, P:N and C:N

These variables were determined at Debrezeit Agricultural Research Center, Ethiopia. Total nitrogen (N) was determined on 2g air-dried soil subsamples by sulfuric acid-salicylic acid digestion, distillation into boric acid, and titration of the resulting solution with hydrogen chloride (Bremner and Mulvaney, 1982). Plant available phosphorus (P) was determined on 1g air-dried soil subsamples after Bray-II extraction (Bray and Kurtz, 1945) and spectral absorbance measurement (882nm) of the resulting supernatant solution by using a spectrophotometer. Organic carbon (OC) was determined by oxidizing 1g of air-dried soil subsamples with potassium dichromate in sulphuric acid medium, subsequently adding orthophosphoric acid and

titrating the resulting solution with ferrous ammonium sulfate (Walkley and Black, 1934). Organic matter (OM) was computed by multiplying OC by 1.724; the conversation factor (Anderson and Ingram, 1993). Cation exchange capacity (CEC) was determined on 10g air-dried soil subsamples according to Chapman (1965). Hence, soil subsamples were first saturated with ammonium using ammonium acetate. Then using potassium chloride, ammonium was leached out and the leachate was distilled into boric acid. Finally, the resulting solution was titrated with sulfuric acid and CEC was computed. P:N and C:N were determined per plot by dividing P (ppm) and C (%) values with the respective N (%) values.

2.5. AMF spore abundance

Spore abundance was determined by taking 50g air-dried soil subsample of each composite soil sample. Spores were extracted from soil by wet-sieving (1mm, 180 µm, 90 µm, and 53 µm sieve sizes) followed by density gradient centrifugation in 50% sucrose (Brundrett et al., 1996). Extracted spores were counted on a 90mm plastic Petri dish according to INVAM protocol (https://invam.wvu.edu/methods/spores/enumeration-of-spores) using a light stereomicroscope (Swift stereo 80) at 2x magnification. SA in the number of spores per 50 g soil sample was computed from the average spore numbers of 40 random fields of observations per Petri dish. The ocular field diameter of the microscope was determined to be 9mm and hence, 100 observations were needed to cover the 90 mm Petri dish. Rarely, spores covered with soil, clusters of spores, and sporocarps were observed and were also counted. SA values per 50 g were finally converted to SA (g^{-1}).

2.6. Greenhouse bioassay

197 AMF infectivity was determined by the greenhouse bioassay method (Moorman and Reeves,
198 1979; Abbott and Robson, 1991) using the INVAM recommended host plant, *Zea mays* L.
199 (https://invam.wvu.edu/methods/infectivity-assays/mean-infection-percentage-mip). *Zea mays*
200 (Melkassa-4 variety) seeds were carefully disinfected with 5% household bleach for 10 minutes
201 and allowed to germinate on filter paper. Then, one germinated seed was sawn on a 500g soil
202 subsample collected from each plot (S1). After growing the *Zea mays* for 6 weeks, the shoot was
203 cut off and the soil carefully washed off the roots to prepare them for AMF root colonization
204 determination. AMF colonization was determined following the ink and vinegar technique as
205 described by Vierheilig et al. (1998) and using black Hero fountain pen ink (Asmelah et al.,
206 2020). Roots were cleared in 10% KOH in an autoclave for 10 minutes (Brundrett et al, 1996)
207 and cleared roots were stained overnight at room temperature in 5% ink (black Hero fountain pen
208 ink, made in China) in vinegar (common food grade white vinegar or 5% acetic acid). De-
209 staining was done by rinsing the stained root in tap water acidified with a drop of vinegar for a
210 minimum of 20 minutes and further rinsing it in tap water until colonization is determined in a
211 few minutes or hours (Vierheilig et al., 1998). AMF root colonization was determined by the
212 gridline intersection method (Giovanetti and Mosse, 1980) by observing roots under a light
213 stereomicroscope (CETI Steddy Stereo Binocular Microscope) at 5.5x magnification (S2).
214

215 *2.7. Data analysis*

216 Nonmetric multidimensional scaling (NMDS) using similarity ratio and ward method was
217 plotted to determine the soil physicochemical property resilience. NMDS was also plotted to
218 explore which physicochemical variables discriminated against the land uses. One way ANOVA
219 was computed to know if forest degradation and deforestation had a significant effect on each of
220 the soil physicochemical variables (%sand/silt/clay, BD, pH, EC, N, P, OM, CEC P:N and C:N).

When significant ($p \leq 0.05$) effect was present, mean values per land use were compared by Tukey's HSD ($p \leq 0.05$) and Dunn-Bonferroni ($p \leq 0.05$) tests after parametric and nonparametric one-way ANOVA respectively. Nonparametric Kruskal Wallis tests were carried out to know the effect of forest degradation and deforestation on fungi spore abundance (SA) and infectivity. When a significant effect was present, Dunn-Bonferroni ($p \leq 0.05$) pairwise mean comparison was made between land uses. For each land use separately, nonparametric Kruskal Wallis test and Dunn-Bonferroni ($p \leq 0.05$) pairwise mean comparisons were also carried out to know the effect of elevation and location on SA and infectivity. Spearman's rank correlations between SA, infectivity, and the various soil physicochemical variables was computed to determine the impact of soil physicochemical property on SA and infectivity. R version 3.6.1 was used to plot NMDS ordinations. SPSS version 20 was used to compute one-way ANOVA, mean pairwise comparisons, and spearman's rank pairwise correlations. Bar graphs were plotted by using SYSTAT version 13.1.

3. Results

3.1. The soil physicochemical property across the land-use gradients

The NMDS ordination depicted that soil physicochemical property was resilient to forest degradation (NF to ShL conversion) but not to deforestation (both NF to CrL or NF to GrL conversions). It also indicated that within the grazing land, the soil physicochemical property had low similarity between GrL(a) and GrL(b) (Fig. 3a). N, EC, pH, OM, sand, and CEC positively contributed to the discrimination of NF and ShL from CrL and GrL while BD, C:N, Clay, and silt contributed negatively. P and P:N had no role in the discrimination of NF and ShL from CrL and GrL but were responsible for the discrimination of Grl3b against GrL1a and GrL2a (Fig. 3b).

The one-way ANOVA results showed that except P, P:N and C:N, all the other soil physicochemical variables were significantly affected by the land-use change (Table 2). Bulk density significantly (p<0.05) increased both by degradation and deforestation. The pH ranged between slightly acidic (NF and ShL) to acidic (CrL and GrL) and it significantly (p<0.05) reduced by degradation and deforestation. EC and N decreased by degradation but a significant (p<0.05) reduction resulted only due to deforestation. P showed a variable trend but there were no significant (p<0.05) degradation and deforestation effects. The P:N increased both by degradation and deforestation but not significantly (p>0.05). The OC (OM) were not affected by degradation but decreased significantly (p<0.05) by deforestation. The C:N, although not significantly (p>0.05), decreased by degradation while it, on the contrary, increased by deforestation. The CEC significantly (p<0.05) increased due to degradation but it more or less remained similar despite deforestation (Table 2).

Figure 3: NMDS ordination plots showing the resilience of the soil physicochemical property to forest degradation and deforestation. The soil physicochemical properties of natural forest and shrubland were similar while they were distinct between natural forest, cropland, and grazing land. The soil physicochemical properties were also different within the grazing land with GrL3b, i.e., plots 51-55, discriminated by P and P:N.

Table 2: Mean (±SE) soil physicochemical variables across the land-use gradients.

	Land uses				**ANOVA**	
Variables	**NF**	**ShL**	**CrL**	**GrL**	**F**	**Chi-square**
BD	0.61±0.02^{c}	0.71±0.02^{b}	0.88±0.01^{a}	0.83±0.02^{a}	46.951***	-
pH	6.64±0.08^{a}	6.25±0.09^{b}	5.70±0.06^{c}	5.36±0.06^{d}	54.852***	-
EC	0.21±0.02^{a}	0.19±0.03^{a}	0.07±0.004^{b}	0.08±0.01^{b}	-	33.8622***
N	0.35±0.01^{a}	0.44±0.10^{a}	0.15±0.01^{b}	0.20±0.02^{b}	-	38.962***
P	15.44±2.13	17.18±3.61^{ns}	15.10±3.77^{ns}	19.96±3.68^{ns}	0.434	-

	ns					
P:N	44.90±6.32 ns	53.10±14.45 ns	120.76±35.18 ns	124.72±29.51 ns	-	5.909
OM	11.52±0.52 a	11.08±0.75[a]	5.99±0.60 [b]	7.46±0.48 [b]	20.791***	-
C:N	19.26±0.76 ns	18.26±1.68[ns]	24.43±3.39[ns]	22.79±1.48[ns]	1.977	-
CEC	23.21±1.27 b	30.75±1.39[a]	21.69±1.27[b]	24.59±1.41[b]	8.845***	-
Sand	80.60±1.24 a	75.25±0.85[b]	64.29±1.16[c]	64.35±1.17 [c]	53.495***	-
Silt	12.10±0.74 b	15.30±0.60 [ab]	16.72±0.99 [a]	12.32±1.53[b]	-	14.970***
Clay	7.30±1.00[b]	9.45±0.54[b]	18.99±1.89[a]	23.33±2.24[a]	-	40.510***

Means with different letters are statistically ($p \leq 0.05$) significant after Tukey or Dunn Bonferroni tests. "ns" indicates there was no land use effect.*** significant at $p \leq 0.001$.

3.2. The AMF spore abundance (SA) and AMF infectivity across land uses

Spore abundance and AMF infectivity ranged 3.4-25.3g^{-1} and 12.0-82.5% respectively (S3) and land-use change had a significant effect on both SA (Chi-square =35.403, p= 0.0001) and infectivity (Chi-square =23.245, p= 0.0001). However, the effect of forest degradation and deforestation was not necessarily similar to both SA and infectivity. Hence, both forest degradation and deforestation had significant effects on SA whereas deforestation did not have a significant effect on infectivity when NF converted to CrL (Figure 4). The highest SA was in the GrL and the lowest in NF. SA in the ShL, CrL, and GrL were significantly 92.7%, 105.3%, and 148.9% greater than the SA in the NF. The highest infectivity, contrary to SA, was in the NF while the lowest was in ShL. Infectivity of the NF was significantly 56.4% and 52.2% higher than the infectivity of the ShL and GrL respectively. Infectivity of the NF, however not significant, was also 11.0% higher than the infectivity of CrL (Figure 4).

Figure 4: Mean SA and infectivity across the land uses. Means with significant ($P \leq 0.05$) differences are indicated with different letters. NF=Natural forest, ShL=Shrubland, CrL=Cropland, GrL=Grazing land.

Elevation and/or location did not have significant effects on SA in all land uses but were found to have an impact on the infectivity of ShL and GrL soils (Table 3). In the shrubland, the highest infectivity was found for ShL1 found at the low elevation while the lowest was found for ShL2 found at the mid-elevation and very closely located to ShL1 compared to ShL3 which is found at the high elevation and very far away from both ShL1 and ShL2. The infectivity of ShL1 soil was significantly and 140.23% higher than the infectivity of the ShL2 soil. It was also 12.1% higher than the infectivity of ShL3 soil, but not significantly (Fig. 5-I). In the grazing land, the highest infectivity was found for GrL1a which is grazing land located at 2800 m elevation and very far away from the remaining grazing lands (GrL2a and GrL3b) which were both located at 2400 m elevation, comparatively close to each other, but with different vegetation cover. The infectivity of GrL1a soil was significantly and 123.35% higher than the infectivity of GrL3b soil. Although not significantly, it was also 12.77% higher than the infectivity of GrL2a soil (Figure 5-II).

Table 3: Effect of elevation and location on AMF spore abundance and infectivity across the land uses

Statistics	**Spore abundance**				**Infectivity**			
	Natural forest	**Shrub land**	**Cropland**	**Grazing land**	**Natural forest**	**Shrub land**	**Cropland**	**Grazing land**
Chi-square	1.044	0.606	2.240	3.440	5.049	8.340	1.340	8.960
df	2	2	2	2	2	2	2	2
P-value	0.593	0.739	0.326	0.179	0.080	0.015*	0.512	0.011*

*Significant at $P<0.05$

Figure 5: Mean infectivity across elevation and location in Shrubland (I) and Grazing land (II). ShL1=Shrubland at 2400m elevation, ShL2 at 2500m, and ShL3 at 2600m; GrL1a=Grazing land at 2800m elevation and with *Pennisetum clandestinum* the dominant cover, GrL2a at 2400m with grass *Pennisetum clandestinum* the dominant cover, and GrL3b at 2400 m but with *Pennisetum sphacelatum* being the dominant cover.

3.3. The correlation of spore abundance (SA) and infectivity with the measured soil physicochemical variables

AMF spore abundance and infectivity were weakly, significantly, and negatively correlated (r_s= -0.29, p< 0.05). SA was significantly correlated with most of the soil physicochemical variables determined. The significant positive correlations were, SA//BD (r_s = 0.68, p<0.01), SA//P:N (r_s = 0.37, p< 0.01), SA//C:N (r_s= 0.35, p< 0.01), SA//silt (r_s= 0.26, p< 0.05), and SA//clay (r_s= 0.53, p< 0.01) while the significant negative correlations were, SA//pH (r_s = -0.57, p< 0.01), SA//EC (r_s = -0.50, p< 0.01), SA//N (r_s = -0.56, p< 0.01), SA//OC or SA//OM (r_s = -0.48, p< 0.01), and SA//sand (r_s= 0.61, p< 0.01). Infectivity correlated significantly, only with pH (r_s= 0.39, p< 0.01), P:N (r_s= 0.26, p< 0.05), and clay (r_s= -0.27, p< 0.05). Whereas, P did not significantly correlate with neither SA nor infectivity, P:N significantly correlated with both SA and infectivity. Moreover, SA correlated with OC (OM) and C:N differently (Table 4).

Table 4: Spearman's rank correlation between SA, infectivity, and soil physicochemical variables

	SA	Inf	BD	pH	EC	N	P	OM	P:N	C:N	CEC	%Sand	%Silt	%Clay
SA	1.00													
Inf	**-0.29***	1.00												
BD	**0.68****	-0.11	1.00											
pH	**-0.57****	**0.39****	-0.70**	1.00										
EC	**-0.50****	0.25	-0.73**	0.72**	1.00									
N	**-0.56****	-0.001	-0.80**	0.67**	0.80**	1.00								
P	0.16	0.22	-0.01	0.16	0.19	-0.03	1.00							
OM	**-0.48****	0.08	-0.77**	0.66**	0.75**	0.79**	0.17	1.00						
P:N	**0.37****	**0.26***	0.37**	-0.14	-0.21	-0.52**	0.82**	-0.28*	1.00					
C:N	**0.35****	-0.02	0.22	-0.34**	-0.30*	-0.56**	0.22	-0.03	0.43**	1.00				
ECE	-0.07	-0.23	-0.27*	0.14	0.19	0.30*	0.07	0.231	-0.10	-0.14	1.00			
%Sand	**-0.61****	0.25	-0.69**	0.79**	0.65**	0.68**	0.14	0.60**	0.27*	-0.20	-0.42**	1.00		
%Silt	**0.26***	0.12	0.43**	-0.07	-0.33*	-0.32*	0.26*	-0.31*	0.15	0.42**	-0.04	-0.19	1.00	
%Clay	**0.53****	**-0.27***	0.60**	-0.79**	-0.59**	-0.63**	-0.23	-0.59**	-0.29*	0.10	0.40**	-0.93**	-0.07	1.00

SA=AMF spore abundance, Inf=infectivity, BD=bulk density, EC=electrical conductivity, OM=organic matter, N= total nitrogen, P= available phosphorus, CEC=cation exchange capacity.

4. Discussion

4.1. The resilience of soil physicochemical property to forest degradation and deforestation

The level of soil physicochemical property resilience to forest degradation and deforestation could potentially indicate the restorability of forest ecosystems (Schoenholtz et al, 2000). Therefore, the soil physichochemical resilience study we carried out for the highly threatened Chilimo dry evergreen Afromontane forest (Tolessa et al., 2017) was very timely. Previously, Tolessa and Senbeta, (2018) and Mammo et al. (2019) studied the physicochemical property of the natural forest (NF) while Tesfaye et al. (2016) determined soil fertility dynamics due to the degradation and deforestation of Chilimo forest by considering only N & OC. Our study, however, considered as much land-use gradient and physicochemical variables as possible. Hence, it is the first comprehensive soil physicochemical resilience study of the Chilimo forest.

The mean values of the soil physicochemical variables found in this study were in most cases not comparable to the mean values previously reported for Chlimo and other DAF (S4). The observed differences with the previous studies on the natural forest (NF) and cropland (CrL) of the Chilimo forest and environs could mainly relate to the differences in the season of data collection in the case of N & OC, or methodology in the case of BD & P, or the types of soil sampled in the case of Hailu et al. (2015). Total nitrogen and OC values of DAF soils were found to significantly vary due to season (wet vs. dry seasons) of soil sampling (Birhane et al., 2018) and it was also determined that P values of Olsen and Bray-II extractions could significantly vary for acidic or slightly acidic DAF ecosystem soils (Mamo et al., 2002). Moreover, while Hailu et al. (2015) sampled vertisol, only a few of our samples were vertic (S3). The difference in the BD values compared to Mammo et al. (2019), could most likely be related to the fact that we report BD determined from sieved soil samples with no gravel and less compaction while Mammo et al. (2019) reported values from in-situ samples.

Our result indicated that soil physicochemical property was resilient to the degradation but not to deforestation of the Chilimo forest. Similarly, Tesfaye et al. (2016) have observed that OC and N reduced due to the deforestation of the Chlimo forest. Lemenih et al (2005), Delelegn et al. (2017), and Birhane et al. (2018), similar to our results, had also reported the reduction of pH and soil nutrient levels due to degradation and deforestation of other DAF in Ethiopia. Therefore, it could be likely that DAF restoration is more challenging as the result of low soil physicochemical property resilience. However, this could be known if the soil functional resilience is evaluated by using DAF characteristic tree species. Lemenih et al (2005) evaluated soil functional resilience to deforestation by considering Maize yield and found that although there was low resilience of soil physicochemical property to deforestation, it was potentially resilient functionally for more than 25 years after deforestation.

359 Soil physicochemical property resilience could potentially predict resilience of AMF
360 community composition (Oehl et al. (2010). This is particularly possible in our case owing to the
361 fact that the landuse changes are associated with distinct vegetation cover and land management.
362 Hence, based on our results, we may consider AMF community composition to be resilient to
363 degradation but not deforestation of the Chilimo forest. Considering only pH and P the most
364 important soil chemical variables determining AMF community composition (Garcia de Leon et
365 al., 2018), we may conclude differently about the resilience of AMF community composition due
366 to degradation or deforestation. This is because while pH showed a marked change due to
367 degradation and deforestation, P did not change significantly in both cases. Schechter and Bruns
368 (2012) have remarked that soil physicochemical property resilience could be an important proxy
369 to AMF community composition if there is a wider difference in a physicochemical property.
370 Hence, it may not be possible to conclude whether or not the AMF community composition was
371 resilient to the deforestation of the Chilimo forest based on our soil physicochemical property
372 result alone. AMF community composition resilience could also be greatly dependent on the
373 AMF species pool. Hence, in areas where the species pool is dominated by generalists, AMF
374 community composition could potentially be resilient despite a significant soil physical and
375 chemical property changes (Hawkes and Keitt, 2015). Our results have indicated that degradation
376 (NF-ShL conversion) and deforestation (the NF-GrL conversion) of Chilimo forest have resulted
377 in a significant AMF functional changes (infectivity) and this could indicate that there were
378 considerable changes in AMF community composition possibly fast colonizer species
379 dominating the NF and CrL and the slow colonizers dominating the ShL and GrL (Oehl et al.,
380 2003). Moreover, from the factors which could determine infectivity, viz., host species, climate,
381 edaphic factors, and soil AMF community composition (Moreira et al., 2006), diffrences in

382 infectivity in our case may most likely be related to the soil factors and AMF communities. This
383 is because, we determined infectivity from trap culture of single host species grown in a similar
384 greenhouse microclimate.
385
386 *5.2.2. The resilience of soil AMF spore abundance (SA) and infectivity to Chilimo forest*
387 *degradation and deforestation*
388 The degradation and deforestation of the Chilimo forest have reduced the canopy cover to
389 exposed the soil to elevated temperature potentially resulting in soil moisture and heat stress. We
390 have also found substantial reductions in soil nutrients. Hence, the resilience (increase in SA) we
391 found despite forest degradation or deforestation could be related to the fungi stress avoidance
392 strategy. When fungi are stressed due to scarcity of carbohydrates, soil nutrients, moisture, and
393 heat among others, AMF sporulation increase to avoid the stress period at the resting phase, the
394 spore (Violi et al., 2008; Silva-Flores et al., 2019). On the contrary, AMF infectivity was not
395 resilient to the degradation and the conversion of natural forest to grazing lands. These results are
396 in perfect agreement with Abbott and Robson (1982) who, in their review, had demonstrated that
397 while SA could be considerably lower in virgin soils compared to disturbed soils, infectivity
398 could, on the contrary, be significantly higher. Previously, Birhane et al. (2018) have also
399 reported a similar increase and reduction of SA and infectivity respectively due to degradation of
400 a dry evergreen Afromntane forest in north Ethiopia. Hence, the fact that we found SA increase
401 along with the increase in soil nutrient stress gradient is corroborated. However, Delelegn et al
402 (2017) reported a reduction of SA due to DAF deforestation and degradation in north Ethiopia.
403 The change in infectivity, in the case of NF-GrL conversion, most probably, have
404 resulted due to soil physicochemical property-change-induced AMF community composition

shifts. In the case of NF-ShL, it could have resulted due mainly to vegetation and land management changes. It could as well be the case that some of the soil micronutrients such as zinc and copper not considered in this study have not been resilient to NF-ShL conversion and hence, resulted in AMF composition change (Xu X et al., 2017) and reduced infectivity. Moreover, despite the significant soil physicochemical property change due to NF conversion to CrL, the reduction in infectivity was very small and not significant. This may be related to the NF legacy effect (Fichtner et al., 2014; Hawkes and Keitt, 2015). Since most of the CrL sampled were converted from NF much recently compared to the ShL and GrL, AMF community composition and function may have been retained due to the NF legacy.

Our results have shown that within each land use, elevation and location did not significantly affect SA. This may indicate that land use was a much more important factor determining SA than elevation or any other related factor. Infectivity was also not affected by elevation and location in the NF and CrL but it was significantly affected in the ShL and GrL. The reason why infectivity was significantly low in ShL2 which is in very close proximity to ShL1 and at mid-elevation is not clear. However, it could be possible that soil factors not considered in this study may have played a role. However, in the case of GrL, the significantly low infectivity in GrL3b coincides with lower P and P:N values (Fig. 3b; Fig. 5). Hence, it could be possible that these soil factors are responsible. However, P stress could, on the contrary, promote mycorrhization (Gutjahr, 2014). Therefore, the infectivity reduction could most likely be related to *Pennisetum sphacelatum* dominance. Low P level in GrL3b may have resulted in *Pennisetum sphacelatum* dominance (Walter, 1985), and the *Pennisetum sphacelatum* dominance in return, could have significantly modified AMF species composition and thereby infectivity. The predominance of ruderal plants for a longer time in a given site has been

identified to be an important factor resulting in the predominance of certain AMF species and loss of some other AMF species and leading to a significant AMF species composition shift (Faggioli et al., 2019). The fact that we found *Pennisetum sphacelatum* to be comparatively with very low infectivity has an important implication in DAF restoration. This is because a substantial part of the DAF ecosystem is covered with the non-palatable Pennisetum spp. including *Pennisetum sphacelatum* potentially with low AMF infectivity and hence requiring AMF inoculation from the target forest to succeed with DAF restoration.

The SA found in this study, across the land uses (3.4-25.3 spores g^{-1}), was comparable to the SA reported (3.6-9.9) & (0.9-14.6) spores g^{-1} by Birhane et al. (2018) & (2020) for two of the remnant DAF of north Ethiopia. It was also comparable to the values (1.3-24.6 spores g^{-1}) reported from DAF nurseries of central and northern Ethiopia (Asmelash et al, 2020) but was much lower than the SA values reported (41.0-129.0 g^{-1}) by Delelegn et al. (2017) across landuses in the DAF ecosystem in north Ethiopia. This difference could, to some extent, be related to the difference in the lowest sieve size used to separate spores (53µm used in this study vs. 38 µm). Our results have shown that SA was significantly correlated with most of (+BD, -pH, -EC, -N, -OM, +P:N, +C:N, -Sand, +Silt, and +Clay) soil physicochemical variables while infectivity significantly correlated to few (+pH, +P:N, and -Clay) of the soil physicochemical variables. This is perfectly aligned with previous reports including the one by Silva-Flores et al. (2019). Relatively similar to our results, Birhane et al. (2018) also reported a slightly negative but statistically non-significant SA correlations (Pearson) with pH, EC, and soil nutrients (N, P, OC). However, Delelegn et al. (2017) reported that no correlation (Spearman) existed between SA and pH, P, and, contrary to our results, reported a positive and statistically significant correlation (spearman) of SA with OC and N.

We have found a negative SA//Infectivity correlation similar to the one reported by Moreira et al. (2006) and this correlation could have resulted due to clay content which is also correlated with SA and infectivity inversely. From several previous studies it was found that clay content affects SA and infectivity differently and the most probable reason provided was the clay role on moisture (Silva-Flores et al., 2019). However, in our case, infectivity was determined on trap cultures which were irrigated regularly. Hence, moisture could less likely be the reason for the observed SA, infectivity, and clay relationships. The main reason could be the clay content effects and/or relationships with BD, CEC, OM, and other soil factors.

5. Conclusion

This study has sufficiently tested the initial hypotheses. Hence, except our H2 which considered AMF spore abundance (SA) to be not resilient to the degradation and deforestation of the Chilimo dry evergreen Afromontane forest, H1 and H3 were proved to hold wholly or partly. According to our finding, infectivity was lowered due to NF-ShL and NF-GrL conversions but not NF-CrL conversion. Moreover, both the degradation and deforestation of Chilimo forest resulted in incresease of SA. Despite increase of SA due to NF-GrL conversion, since both infectivity and soil physichochemical property (a potentially proxy to AMF community composition resilience) were not resilient, more importantly, when the land-use changed from natural forest to *Pennisetum sphacelatum* dominated grazing land, forest restoration projects on such grazing lands could potentially consider AMF inoculation. However, when the planting sites are croplands, there maybe little or no benefit of AMF inoculation.

In the future, AMF community composition resilience should be investigated by detrmining AMF species composition of these four land uses morphologically from field soil and

trap culture or molecularly. Doing so is also very important because it will enable us to know to what extent AMF community composition and physichocemical property resilince are correlarted. Similar studies should also be carried considering the various DAF in Ethiopia so that better AMF inoculation strategies could be formunalted for successful restoration of the severely degraded dry evergreen Afromontane forests of Ethiopia.

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7. Supplementary materials

S1: Greenhouse bioassay setup

S2: Root AMF infection; **N** not infected, **V** vesicle, **IRM** intraradical mycelium, **ERM** extraradical mycelium.

S3: Measured soil variables across land uses, altitude and per plot

S4: The values of soil physicochemical variables previously reported comparative to the values found in this study. When previously reported values are ±10% (for pH) or ±25% (for the other variables) of our results, we consider them to be comparable. NF natural forest, CF Chilimo forest, CrL crop land, DAF dry evergreen Afromontane forests