The potential of native arbuscular mycorrhizal fungi inoculation to improve the dry evergreen Afromontane forests restoration efforts in Ethiopia

Dissertation

For the partial fulfillment of a Doctoral (Ph.D.) degree in Plant Biology and Biodiversity Management (Botanical Sciences), College of Natural and Computational Sciences,
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This is to certify that the thesis prepared by Fisseha Asmelash Belay, entitled: **The potential of native arbuscular mycorrhizal fungi inoculation to improve the dry evergreen Afromontane forests restoration efforts in Ethiopia**, and submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Plant Biology and Biodiversity Management (Botanical Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

The potential of native arbuscular mycorrhizal fungi inoculation to improve the dry evergreen Afromontane forests restoration efforts in Ethiopia

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Currently, the restoration of dry evergreen Afromontane forests (DAF) is one of the most important environmental agendas in Ethiopia. However, DAF restoration is very challenging and so far, there has been limited success. Therefore, this study aimed to evaluate if there are potential benefits of arbuscular mycorrhizal fungi (AMF) inoculation to improve DAF restoration. Firstly, we investigated the AMF status of nine native tree species raised in ten tree nurseries of central and northern Ethiopia. Secondly, we determined the AMF spore abundance (SA) and infectivity and the soil physicochemical property (a proxy to AMF composition) across four land uses, viz., natural forest (NF), shrub land (ShL), cropland (CrL), and grazing land (GrL) of the Chilimo forest (CF). Thirdly, in a mesh-house experiment, we evaluated the whole-soil AMF inoculation effects on the growth and quality of *Cordia africana* Lam., *Juniperus procera* Hochst. ex Endl., and *Podocarpus falcatus* (Thunb.) R. Br. ex Mirb seedlings grown on sterile and non-sterile degraded DAF ecosystem soil. SA in the nurseries ranged from 1.30-24.63 spores g^{-1} of soil and was comparable with the SA range found in the CF (3.4-25.3 g^{-1} of soil). Root AMF colonization (RC) of seedlings ranged from 8-97.67%. Our results indicated that none of the seedlings were with low

SA and RC simultaneously. We also found that land-use changes in the CF, significantly (p<0.05) affected most of the soil physicochemical variables. The nonmetric multidimentioanl scaling ordination plot depicted that the soil physicochemical property was resilient to CF degradation (NF-ShL conversion) but not deforestation (NF-CrL or GrL conversions). SA was resilient to both the degradation and deforestation of CF but infectivity was only resilient to NF-CrL conversion. The mesh-house experiment results indicated that *C. africana* had significantly (p<0.05) higher mycorrhizal responsiveness compared to *J. procera* and *P. falcatus*. Moreover, AMF inoculation significantly (p<0.05) increased *C. africana* growth, while little or no effect was found for *J. procera* and *P. falcatus*. Based on our findings, we conclude AMF inoculation to be merited in some conditions (selected tree species and planting sites). However, additional studies are required on more tree species, AMF variables, and DAF.

Keywords: arbuscular mycorrhizal fungi (AMF); *C. africana*; Chilimo forest; dry evergreen Afromontane forests (DAF); forest restoration; *J. procera*; *P. falcatus*

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LIST OF ACRONYMS

AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
CEC	Cation exchange capacity
CrL	Cropland
DAF	Dry evergreen Afromontane forests
EC	Electrical conductivity
ERM	Extraradical mycelium
GrL	Grazing land
I	Inoculted with whole-soil AMF
LA	Leaf area
LN	Leaf number
MR	Mycorrhizal responsiveness
N	Total nitrogen
NF	Natural forest
nI	Not inoculated with whole-soil AMF
nS	Non-sterile soil
OC	Organic carbon
OM	Organic matter
P	Plant available phosphorus
P:N	Available phosphorus to total nitrogen ratio
ppm	Parts per million
R:P	Root to plant drymass ratio
R:S	Root to shoot dry masss ratio
RC	Root AMF colonization
RCD	Seedling collar diameter per age
RdM	Root dry mass
RfM	Root fresh mass
RGR	Relative growth rate
RGR-CD	Relative collar diameter growth rate
RGR-H	Relative height growth rate
RGR-LN	Relative leaf number growth rate
RSHI	Seedling height per age

SA	AMF spore abundance
SdM	Shoot dry mass
SfM	Shoot fresh mass
SHI	Seedling shoot height
ShL	Shrub land
TdM	Total seedling dry mass
TfM	Total seedling fresh mass

1. INTRODUCTION

1.1. Background and justification

The dry evergreen Afromontane forests (DAF) ecosystem, which comprises the large proportion of Ethiopia's landmass, was once predominantly covered by the DAF (Tamrat Bekele, 1993; Demel Teketay and Anders, 1995; Friis et al., 2010). Due to human-induced land-use changes, however, most of the DAF have been deforested and only a few degraded fragments are now remaining (Demel Teketay and Anders, 1995; Mengeste Kindu et al., 2013; Terefe Tolessa et al., 2017). Currently, the DAF ecosystem is characterized by extensive crop cultivation, free grazing, and land degradation (IBC, 2005; Mulugeta Lemenih and Bongers, 2011). Hence, the restoration of the DAF has emerged to be the most important environmental agenda of our time.

Accordingly, in 2011, the Ethiopian government declared it was perusing a green economic growth model (ECRGE, 2011) and committed to restoring of 15 million hectares of forests (UNDP, 2016) and mainly dry evergreen Afromontane forests (MEFCC, 2018) by 2025. To that effect, billions of tree/shrub seedlings are being planted every year and billions of birr[1] in seedlings production and labor, among others, is being invested each year. However, most of these tree-planting projects, especially those within the DAF ecosystem are observed to fail or succeed very rarely. Previous massive DAF restoration programs had also, due mainly to poor seedlings survival, achieved limited success (Badeg Bishaw, 2001). Therefore, the identification of practices that could significantly improve the survival and establishment of planted DAF native trees is

[1] Ethiopian currency

crucial. Improved seedlings field survival is not only important to ensure DAF restoration but it is also crucial to save hundreds of millions of birr that could be lost annually due to seedlings death.

African soils in general (Walter, 1985) and Afromonatne soils in particular (Dalling et al., 2016) are too deficient in the essential nutrients (e.g. N&P) required for seedlings survival, growth, and forest development. The DAF ecosystem is further constrained by the dry climate (Mulugeta Lemenih and Bongers, 2011). Moreover, DAF restoration requires the planting of DAF characteristic tree/ shrub species since passive restoration is hardly possible (Mulugeta Limenih and Demel Teketay, 2004). Therefore, due mainly to these facts, DAF restoration is a very challenging task.

Through long years of research and experience, Legesse Negash (2010) has determined that in order to ensure the field survival and establishment of DAF characteristic tree species seedlings, after planting care (hoeing, weeding, watering, manure application, and mulching) for at least two-three years was a requirement. Although after planting care could be indispensable, it could as well be expensive and less-likely practical in large scale DAF restoration programs. Therefore, the search of other cheaper and yet, effective techniques such as the use of nurse plants as demonstrated by Tesfaye Bekele (2005), Aerts et al. (2007), Legesse Negash and Birhanu Kagnew (2013), and Abrham Abiyu et al. (2017) is highly relevant. Meanwhile, the potential role of native arbuscular mycorrhizal fungi (AMF) inocula should also be explored in this regard.

Arbuscular Mycorrhiza (AM) is an ancient, adaptive symbiosis that probably enabled land colonization of plants (Smith and Read, 1998). When the soil is deficient with phosphate and nitrates, plants initiate root infection by AM fungi (Gutjahr, 2014). Then, the fungi colonize the root cortex and develop extraradical mycelia (ERM) which are very extensive; as long as 4200 m per gram of soil (Leake et al., 2004). 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). AMF could supply up to 100% of host plants phosphorus requirement (Smith et al., 2011) and could reduce more than 40% of irrigation requirement (Gianinazzi et al., 2010). Hence, AMF inoculation has been shown to improve tree seedlings' growth (Urgiles et al. 2009; Urgiles et al., 2014; Schüßler et al., 2016), field survival, and establishment (Pouyu-Rojas and Siqueira, 2000; Dag et al, 2009; Mitiku Habte et al., 2001; Kapulnik et al., 2010; Karthikeyan and Krishnakumar, 2012). Therefore, AMF inoculation could be an appropriate DAF restoration technology.

AMF are ubiquitous in existence found almost in every soil (Abbott and Robson, 1991; Brundrett and Abbott, 2002) including mine soils (Wang, 2017) and also, a very small amount of infective AMF propagule could be enough (3 spores being as good as 225) to cause sufficient root infection (Daft and Nicolson, 1969). Moreover, there are reports indicating that AMF abundance (Zangaro et al., 2013; Stürmer and Siqueira, 2011; Emiru Birhane et al., 2018) and species richness (Picone, 2000; Zhang et al., 2004; Stürmer and Siqueira, 2011) could increase despite the increase in forest degradation. Soil AMF

communities were also found to be resilient to forest degradation and deforestation in various forest ecosystems (Johnson and Wedin, 1997; Picone, 2000; Zhang et al., 2004; Violi et al., 2008; Carrillo-Saucedo et al., 2018). Therefore, the importance of AMF inoculation in forest restoration projects has become a matter of debate (Rillig et al., 2019; Ryan and Graham, 2018). According to Verbruggen et al. (2013), AMF inoculation success or failure greatly depends on the type of plant species inoculated and the AMF status, viz., AMF abundance, AMF species composition, and infectivity, of the planting material and the planting sites. Therefore, the knowledge on the AMF status of native tree species seedlings being raised in the tree nurseries suitable for DAF restoration (DAF nurseries) and the AMF status of the degraded planting sites comparative to DAF, are prerequisite for planning AMF inoculation in DAF restoration projects. Moreover, AMF responsiveness of the most important DAF native tree/shrub seedlings should also be evaluated. However, to this date, little knowledge is available in these regards.

In the past, Michelsen (1992) has surveyed the AMF status in four DAF nurseries of central Ethiopia and found out that those nurseries were deficient in infective AMF propagule. Regarding the AMF status of planting sites, there have only been two relevant studies carried out by Emiru Birhane et al. (2018) and Yoseph Tewodros et al. (2017). Whereas Emiru Birhane et al. (2018) have found increased AMF spore abundance but reduced AMF infectivity due to DAF degradation Yoseph Tewodros et al. (2017) on the contrary, have reported a decreased AMF spore abundance. This indicates that the effect of land use and management changes should further be investigated. The AMF status of DAF characteristic tree species has also been very well studied (Tesfaye Wubet et al.,

2003a; 2003b; 2006; 2009). Accordingly, DAF characteristic tree species were found to be arbuscular mycorrhizal (Tesfaye Wubet et al., 2003a). Moreover, the AMF composition in roots of seedlings and conspecific adult DAF characteristic tree species was found to significantly vary (Tesfaye Wubet et al., 2009) and distinctive AMF communities were observed to exist in the root systems of co-occurring DAF characteristic tree species (Tesfaye Wubet et al., 2006). However, the only AMF inoculation study so far available that is relevant to DAF restoration is the one by Fisseha Asmelash et al. (2019) which evaluated whole-soil AMF inoculation effects on the field survival and growth of the most important Ethiopian native tree species; *C. africana*, *J. procera*, and *P. falcatus*. This field experiment was unfortunately carried out on fallowed farmland with a very high phosphorus fertilizer legacy. Moreover, the seedlings used were collected from nearby nurseries and could have had sufficient inoculum already. Hence, AMF inoculation did not have effects on either the filed survival or growth (height and collar diameter) of none of the three species investigated. Therefore, there is the need for carrying out such AMF inoculation experiments and more importantly, on degraded (particularly phosphorus poor) DAF ecosystem soils and under a controlled environment.

Therefore, this Ph.D. work was carried out to answer these outstanding questions regarding the potential role of AMF inoculation concerning DAF restoration. Hence, we investigated the AMF status of nine Ethiopian native tree species in ten DAF nurseries. We also investigated the effects of DAF degradation (natural forest conversion to shrubland) and deforestation (natural forest conversion to cropland or grazing land) on

AMF abundance and infectivity. Thirdly, in a mesh-house experiment, using the three native tree species namely *C. africana, J. procera*, and *P. falcatus* we determined the growth responses of seedlings grown on a sterile and non-sterile degraded DAF ecosystem soil.

Arbuscular mycorrhizal fungi are obligately symbiotic organisms and cannot complete their life cycle without host plants (Parniske, 2008). Therefore, unlike the free-living or facultative microbes, AMF spore abundance (SA), AMF infectivity, and AMF community composition of the soil could be greatly influenced by vegetation (Kivlin et al., 2011) and host plants (Eom et al, 2000). Plant factors were found to equally (Johnson et al., 1992; Song et al., 2019) or comparably (Wang et al., 2015) influence AMF community composition as that of soil factors. However, there are variable reports regarding the relative importance of vegetation and soil factors in shaping soil AMF community composition. According to Goldmann et al. (2019), plant factor was found to be more important in shaping soil AMF community composition while Helgason and Fitter (2009), Oehl et al. (2010), and Klichowska et al. (2019) have found otherwise. Interestingly enough, soil factors could be more important than plant factors even for the AMF community composition in the roots (Schechter and Bruns, 2012). Meanwhile, for land-use changes such as forest degradation and deforestation that are characterized by a distinct change in vegetation and land management, AMF communities could most likely be significantly affected if the land-use change is also accompanied by a distinct change in the soil physicochemical property. Oehl et al. (2010) have indicated that the soil

physicochemical property could serve as a potential proxy to AMF community composition and vice versa.

Therefore, in this Ph.D. study, we also determined soil physicochemical property resilience to the degradation and deforestation of the Chilimo forest to use it as a proxy to AMF community composition resilience. Resilience, according to Hodgson et al. (2015), is the ability of an ecosystem and/or its components to resist change to (resistance) or recover from (recovery) disturbance or both. Hence, in this study, we evaluated resilience by determining resistance only, and hence, recovery was not considered.

1.2. Hypotheses

H1: *Ethiopian native tree species seedlings raised in DAF nurseries have low levels of infective arbuscular mycorrhizal fungi abundance*

H2: *AMF spore abundance and infectivity are not resilient to the degradation and deforestation of DAF*

H3: *The soil physicochemical property is not resilient to the degradation and deforestation of DAF and hence,*

H4: *Soil AMF community composition may not be resilient to the degradation and deforestation of DAF*

H5: *Native whole-soil AMF inoculation significantly improves the growth and quality of Cordia africana, Juniperus procera, and Podocarpus falcatus seedlings planted on a degraded DAF ecosystem soil*

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to determine the potentoial of arbuscilar mycorrhizal fungi inoculation to improve the dryevergreen Afromontane forests restoration success in the Ethiopian context.

1.3.2. Specific Objectives

The specific objectives of this project were to:

1. Determine the AMF spore abundance and AMF root colonization of pot soils and roots of nine native Ethiopian tree species seedlings in ten of the DAF nurseries.
2. Determine the effect of nursery management on the AMF spore abundance and AMF root colonization of native Ethiopian tree species seedlings
3. Determine soil physicochemical variables, AMF spore abundance, and AMF infectivity across four land uses, viz., natural forest, shrub land, cropland, and grazing land, of the Chilimo forest.
4. Determinine the AMF responsiveness of *C. africana*, *J. procera*, and *P. falcatus* at the seedling stage.
5. Determinine the effects of whole-soil AMF inoculation on the growth (e.g. height, collar diameter, and dry mass) and quality (rooting depth, root to shoot (R:S) and to total plant (R:P) ratios) of *C. africana*, *J. procera*, and *P. falcatus* seedlings growth on a sterile and non-sterile degraded DAF ecosystem soil.

2. LITERATURE REVIEW

2.1. The dry evergreen Afromontane forests (DAF) and the DAF ecosystem of Ethiopia

The dry evergreen Afromontane forests are among the dry land vegetation types of Ethiopia characterized by the tree species; *Acaca abyssinica*, *Junipperus procera*, *Olea europaea* subsp. *cuspidata*, and *Podocarpus falcatus* (Mulugeta Limenih and Bongers, 2011). Hence, the DAF ecosystem of Ethiopia refers to all the area that is potentially or currently covered by the DAF (Friis et al., 2010). These are most of the landmasses of the central, southeastern, and northern Ethiopia falling within the altitudinal ranges of 1500-3200 m (Zerihun Woldu, 1999) or 1900-3400 m (Mulugeta Lemenih and Bongers, 2011). Hence, the DAF ecosystem constitutes most of the Ethiopian highlands which cover more than 50% of the country's land area. The mean annual rainfall and temperature characteristic of DAF ecosystem are 700-1100 mm and 14-20°C, respectively (Demel Teketay, 1996), and in this ecosystem resides the majority of the Ethiopian population (Zerihun Woldu, 1999; IBC, 2005). The DAF ecosystem of central and eastern Ethiopia is characterized by the cereal-based farming system, while in the western and central-southern of its range mixed root crop farming system is predominant (Zerihun Woldu, 1999).

The DAF can be divided into five namely; *Mimusops kummel* forest (north-west Ethiopia), *Podocarpus falcatus* forest (now found rarely in southern Ethiopia), *Juniperus procera* forest (the most widespread type), *Juniperus procera* and *Podocarpus falcatus* forest (found in Shewa, Arsi, Harar, and Sidamo), and *Acacia abyssinica* forest that

occupies the driest range of the ecosystem and is found in the north-western part of Ethiopia (Zerihun Woldu, 1999). Within forest ecosystems, grasslands could also be prominent mainly due to poor soil nutrient conditions and impermeable layers in the soil profile both inhibiting forest development (Walter, 1985). Similarly, within the DAF ecosystem, there are land units that are covered by *Pennisetum* grass hence, the DAF ecosystem is more appropriately referred to as the dry evergreen Afromontane forest and grassland complex (Friis et al., 2010). Research findings have revealed that AM fungi are predominant in this ecosystem (Tesfaye Wubet *et al.*, 2003a).

2.2. Mycorrhiza and the arbuscular mycorrhizal fungi

Although the knowledge on a new king of fungus-root association has been there much earlier, it was in 1885 that Albert Bernard Frank (a German botany professor) sufficiently described the association and named it "mycorhiza" (Trappe, 2005); to mean "fungus roots" in Greek (Siddiqui and Pichtel, 2008). Later on, it was renamed as "mycorrhiza" after the letter "r" was added (Koide and Mosse, 2004). Currently, mycorrhiza is defined as a symbiotic relationship between most plant species and intimate fungi of mostly, the root and rarely, the subterranean stems and thallus of bryophytes (Brundrett, 2004). Mycorrhiza facilities the bidirectional movement of nutrients whereby carbon and inorganic nutrients respectively, are supplied to the fungi and the host plant (Al-Karaki, 2013). However, about 500 plant species are "myco-heterotrophic" that obtain carbon from the fungal hyphal networks in the soil (Gomes et al., 2019).

There are three main types of mycorrhizas namely; the endomycorrhiza, ectomycorrhiza, and ectendomycorrhiza (Barea *et al*, 2011; Al-Karaki, 2013). These are further categorized into seven, viz., ectomycorrhiza, monotropoid mycorrhiza (a kind of ectomyccorrhiza), arbutoid mycorrhiza (a kind of ectomyccorhiza), ericoid mycorrhiza (a kind of endomycorrhiza), orchid mycorrhiza (a kind of endomycorrhiza), arbuscular mycorrhiza (a kind of endomycorrhiza) and ectendomycorrhiza is a kind of mycorrhiza that has both the characteristics of ectomycorriza and endomycorriza (Finlay, 2008). There are only a few plant species within the families Amaranthaceae, Cyperaceae, Chenopodiaceae, Caryophyllaceae, Polygonaceae, Brassicaceae, Proteaceae, Zygophyllaceae, Orobanchaceae, Commelinaceae that do not form any of these mycorrhizas (Brundrett *et al*., 1996).

Arbuscular mycorrhiza (AM) and ectomycorrhizal (ECM) are the most important types of mycorrhiza. AM is formed in more than 80% of the terrestrial plants (Barea *et al*, 2011) while ECM is formed in only 3% of plant species which, however, are most of the very important forest tree species of the temperate and boreal regions. ECM fungi develop sheath or mantle around the feeder roots, the mycelium penetrates between the cells (intercellularly) of the root and forms the Hartig net but do not form intracellular penetrations. The fungi involved are mostly Basidiomycota and Ascomycota. AMF, on the other hand, colonize the root cortex both intercellularly and intracellularly and with no sheath formation (Barea *et al*, 2011). Inside cortical cells, special tree-like symbiotic structures called the arbuscules, peculiar features of AMF, are formed (Fig. 1). Formerly, AM fungi used to be called Vesicular-Arbuscular Mycorrhiza fungi due to the presence

of a storage body called vesicles. However, since it is now known that not all AM fungi possess vesicles, the name arbuscular mycorrhiza fungi is the accepted naming currently (Koide and Mosse, 2004). AMF had been grouped under the phylum Zygomycota but were latter regrouped in to the monopylum Glomeromycota (Schüßler et al., 2001). Currently, the AMF are regrouped in to the phylum Mucromycota, subphylum Glomeromycotina (Spatafora et al., 2016). Currently, there are 41 AMF genera and only about 333 validly described species of AMF (http://amf-phylogeny.com/amphylo_species.html, accessed on 3/30/2020). In arbuscular mycorrhiza, the plant could partition 4-20% of the photosynthate to the fungi (Lerat et al., 2003) in the form of sugar (hexose) and lipids (Bago et al., 2003; Keymer et al., 2017) and therefore, if the fungi are not able to provide a net benefit to the plant, the association could become parasitic (Johnson et al., 1997).

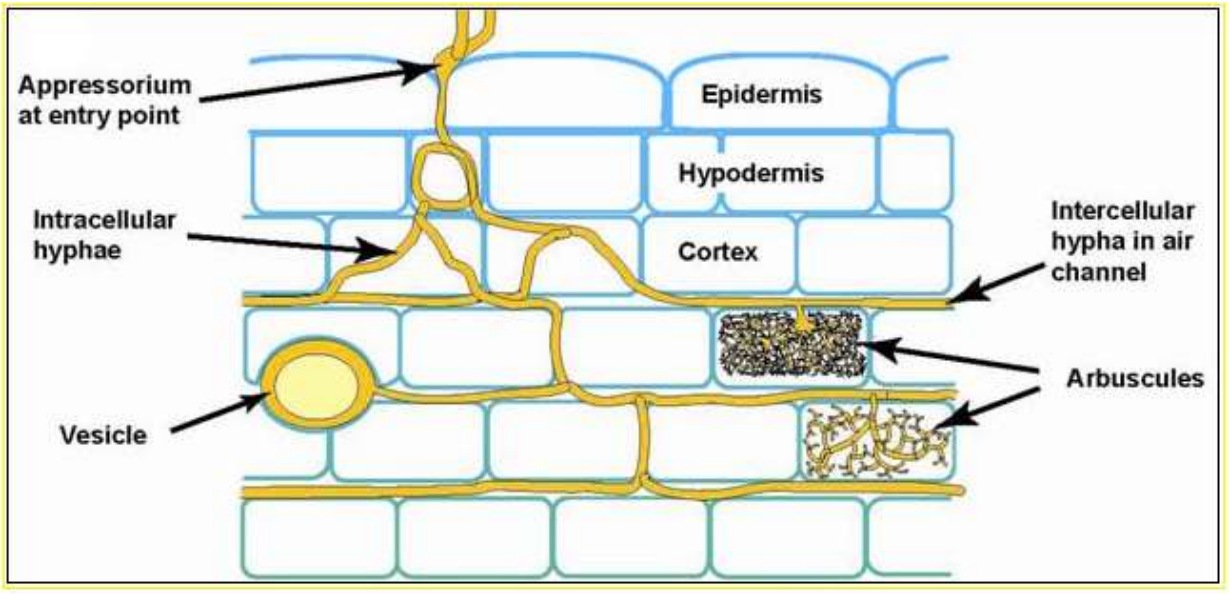

Figure 1: Vesicular-Arbuscular Mycorrhiza (adopted from Brundrett and Abbott, 2002)

2.3. The potential role of AMF in forest restoration

2.3.1. The concept of forest restoration

The international society for ecological restoration (SER) defined forest restoration as, the process of assisting the recovery of a forest ecosystem that has been degraded, damaged or destroyed (SER, 2004) and according to Harris et al. (2006) and Higgs et al., (2014) this definition is considered to be the authentic and the widely accepted one. Based on the restoration goal, three concepts of forest restoration are recognized and these are (Aronson et al., 1993; SER, 2004):

1. True forest restoration: when the goal is to return to the climax, pre-existing forest status,
2. Forest rehabilitation: when the goal is to return to an intermediate successional status of the given forest ecosystem or otherwise known as an alternative steady-state, and
3. Forest reclamation: when the goal is neither to return to the climax forest nor an intermediate successional stage but is rather to increase forest cover including novel forest ecosystem development by using also exotic trees.

With the progression of forest degradation level, forest restoration becomes more complex and potentially impossible while forest reclamation, on the other hand, becomes more feasible (Aronson et al., 1993). Moreover, since novel climatic conditions are anticipated in the future due to the global climate change, to have forest reclamation as a goal is considered to be relevant (Choi, 2004). In this Ph.D. dissertation, the term forest restoration considers the true forest restoration and not forest reclamation or rehabilitation.

Since degraded ecosystems lost multiple species and forest restoration tries to revert that, forest restoration is mainly based on community ecology (Young et al., 2001). Hence, to better grasp the concept of forest restoration, understanding the definition following Hobbs et al. (2007) is very relevant. According to Hobbs et al. (2007), forest restoration is a process of plant community assembly and succession mediated by disturbance. Whereas succession refers to the more or less regular and predictable replacement of seral plant communities (pioneers to the climax), community assembly refers to the species dynamics within each of the seral communities (Fisseha Asmelash et al., 2016). There are three basic causes of succession, viz., site availability, differential species availability, and differential species performance and designed disturbance is therefore needed to manipulate site availability. When non-native species prevent the regeneration of natives and where a structural decline of soil has occurred, a physical disturbance is needed respectively to force the jump from one metastable state to another and to encourage the germination of desired species (Hobbs and Norton, 1996)

Community assembly is a hierarchical process with local species assemblages representing subsets of the larger species pool. These subsets are established by filtering through local abiotic and biotic factors such as microclimate, soil type, plant interactions, herbivores, and pollinators which sometimes can vary between plots (Kikvidze et al., 2015). A typical filter effect was observed by Henry Gleason in 1927 whereby ponds at a similar locality having similar environmental conditions formed different wetland communities (Hobbs et al., 2007). Moreover, since it can determine the later seral community assemblage and thereby succession trajectory, the initial community

assemblage is very important. Accordingly, in 1954, Egler argued that ecosystem development can be accelerated by controlling initial species composition and succession to achieve the desired endpoint (Hilderbrand et al., 2005). Hence designing the composition of pioneer species is an important technique to succeed in forest restoration (Angelini et al., 2011) and it has been proved practically (Cortines and Valcarcel, 2009). Therefore, forest restoration strategies usually center on the manipulation of assembly, i.e., plant species order of arrival and modification of filters, to accelerate and/or jump-start succession (Young et al., 2001; Hobbs et al., 2007; Gómez-Aparicio, 2009).

In essence, forest restoration, guided by simplified principles, strives to (re)create complex systems in a matter of years what actually, under natural conditions, takes decades or centuries (Hilderbrand et al., 2005). When applicable, removing a perturbation and allowing the ecosystem to recover through the natural ecological processes (passive restoration) is the simplest way of forest restoration. However, to jump-start the process, climax tree species could be planted. Hence, forest restoration activities include the range of activities from area enclosure to planting of as much reference site species as possible (Palmer et al., 2006). With the growing appreciation of plant-plant facilitation, and since they are better adapted to resource-limited conditions, early-mid successional shrubs could be more sutable than the late-successional tree/shrubs for reforestation plantation (Gómez-Aparicio, 2009; Padilla et al., 2009).

2.3.2. AM Fungi and measurable forest restoration attributes

The nine succession traits which were originally proposed by Odum (1969) were adopted by Aronson *et al.* (1993) to be used as forest restoration attributes (Table 1). These concepts of forest restoration attributes are currently incorporated into international society for ecological restoration (SER) standards of measuring forest restoration success (SER, 2004). Ruiz-Jaen and Aide (2005) have divided these forest restoration attributes into three categories, viz., diversity (mainly plant), vegetation structure (including density and phytomass) and ecological processes such as nutrient cycling, soil organic matter, and mycorrhizal abundance and infectivity. Therefore, the possible effect of AMF inoculation towards improving forest restoration could best be evaluated based on these measurable restoration attributes. Accordingly, Fisseha Asmelash et al. (2016) have reviewed the potential role of AM fungi in forest restoration by considering their roles in relation to the Aronson et al. (1993) restoration attributes. Hence, below we have presented some of these AMF roles as discussed by Fisseha Asmelash et al. (2016).

Table 1: Features of degraded lands compared to the reference forest ecosystems (Based on Aronson *et al*., 1993 cited in Fisseha Asmelash, 2016)

<table>
<tr><th>Structural indicators of land degradation</th><th colspan="2">Functional indicators of land degradation</th></tr>
<tr><td>1)Low total plant cover</td><td colspan="2">Low biomass productivity</td></tr>
<tr><td>2)Low perennial and 3) annual plant species richness</td><td colspan="2">Low soil organic matter</td></tr>
<tr><td>4)Low aboveground phytomass</td><td rowspan="3">Poor soil water relation</td><td>Lowered soil water reserves</td></tr>
<tr><td>5)Low beta diversity (species turnover along environmental gradient)</td><td>Low coefficient of rainfall efficiency (the amount of water infiltrating to middle and deep soil layers)</td></tr>
<tr><td>6)Decreased life form spectrum (Decreased number of species with different modes of adaptation)</td><td>Reduced length of water availability period</td></tr>
<tr><td>7)Reduced number of keystone species</td><td colspan="2">Low rain use efficiency (RUE)</td></tr>
<tr><td>8)Low soil microbial biomass</td><td colspan="2">Poor nutrient cycling index (the ratio of the amount of nutrients mainly N&P recycled to the amount leaching or lost)</td></tr>
<tr><td>9)Low soil microbial diversity</td><td colspan="2">Low nitrogen use efficiency (NUE)</td></tr>
</table>

2.3.2.1. The AM fungi role in soil aggregation, soil organic matter, and soil water relation

AMF hyphae grow into the soil matrix and create the skeletal structure that is able to hold primary soil particles thereby forming the soil aggregates (Augé, 2004; Al-Karaki, 2013). Moreover, by influencing the soil aggregate former bacterial communities, AMF improve soil aggregation (Rilling, 2004). The glomalin that is produced by dead AMF hyphae is also an important hydrophobic soil aggregate former (Barea *et al.*, 2002; Simard and Austin, 2010). Hence, since the AMF are important soil aggregate formers, they are considered to be the most effective fungi groups playing a major role in soil structure stabilization (Augé, 2004) and have been shown to stabilize soil even up to 5 months after their host's death (Soka and Ritchie, 2014).

Due to the fact that AMF improve aggregation and stability and due to the fact that they drain significant amount of carbon from the host plant and add to the soil, AMF increase soil organic matter content and stability (Rilling, 2004; Leifheit *et al*., 2014). Moreover, AMF by improving soil aggregation and soil organic matter, they do significantly improve the soil water relations. Accordingly, when non-mycorrhizal plants were planted on mycorrhizal soils, they were observed to tolerate drought more compared to the instances when they were planted on non-mycorrhizal soils (Marschner, 1995).

2.3.2.2. AM fungi and the nutrients cycling index of the major soil nutrients

The most important role of AMF is their role in plant phosphorous nutrition (Skujins and Allen, 1986). According to some reports, AMF were observed to transfer nitrogen from N-fixing plant to the nearby non-N-fixing plant (Requena *et al*., 2001). They were also observed to increase the utilization of different forms of nitrogen by plants (Govindarajulu et al., 2005). AMF could also take up nitrogen directly and transfer it to host roots (Govindarajulu *et al*., 2005). However, the cost-benefit of AMF-plant relations in nitrogen nutrition may be negative and further assessments are required in this regard (Smith and Smith, 2011). Although few data exist, AMF could also potentially improve potassium nutrition in plants (Dag *et al*., 2009; Garcia and Zimmermann, 2014). The beneficial role of AMF in other plants macro and micronutrients nutrition has also been reported (Emiru Birhane *et al*., 2012).

The external mycelia of AMF establish an underground network and link the different plants and sequester carbon, nitrogen, and phosphorous while it also allows the transfer of these nutrients among plants (Rodriguez-Echeverrıa *et al*., 2007). Hence, due to these important nutrients cycling functions, AMF could also significantly reduce the fertilization requirement (Gianinazzi *et al*., 2010). In the meantime, AMF could also reduce the leaching of the major nutrients from the soil (Rodriguez-Echeverrıa *et al*., 2007). In a comprehensive assessment done by Bender *et al*. (2015), it was possible to determine that whereas AMF inoculation could increase the nutrient uptake of plants, it also at the same time significantly reduced leaching of dissolved organic N and un-reactive phosphorus. Hence due to the increased nutrient uptake by plants and reduced leaching of these nutrients, AMF could significantly improve the nutrient cycling of a site.

2.3.2.3. AM fungi and plant tolerance to abiotic stresses

The role of AMF in relation to the increase of plant drought and salinity tolerance has been demonstrated several times (Al-Karaki, 2013). The AM fungi role in alleviating heavy metal stress in plants is also very well-known (Leyval *et al*., 1997; Hildebandt *et al*., 2007; Soares and Siqueria, 2008; Amir *et al*, 2013). The inoculation of drought-tolerant AM fungi ecotypes has been shown to significantly improve plant water relation and up to 42% reduction in plants' water requirement was observed (Gianinazzi *et al*., 2010). Interestingly enough, Navarro *et al*. (2013) found out that *Citrus* rootstocks inoculated with AM fungi showed a significantly increased growth compared to the non-

inoculated individuals despite the fact that inoculated individuals were irrigated with saline water while the non-inoculated ones were irrigated with non-saline water.

The mechanism by which AM fungi increase plants' tolerance to drought, salinity, and heavy metal stresses is mainly nutritional (Soares and Siqueria, 2008; Navarro *et al*., 2013, Al-Karaki, 2013). Soares and Siqueria (2008) demonstrated that both phosphorus fertilization and AM fungi inoculation of plants significantly improved plant growth on heavy metal polluted soils and based on this observation, they concluded that AM fungi induced plant tolerance of heavy metal stress is mainly through the phosphorus nutrition. However, the immobilization of heavy metals in AMF biomass, i.e., mainly cell wall and the vesicles and in the glomalin, have also been considered to be the non-nutritional mechanisms by which AM fungi improve plant tolerance to heavy metals stress (Hildebandt et al., 2007).

The non-nutritional mechanisms by which AMF increase plant tolerance to drought include hormonal changes, hyphal soil improvement-induced soil water relation improvement, improved plant water relation due to the hyphal ability to scavenge moisture in a greater soil volume including the micro-pores, and increased photosynthetic efficiency of plants (Marschner, 1995; Emiru Birhane *et al*., 2012). AMF induced plants' accumulation of compatible osmolites is also considered to be the other non-nutritional mechanism (Al-Karaki, 2013).

The positive AM fungi effects on plant drought tolerance could in the meantime improve the ability of plants to tolerate salinity stress. This is because the AMF improved moisture acquisition by plants could help to significantly dilute salts within the plant cells (Larcher, 1995). Moreover, AM fungi hyphae in the soil could remove a substantial amount of salt from the soil solution and accumulate it in its tissues thereby helping the plant cells be excluded from accumulated salt. AMF also produce enzymes involved in antioxidant defense, and change in cell wall elasticity and membrane stability which is also very important in plant salinity tolerance (Al-Karaki, 2013).

2.3.2.4. AM fungi and plant resistance and tolerance to the biotic stresses

There are several published articles to show the beneficial effects that AMF have in relation to plant tolerance against biotic stresses. Likewise, Yang *et al*. (2014) have done a meta-analysis using 144 published papers. Considering the role that AM fungi have in bio-protection, they were described as 'health insurance' of plants (Gianinazzi *et al*., 2010). One of the mechanisms by which AM fungi could increase plant tolerance to pathogens could be the synergetic interactions they forge with the Plant Growth Promoting Rhizobacteria (PGPR) and it is very well documented that PGPR are able inhibit the plant-pathogen interaction and hence, disease progression (Figueiredo *et al*., 2010). Plants' secondary metabolites are known to have a role in plants' defense against herbivores (Larcher, 1995) and AMF are known to stimulate the synthesis of plant secondary metabolites (Gianinazzi *et al*., 2010). Therefore, the AMF role in defending herbivory could be related to their role on the secondary metabolites. The other reason by

which AM fungi increase plants' herbivory tolerance could relate to the compensatory growth (Kula *et al*., 2005).

2.3.2.5. The role of AM fungi in tree/shrub seedlings growth, field survival and establishment on degraded lands

Lekberg and Koide (2005) carried out a meta-analyses based on 290 of the published experiments to determine the AM fungi roles on the plant growth and productivity. The analysis also determined the effects of the three common AMF management methods, viz., inoculation, short fallow, and reduced soil disturbance. The result of the meta-analysis indicated that generally, AM fungi increase individual plant's growth and productivity. Moreover, the results revealed that inoculation and short fallow result in significant positive effects on plant growth and productivity (Lekberg and Koide, 2005). Similarly, a recent meta-analysis by Lin *et al*. (2015) which used 304 papers for analysis also concluded that AMF inoculation increases the growth and productivity of plants grown alone. An experiment in Ethiopia has also shown that AMF inoculation was more effective for individuals planted alone (Emiru Birhane *et al.*, 2014). Huante *et al*. (2012) in their experiment have recorded a significant effect of AMF inoculation on seedlings growth and more importantly the growth of the slow-growing tree species. Figure 2 shows how AMF inoculation could significantly increase tree seedlings growth.

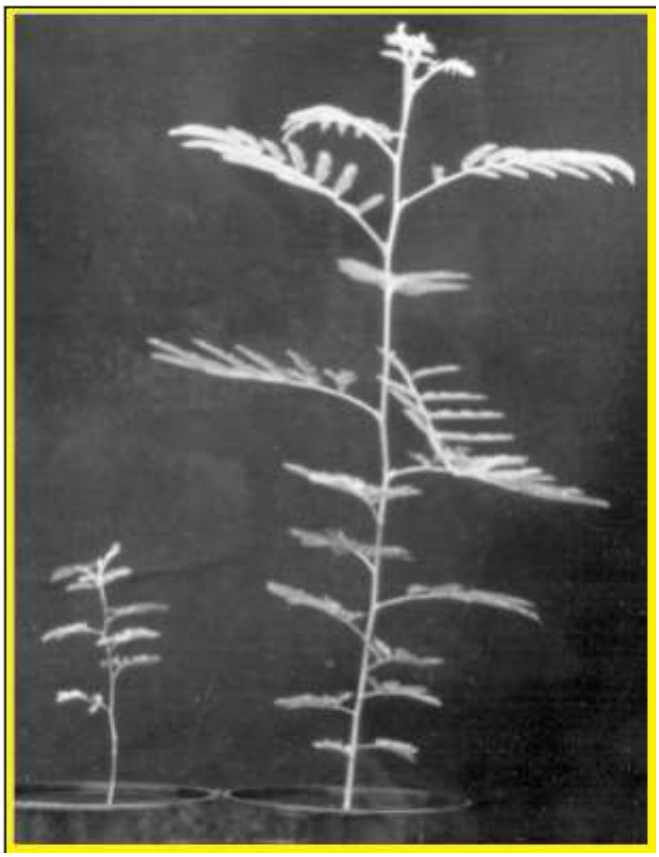

Figure 2: AMF inoculated *Acacia koa* grew significantly tall in a phosphorus limited soil compared to the non-inoculated ones (adopted from Miyasaka *et al*., 2003)

The success of forest restoration greatly depends on the rate of tree seedlings field survival and establishment. Hence, since AM fungi can significantly improve tree seedlings survival and establishment (Pouyu-Rojas and Siqueira, 2000; Ouahmane *et al*., 2006; Dag *et al*., 2009; Mitiku Habte *et al*., 2001; Kapulnik *et al*., 2010; Karthikeyan and Krishnakumar, 2012). AMF inoculation is considered to be an important forest restoration biotechnology. Pouyu-Rojas and Siqueira (2000) have investigated the AM fungi inoculation effects on the survival and establishment of seven tree species seedlings planted on degraded pot soils and found that AM fungi inoculation both in the nursery and during field planting was equally effective way of improving seedlings' field survival and establishment. Mitiku Habte *et al*. (2001) have also determined the effect AMF

nursery inoculation has on the field establishment of *Acacia koa* and accordingly, AMF by their effect on the phosphorus nutrition, was shown to improve transplanted seedlings growth and establishment. Ouahmane *et al*. (2006) demonstrated that AMF inoculation had significant positive effects on the field survival and establishment of *Cupressus atlantica* seedlings planted on a degraded Moroccan field site.

Similarly, Kapulnik *et al*. (2010) have evaluated the effect of AM fungi nursery inoculation that enhanced field establishment and growth of *Olea europaea* seedlings. Hence, the authors further remarked, AM fungi inoculation improved seedlings field performance significantly and most importantly for the first 2.5 years after plantation. Interestingly, they observed that AM fungi growth effects decreased with increasing seedlings age. Karthikeyan and Krishnakumar (2012) have also determined AM fungi inoculation effect on the survival and establishment of *Eucalyptus tereticornis* on pot soil of highly degraded origin and were able to record a doubled seedlings survival and a significantly increased establishment.

2.3.2.6. AM fungi shape plant communities and could drive succession

The fertility and the mycorrhizal fungus status of soil influence the occurrence of plant species (Janos, 1980). The mycorrhizal status of a site determines the composition of pioneer plant communities, the composition of the pioneer plant communities will further determine the amount and composition of infective AMF which will influence the composition of seral communities to the climax (Janos, 1980; Renker *et al*., 2004). Thus, if specific compatible relationships between certain plant taxa and AMF are required for

mutual symbiont survival, the loss of compatible AMF species or ecotypes may limit the distribution of a particular plant species. In such a manner, the availability of AMF taxa may influence the composition and function of the plant community (Renker *et al*., 2004).

The other way in which AMF could influence plant community structures is their effects on the richness or evenness of coexisting plants (Brundrett and Abbott, 2002). The fact that only some 240 AMF morphospecies are responsible for the AM association formed with more than 200,000 host plants species (Lee *et al*., 2013) have indicated that AM fungi have no or low host specificness. Therefore, a single mycorrhizal fungus can link different plants together by forming mycorrhizal networks (Song *et al.*, 2014). These networks, by facilitating new seedlings regeneration, altering species interactions, and changing the dynamics of plant communities, have been shown to increasing plant diversity (Simard and Austin, 2010). Likewise, in microcosm experiments of temperate zone grassland community, Van der Heijden *et al*. (1998), Vogelsang *et al*. (2006), and Schnitzer *et al*. (2011) found that by increasing plants' fitness and evenness, AMF inoculation improved plant community diversity. The other important AM fungi role is inhibition of invasion by alien species. This could be directly by the inhibition of invasives as shown by Janos *et al*. (2013) or indirectly by reducing the vacant niche through increased native plants' survival, establishment, and diversity.

2.3.2.7. AM fungi and the soil microbial diversity and abundance

AMF-derived carbon, the AMF hyphae and root litter are the most abundant carbon sources in the soil (Brundrett and Abbott, 2002; Rilling, 2004). Hence, increasing the supply of energy in the soil, AMF significantly influence the soil microbial populations. Moreover, since AMF could influence plant communities, they also in a way influence the soil microbial communities (Rilling, 2004). Furthermore, AMF hyphal exudates and mucilages could be responsible to the maintenance of microbial populations in the soil. Moreover, since hyphal exudates and mucilages may stimulate some microorganisms but still suppress others (Herman *et al.*, 2012), it could be the case that AMF increase the diversity and abundance of plant beneficial microbes and inhibit the harmful ones.

2.3.2.8. AM fungi and the plant community primary productivity and plant competition

In accordance with the ascending but asymptotic diversity-productivity pattern, AM fungi inoculation has been shown to increase plant productivity at community level and the effect increases with increase in plant species richness (Schnitzer *et al*., 2011). At low plant diversity, soil microbes tend to be pathogenic suppressing plant productivity. With increasing plant species number however, soil microbes have positive effect on plant productivity up to 5 fold (Figure 3; circle and triangle). In the absence of soil microbes, plant productivity could have a weak positive linear relationship with plant diversity (Figure 3; square).

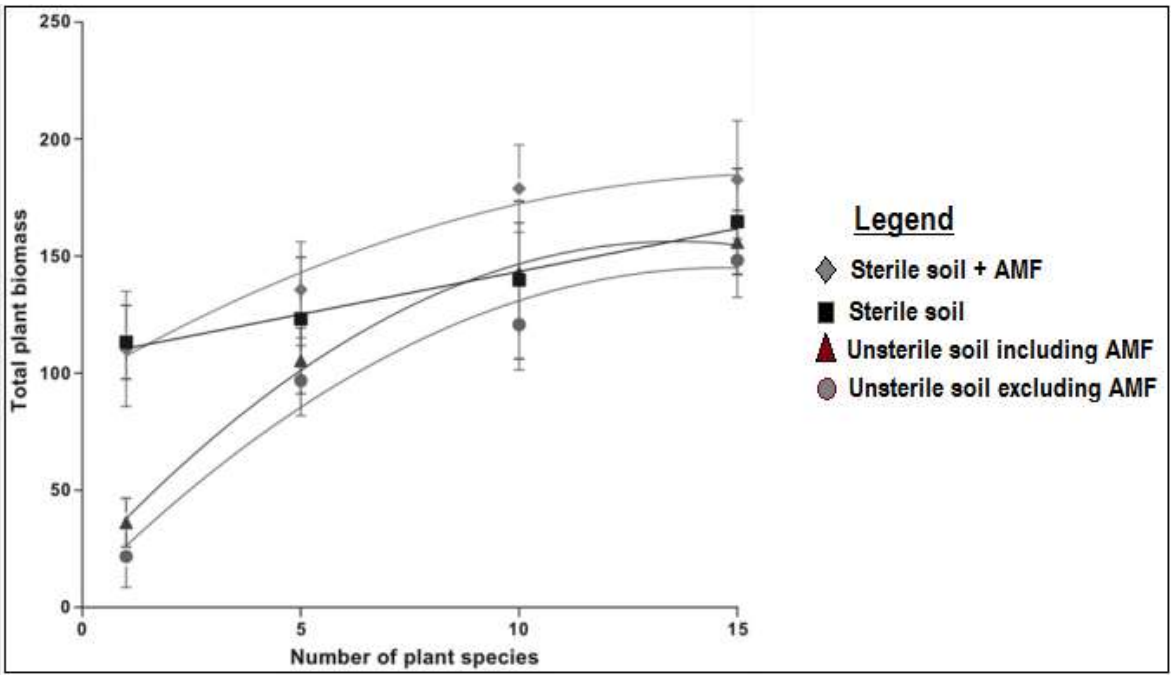

Figure 3: Soil microbes, AM fungi, and plant diversity-productivity relationship (adopted from Schnitzer *et al*., 2011)

In the microcosm experiments, Van der Heijden *et al*. (1998) and Vogelsang *et al*., (2006) have shown that AMF inoculation could significantly improve the plant community productivity. Moreover, according to Van der Heijden *et al*. (1998) observation, increased plant productivity could be the function of AMF richness. However, according to Vogelsang *et al*. (2006), no significant plant productivity effect was observed due to AMF richness. Van der Heijden *et al.* (1999) explained these contrasting results by attributing them to niche complementarity and sampling effect respectively. The "niche complementarity" theory argues that the presence of many species and functional types results in more complete utilization of resources because different species specialize on different resources, resulting in higher overall productivity while the "sampling effect" theory argues species identity is more important than diversity and asserts that productivity increases with diversity solely due to an increased

probability that communities with more species contain a few very productive species that disproportionately contribute to community-wide productivity (Schnitzer *et al*., 2011).

Contrary to both of the above reports, in the recent meta-analysis on 304 study results, it was found thet AMF inoculation either has no effect on plant productivity or even has a negative effect (Lin *et al*., 2015). According to the results, AMF inoculation increased plant productivity only when experiments were conducted in the greenhouse. Moreover, according to the greenhouse experiment by Klironomos *et al*. (2000), it has been shown that AMF inoculation increased plant productivity but not for all AMF species; with lower plant productivity recorded when the plant community was inoculated with *Glomus intraradices*.

Similarly, variable AMF effects were observed for plant competition. According to Emiru Birhane *et al.* (2014), AMF inoculation did not have a positive effect on the competitive ability of the tree species; *Acacia etbaica* and *Boswellia papyrifera.* The explanation for such a phenomenon is that mycelial network formed in the soil could share resources among the competing plants evenly (Rodriguez-Echeverrıa *et al*., 2007*).* Hence, the AMF-plant competition effects could be governed by the plant functional group, life history, mycorrhizal status (Scheublin *et al*., 2007; Lin *et al*., 2015), and the below ground functional traits (Emiru Birhane *et al.*, 2014). According to the meta-analysis conducted by Lin *et al*. (2015), AMF inoculation significantly increases N-fixing forbs, decreases C3 grasses and non-N-fixing forbs and woody plants, and has no effect

on C4 grasses competitive ability whether these functional groups compete intra or inter-specifically (Lin *et al*., 2015).

2.4. The status and potential of the AM fungi biotechnology in forest restoration

AM fungi inoculation in forest restoration can be considered as an important biotechnological tool. Accordingly, AMF inoculation has proved to be merited under the wide range of soil conditions (Janos, 1980; Brundrett and Abbott, 2002), also on soils of abundant AMF (Banerjee *et al*., 2013). Positive AMF effect is ensured not only by AMF abundance but by both abundance (quantity) and composition and infectivity (quality) of AM fungal communities (Onguene and Kuyper, 2005). Another important observation was the AMF suppressive effect on the ruderal plants (Veiga et al., 2011). This is important because, ruderal plants could invade plantation sites and AMF suppression could lower competition by these weeds againest tree/shrub seedlings planted. Koide and Mosse (2004) on the contrary, suggested that instead of going for AMF inoculation, it would be more appropriate and economical to focus on indigenous AMF population management of the planting site. Likewise, Renker *et al*., (2004) recommended AMF to be important but only as the last option.

Although there are different opinions on the importance of AMF inoculation, in situations where AMF inoculation proposed, the first questions to answer is, what kind of inoculum to use/prepare? We have to bear in mind that AMF show a wide range of functional diversity (Johnson *et al*. 1997; Kliromonos, 2003; Smith *et al*., 2011). Kliromonos (2003) demonstrated that exotic-native AMF-host or vise-versa combinations resulted in highly

parasitic interaction. Therefore, the use of native inocula should be preferred to the use of exotic inocula. Early-successional AMF should be used when seedlings are inoculated for restoration, even for late-successional tree species (Allen *et al*., 2003). Their argument is that, late-successional AMF have big spores and demand much carbon while early-successional AMF have small spores and lower carbon demand and they conclude, seedlings may not benefit from late-successional AMF inoculation. According to Fischer *et al*. (1994), AMF abundance in grasslands could be ten times more than the abundance in the forest, and hence, grassland AM could have a significantly high inoculation effect. Similarly, Onguene and Kuyper (2005) demonstrated that early successional grass inocula had a positive effect for most of the cases (80%). However, they have also recorded the grass land inoculum had suppressed the growth of *Terminalia superba* and therefore concluded that allochtonous AMF inocula may not be always effective (Onguene and Kuyper, 2005). Hence, the use of site adapted AM inocula may be the best inocula to be used in forest restoration. But one question not yet answered is; whether or not tree species seedlings require host-specific inocula for their better growth, survival, and establishment. There are now data to indicate plant species even those co-occurring may prefer to associate with distinct AM fungi communities (Tesfaye Wubet *et al*., 2006). Even more, one has to ask; does inoculating seedlings with inocula from seedlings rhizosphere or from adults rhizosphere deliver better positive results? This is because; conspecific seedlings and adults could prefer to associate with distinct AMF communities (Tesfaye Wubet *et al*., 2009).

The AMF richness in AMF inocula is considered to improve inocula effectiveness. Plant response is substantially lower when inoculated with single AMF species and the response keeps increasing from multiple fungal species to whole-soil inoculums (Hoeksema *et al*., 2010). Compiling the long years of experience in AMF research, Barea *et al*. (2011) recommend the use of autochthonous foundation shrub inoculated with autochthonous AMF consortia inoculums for the best way of Mediterranean forests restoration. The shrubs not only serve as foundation species, but also serve as AMF resource island (Barea *et al*., 2011).

Selecting a few dominant AMF species, multiplying them, and applying as inoculums (which is the most common way) may result in negative effects on plant growth since the inoculum can disrupt indigenous AMF community structure and create competition among AMF and could result in inoculum failure (Janoušková *et al*., 2013). Therefore, in areas with low levels of indigenous AMF abundance, multiplying all and not only the dominant AM fungi species may be the best way of preparing AMF inocula. However, the application of AMF species-rich inocula is not generally agreed; some argue that better results due to inocula with better AMF species richness is due to sampling effect, and selecting single effective AMF species should get the attention of restoration ecologists. Sampling effect is briefly explained earlier (section 2.3.2.8.).

The other challenge associated with AMF biotechnology is related to inocula production for large-scale applications. This is due mainly to the obligate nature of AMF. AMF cannot be cultured axenically, i.e., without a host (Azcón-Aguilar *et al*., 1999; Fortin *et*

al., 2005). But, monoxenic culturing of AMF (using the root cuttings) has been made possible a few decades ago (Bécard and Fortin, 1988). In India, using the monoxenic *in vitro* culturing, large-scale industrial production of biologically clean AMF inocula has been possible (Adholeya *et al*., 2005). However, monoxenic *in vitro* AMF culturing is not widely practiced since it demands high technology and skill, and not all AMF species are readily culturable using the available technique (Fortin *et al*., 2005). The techniques about large-scale monoxenic *in vitro* AMF culture production are explained by Adholeya *et al.* (2005) and Cranenbrouck *et al.* (2005).

The conventional methods of inocula production like the use of substrate-based pot culture and substrate-free methods of hydroponics and aeroponics techniques are also costly and large scale application of AMF inocula may hardly be possible. Therefore, managing the *in-situ* AMF is sometimes considered to be an effective AMF biotechnology for the restoration of degraded lands. The available research reports also indicate that short fallow could equally improve plants growth and productivity to that of AMF inoculation (Lekberg and Koide, 2005). It also has been shown that an obligately arbuscular mycorrhizal pioneer nurse shrub *Lavandula stoechas*, by increasing among others, *in situ* AMF abundance and infectivity, significantly improved the field survival and establishment of *Cupressus atlantica* seedlings (Duponnois *et al*., 2011). Kumar *et al*. (2010) also compared different plant composition effects on *in situ* management of AMF on a degraded coal mine spoil and they were able to record significantly improved AM fungi abundance, diversity, and infectivity when the cover crops were mainly grasses and N-fixing shrubs. Hence, AMF can be manipulated by fallowing or/and by designing

the plant species composition so that AMF abundance is increased which could be important to facilitates forest restoration. However, some investigations indicated that grass cover can significantly suppress tree/shrub seedlings-saplings growth (Riginos, 2009) or may have seasonally variable effects (Good *et al*., 2014). Therefore, investigation on cover plant management options to effectively manage AMF and facilitate tree/shrub seedlings growth is according to Fisseha Asmelash et al. (2016), an important research topic.

Nowadays, to make AMF inoculation less costly, substrate-free inocula preparation methods and *in vitro* production on excised plant roots are being intensively researched (Ijdo *et al*., 2011). The conventional inocula preparation method, although labor-intensive and costly, can be a source of employment, especially in developing countries. Meanwhile, AMF inoculation biotechnology will remain to be a feasible way of forest restoration in most parts of the world (Fisseha Asmelash et al, 2016). Figure 4 shows the simplified schematic model of forest restoration using AMF.

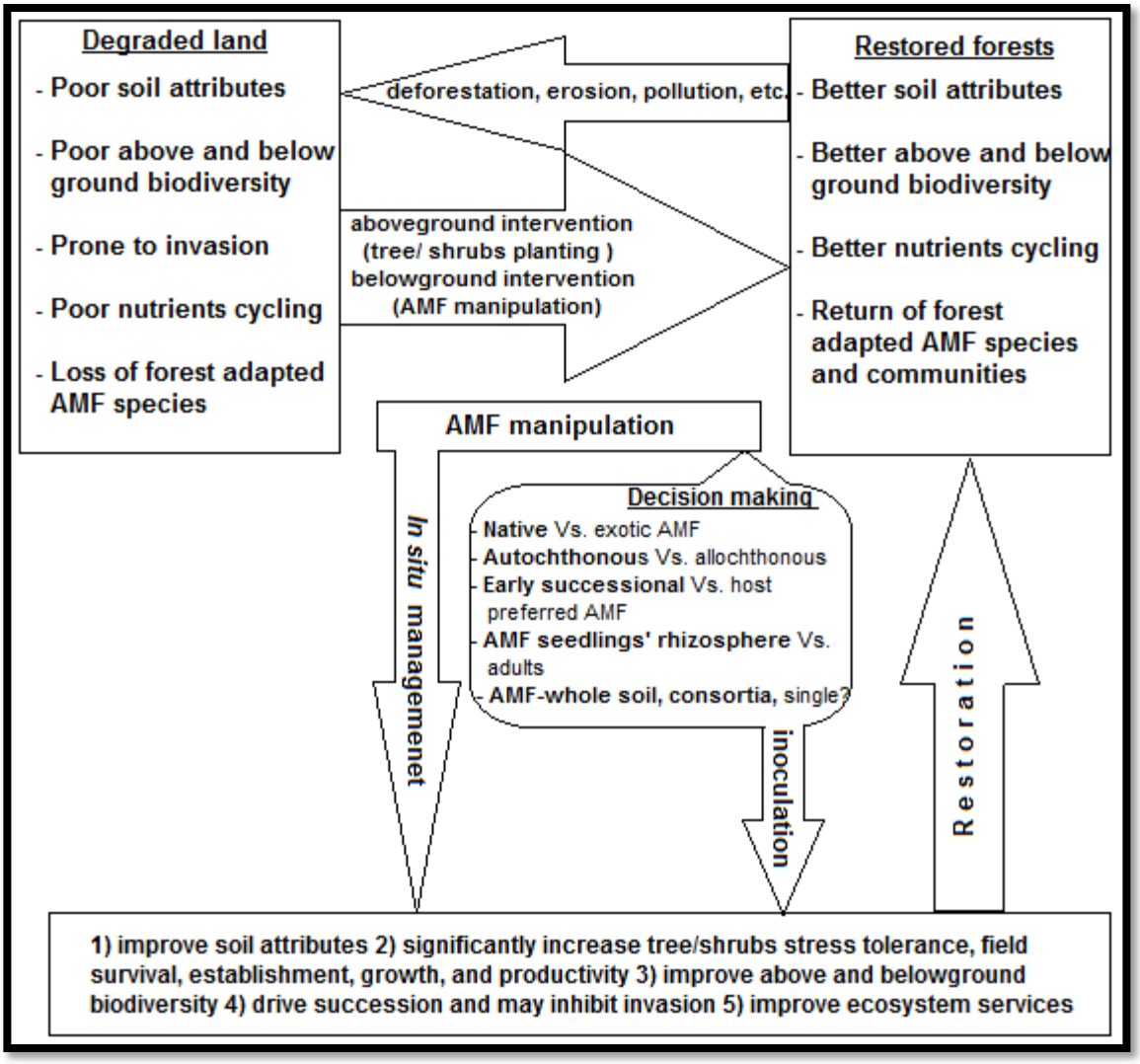

Figure 4: A simple schematic model to show forest restoration through the application of AM fungi (adapted from Fisseha Asmelash et al, 2016).

2.5. The state of arbuscular mycorrhiza research in Ethiopia

In Ethiopia, although mycorrhiza research in agronomy can go back to the mid-1980s (Tekalegn Mamo and Killham, 1987), it was in the early 1990s that Anders Michelsen from Copenhagen University conducted the first research on mycorhiza relevant in forestry. Michelsen conducted nursery AM inoculation trials to know the effects on nursery growth and field survival of *Acacia abyssinica* and *Acacia sieberiana* seedlings

(Michelsen, 1993a). Again, Michelsen and Sprent (1994) examined the effect of AM fungi inoculation on nitrogen fixation of legume trees of Ethiopia.

Since then, not much of tree arbuscular mycorrhiza inoculation studies have been conducted in Ethiopia. The latest of such studies are the studies by Emiru Birhane *et al.* (2012, 2014, and 2015). Emiru Birhane *et al.* (2012) have examined the effects of native AM consortia inoculation on photosynthesis rate, water use efficiency, and growth of *Boswellia papyrifera* seedlings grown under mimicked rainfall shortage. Accordingly, they found out that inoculation had significant positive effects on all the measured variables. Emiru Birhane *et al.* (2014) examined the effects of native AM consortia inoculation on tree seedlings' competitive ability using *Acacia etbaica* and *Boswellia papyrifera* and found out that AM inoculation did not increase the competitive ability of tree seedlings under study. Emiru Birhane *et al.* (2015) have examined the effects of native AM consortia inoculation and moisture availability on seedlings nutrient uptake, biomass, and root mycorrhizal colonisation of *Acacia senegal, A. etbaica,* and *Boswellia papyrifera*. Their finding showed that at high moisture level the fast-growing *Acacia* spp. benefited more from the AM inoculation while at lower moisture level slow-growing *Boswellia papyrifera* benefited.

Michelsen (1993b) has also pioneered ecosystem-level research on AM fungi association in Ethiopian forests. The authors further investigated the mycorrhizal status of 28 epiphytic plants of the Bale Mountains National Park and found out that only 7 of the 28 epiphyte species were colonized by arbuscular mycorrhizal fungi, of which, the three

facultative epiphytic species were with the highest colonization. Latter, Tesfaye Wubet *et al*. (2003a) carried out a research in three of the dry Afromontane forests of central and eastern Ethiopia. Their research focused on 11 of the most ecologically and economically important native tree species of the DAF of Ethiopia intending to morphologically determine if these tree species were mycorrhizal or not. They reported that these tree species were all arbuscular mycorrhizal and they generated data on the mycorrhizalness of the seven species for the first time.

After some seven years, another important ecosystem-level AM research was conducted in three woodlands of northern Ethiopia by Emiru Birhane *et al.* (2010). The authors conducted their research in the combretum-terminalia and acacia-comiphora ecosystem of northern Ethiopia with the main objectives of determining if woody species of these ecosystems were arbuscular mycorrizal or not and morphologically identifying the AM fungi genera associated with the woody plants of the ecosystems. They investigated 43 woody plants and found out that all were mycorrhizal with the *Glomus* genus being the predominant fungus. The investigators further noted also, 17 of the studied species were arbuscular mycorrhizal for the first time.

Morphological studies on AM association in different land uses and species had also been conducted. Michelsen (1992) has investigated the mycorrhizal status of tree nursery seedlings in Ethiopia, Zebene Asfaw (2003) has also morphologically investigated AM associations on traditional agroforestry system of southern Ethiopia. Diriba Muleta et al. (2008) investigated factors affecting the AMF spore abundance in the southwestern

Ethiopia *Coffea arabica* farming systems. Accordingly, they have found that sampling points, sites and depths, shade tree species and shade tree/coffee plant age significantly affected AMF spore abundance. Moreover, they have found that, compared with coffee monoculture system, coffee agroforestry system maintained a higher AMF spore abundance and more importantly, at the lower soil depth. Similarly, Tadesse Chanie and Fassil Assefa (2013) have morphologically investigated the AM fungi abundance and diversity in the rhizosphere of *Coffea arabica* shade trees of southwestern Ethiopia. Zerihun Belay *et al*. (2013) morphologically investigated AM fungi associations with the Acacias of the rift valley. Mengsteab Hailemariam *et al*. (2013) morphologically investigated the role of agroforestry trees to transfer infective AMF to the associated annual crop. Mengsteab Hailemariam *et al*. (2014), using the trap plant maize, investigated the AMF infctivivity of *Faidherbia albida* rhizospheric soil collected from different land-use types, viz., area exclosure, grazing, and cultivated lands. Zerihun Belay *et al.* (2015) investigated the diversity and abundance of AM fungi across different land-use types. They also compared the AMF abundance and diversity of soils from these land uses versus trap culture. Accordingly, they have found out that land uses with diversity in plants had better AMF abundance and diversity. They have also found out that AMF abundance and diversity varied between site collected and trap culture soils.

The use of molecular techniques generated valuable new information to AM fungi research in Ethiopia. The first publication on molecular-based AM fungi resource base assessment study was by Tesfaye Wubet *et al.* (2003b) and they identified the AM communities associated with *Prunus africana* of the dry evergreen Afromontane forest

ecosystem of Ethiopia using the molecular technique. Latter in 2006, another publication of molecular technique based study on AM fungi association of Ethiopian native trees was published (Tesfaye Wubet *et al.*, 2006). The authors compared AM communities associated with *Podocarpus falcatus* and *Juniperus procera* from two forests of the dry evergreen Afromontane forest ecosystem of Ethiopia. The finding showed that AM communities were significantly variable between sites and co-occurring species as well. A similar article was published by Tesfaye Wubet *et al.* (2009) that compared the AM communities associated with *Olea europaea*, *Prunus africana* and *Podocarpus falcatus* in two of the DAF of Ethiopia. Additionally, they compared the AMF communities of the rhizospheres of adults and seedlings of the same species. Their finding showed that AMF communities significantly differed between sites and tree age (seedlings and adults) as well. Using the molecular technique, De Beenhouwer et al. (2015) determined the AMF communities associated with the roots of *Coffea arabica* in the southwest Ethiopian highlands. These authors also evaluated the soil, spatial location and elevation effects on the AMF diversity and composition. According to their results, soil variables were found to be more important determinants of AMF diversity and composition than spatial location or elevation.

In recent years also, there have been a handful of AMF research activities conducted in Ethiopia (e.g. Beyene Dobo et al., 2016; Emiru Birhane et al., 2017&2018; Yoseph Tewodros et al., 2017; Fisseha Asmelash et al., 2019). However, it can be concluded that AMF research and collection in Ethiopia is at its infant stage. Therefore, both AMF research and the collection of beneficial AMF species should get sufficient attention.

Meanwhile, this Ph.D. work could contribute significantly by generating new knowledge regarding AMF pertinent to DAF restoration.

2.6. Factors enhancing tree seedlings field survival and establishment on degraded dry lands

Two of the most important morphological and physiological traits that could significantly improve seedlings' field survival and establishment on degraded dry sites are; having a bigger rooting depth (Lobet et al., 2014; Trona et al., 2015) and the accumulation of more non-structural carbohydrates (NSCs) (O'Brien *et al*., 2014). The other most important morphological trait and that has often been considered to be the best single predictor of field survival and establishment of seedlings' on degraded dry sites is having a bigger collar diameter (Grossnickle, 2012; Haase, 2007). Plants also adjust partitioning and having bigger root:shoot (R:S) or root:plant (R:P) dry mass ratios are also important physiological traits desirable by seedlings in order to improve their survival and establishment on multiple resource-limited sites (Comas et al., 2013).

The NSCs can be directly measured in the laboratory or can be determined indirectly by measuring leaf area, photosynthetic rate, and root system size (Villar-Salvador et al., 2015). Since the root system size is directly proportional to the collar diameter, it can be inferred from the collar diameter (Grossnickle, 2012). Therefore, good quality seedlings desirable in DAF restoration should be having bigger R:S and R:P dry mass ratios and they should also be having a bigger rooting depth, collar diameter, and leaf area among others. Hence, in this Ph.D. work, the effect of whole-soil AMF inoculation on the

performance of *C. africana*, *J. procera*, and *P. falcatus* was evaluated by measuring these seedling growth and quality variables.

3. METHODOLOGY

The AMF nursery survey was carried out in ten of the DAF nurseries (i.e., nurseries that are suitable for DAF restoration) in the central and northern Ethiopia. The AMF resilience study was carried out in the Chilimo forest found in the Oromiya region, central-west Ethiopia. The AMF inoculation experiment was carried out in the mesh-house at the EBI (Ethiopian Biodiversity Institute) after seedlings were trained in the greenhouse at FRC (Forestry research Center), Ethiopia.

3.1. The survey of the AMF status in ten of the DAF nurseries

3.1.1. Seedlings collection

Severe loss of DAF occurs in the central and northern parts of Ethiopia. Massive restoration activities have also been carried out in these areas. Therefore, we selected nurseries from those locations (i.e. Addis Ababa, north Shewa and south Wollo of the Amahara region, west Shewa and west Hararge of the Oromiya region, and south Tigray) to carry out this study (Fig. 5). Since, no nursery raising native tree species was identified in north Wollo, we were, unfortunately, not able to include nurseries from this admistrative zone. Data and seedlings were collected from May 1-30, 2018. Collecting seedlings at this point of the time in the year was relevant such that the RC and SA of seedlings would be determined just before seedlings field planting in June-July.

Nurseries that raised native trees and shrubs were listed in collaboration with relevant actors from the selected districts. Then, from the listed nurseries, one nursery from each of the administrative zones was randomly selected. Moreover, considering the proportion

of nurseries raising native trees, except for south Tigray and south Wollo, one nursery each was added randomly. Therefore in this study 10 randomly selected tree nurseries were surveyed. From the selected nurseries, 16 seedlings were collected per each evaluated species; *Acacia abyssinica* (family Fabaceae), *Cordia africana* (family Boraginaceae), *Dovyalis abyssinica* (family Flacourtiaceae), *Hagenia abyssinica* (family Rosaceae), *Juniperus procera* (family Cupressaceae), *Millettia ferruginea* (family Fabaceae), *Olea europaea* subsp. *cuspidata* (family Oleaceae), *Podocarpus falcatus* (family Podocarpaceae), and *Prunus africana* (family Rosaceae). *C. africana* and *O. europaea* were collected from six different nurseries, *J. procera* from five, *D. abyssinica*, *H. abyssinica* and *P. falcatus* from two while *A. abyssinica*, *M. ferruginea* and *P. africana* only from one nursery. For each of the collected seedlings, age, pot height (PHI), pot diameter (PD), seedling height (SHI), and root collar diameter (CD) were measured and recorded (Annex 2). Furthermore, general information such as the name of the nursery, location, geographic coordinates, elevation, potting substrate ratio, ownership data, and name of all tree and shrub species being raised were recorded (Table 2; Annex 1). Climate data relevant to each of the nurseries was also gathered from online resources (Table 2).

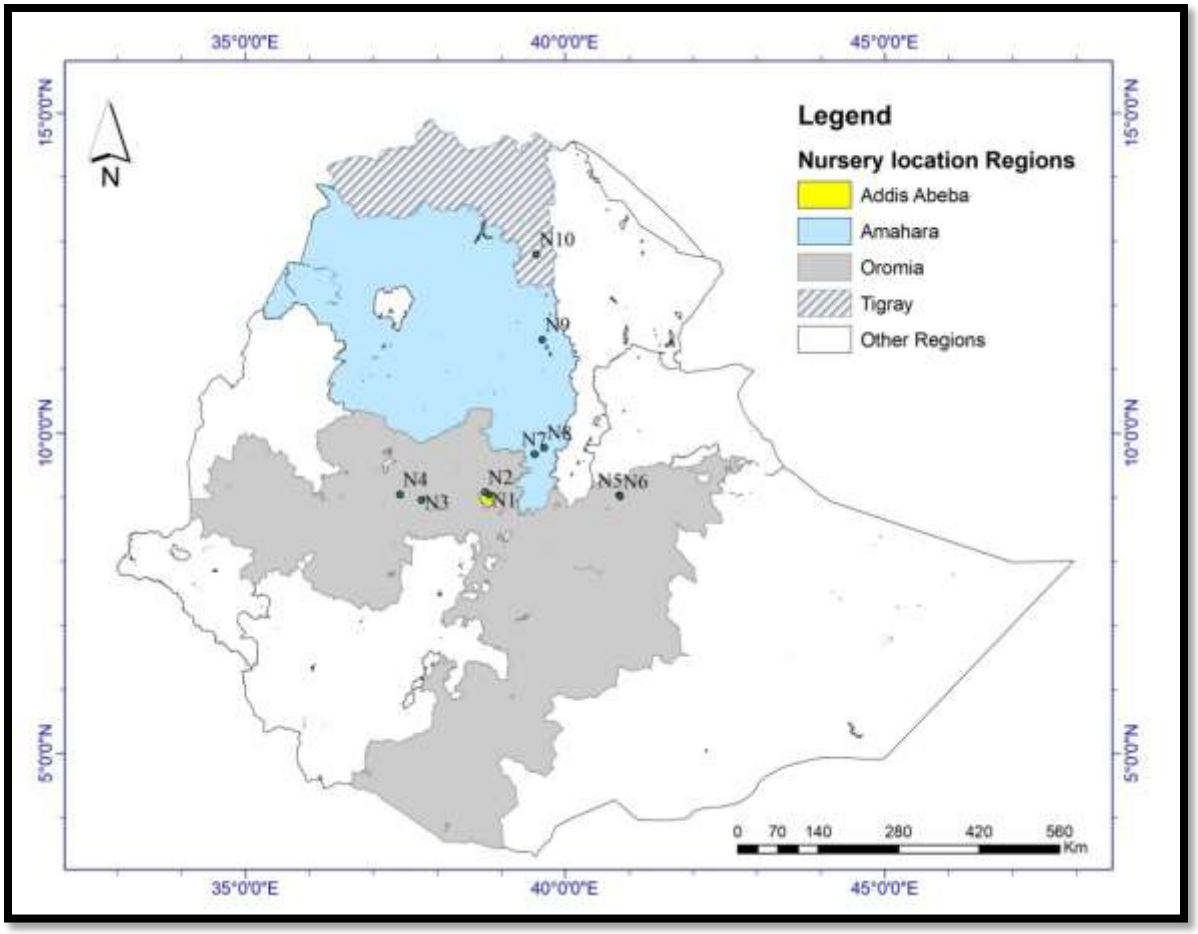

Figure 5: Location of the nurseries selected for this study

Table 2: Description of the ten DAF nurseries surveyed.

Code	Name of the nursery	Location	Geographic coordinate		Elevation	Climate			Pot soil composition	Owner
			North	East		MAT (°C) [1]	MAR (mm) [1]	Köppen climate class [2]		
N1	Susuni	Addis Ababa	9.037944°	38.816°	2515	15.7	1235.9	Monsoon-influenced temperate oceanic (Cwb)	3:1:1 (TS:S:C)	GO
N2	Entoto	Addis Abeba	9.080361°	38.75219°	2687	15.7	1235.9	Monsoon-influenced temperate oceanic (Cwb)	4:1:1 (TS:S:C)	GO
N3	Teferi & Mulugeta	Tokae kutay/west shewa	8.958833°	37.75744°	2028	16.3	1449.4	Warm-summer Mediterranean (Csb)	10:5:3 (TS:M:S)	Private
N4	Sokondo	Cheleya, west shewa	9.042833°	37.42175°	2309	17.1	1623.4	Warm-summer Mediterranean (Csb)	3:2:1 (FS:C:S)	GO
N5	Jello	Chiro, West hararge	9.030611°	40.85044°	2171	23.2	843.4	Tropical savannah (Aw)	12:1:1 (FS:S:C)	GO
N6	Qebenewa	Chiro, West hararge	9.011139°	40.86075°	2509	23.2	843.4	Tropical savannah	10:5:1 (FS:C:S)	GO

								(Aw)		
N7	Debre birhan teachers college	Bosenawerana, north shewa	9.672333°	39.521391°	2759	16.3	1157.7	Monsoon-influenced temperate oceanic (Cwb)	3:1:1 (TS:S:C)	NGO
N8	Mush	Bosenawerana, north shewa	9.773°	39.67225°	2945	16.3	1157.7	Monsoon-influenced temperate oceanic (Cwb)	3:2:1 (FS:C:S)	GO
N9	Tis aba lima	Ambassel, south wollo	11.4605°	39.64417°	1493	18.9	1030.3	Monsoon-influenced humid subtropical (Cwa)	2:2:1(FS:S:C)	GO
N10	Hizba teklehaimanot	Enda mehoni, south Tigray	12.79417°	39.55339°	2341	23.3	600.3	Hot semi-arid (BSh)	3:1:1(FS:S:C)	GO

MAT=Mean annual temperature, MAR=Mean annual rainfall, GO=Government organization, NGO=Non-government organization, FS=Forest soil, TS=Topsoil, S=Sand, C=Compost, M=Manure.

[1]Source: https://climatecharts.net [accessed 02.08.19]; based on 1987-2016 weather data

[2]Source: https://en.wikipedia.org/wiki/K%C3%B6ppen_climate_classification [accessed 02.08.19]

3.1.2. Root AMF colonization determination

Eight seedlings per species were used for root AMF colonization (RC) determination. This was similar to the number of seedlings used in Michelsen (1992). At the field, plastic pots were carefully cut with a cutter and each seedling was soaked in water to gently detach the root system from the soil. Then, the roots were carefully washed with tap water and fine roots were cut into pieces~1 cm long. These fine roots were mixed and preserved in 50% ethanol in tightly sealed vials and were transported to Addis Ababa University ecology and ecophysiology laboratory where they were kept at room temperate (18-23°C in our case) until RC was determined within a month time.

Root colonization was determined on three replicates of 2-3g fine root subsamples per species and for 200 gridline interaction points (Giovanetti and Mosse, 1980). Grid of 2.54 cm x 2.54 cm was first marked beneath the 90 mm diameter plastic Petri dish with a marker. AMF staining was performed by the root staining and de-staining technique using ink and vinegar, based on Vierheilig et al. (1998). To clean the preserved roots from alcohol, they were thoroughly washed with tap water. To clear the root cortical cells materials (nuclear and cytoplasmic) and enable maximal stain penetration (Mitiku Habte and Osorio, 2001), fine root samples were autoclaved for 10 minutes in 10% KOH in tightly sealed vials (Brundrett et al., 1996). Clearing *Juniperus procera* seedlings roots was however not possible by using 10% KOH or by using 10% H_2O_2 or 10% household bleach after 10% KOH. Soaking the roots in 10% KOH for ten days at room temperature did not clear them either. Clearing was only possible by using the modified alkaline hydrogen peroxide method described by Brundrett et al. (1996). Hence, without the use

of 10% KOH, *J. procera* roots were directly soaked in 0.5%NH_4 + 0.5% H_2O_2 (1:1 volume) and were left on a shaker overnight at medium speed. Once the roots were cleared, they were stained by soaking them in 5% ink (Hero black ink, made in China) in vinegar (common food grade white vinegar) overnight at room temperature. They were then de-stained by rinsing the stained roots in tap water acidified with a drop of vinegar for a minimum of 20 minutes and further rinsing it in tap water until RC was determined after a few minutes or hours using a light dissecting microscope (CETI Steddy Stereo Binocular Microscope) at 5.5x magnification. Roots were considered to be colonized with AMF if stained structures of arbuscules, vesicles, aseptate hyphae (extraradical and intraradical mycelia), and/or intraradical spores were observed (Annex 3).

Black Hero ink (China) was used for AMF staining because it was found to give very good contrast (Fig. 6). At the time of this investigation, the only fountain pen inks that were found in the Ethiopian market were permanent black Parker-Quink (France), washable blue Hero (China), and permanent black Hero (China) inks. Hence, these three inks were compared using *Olea europaea* subsp. *cuspidata* seedlings root. *Olea europaea* was chosen because, compared to the remaining tree species, surplus fine root was available. Accordingly, black Hero (China) ink was found to be the most suitable (Fig. 6). Since, Trypan Blue, which has been commonly used to stain AMF structures of roots by most researchers in Ethiopia and worldwide is now considered to be carcinogenic (Vierheilig et al., 2005), our use of ink is justified. Besides, Trypan Blue is much more expensive and is rarely found in Ethiopian markets.

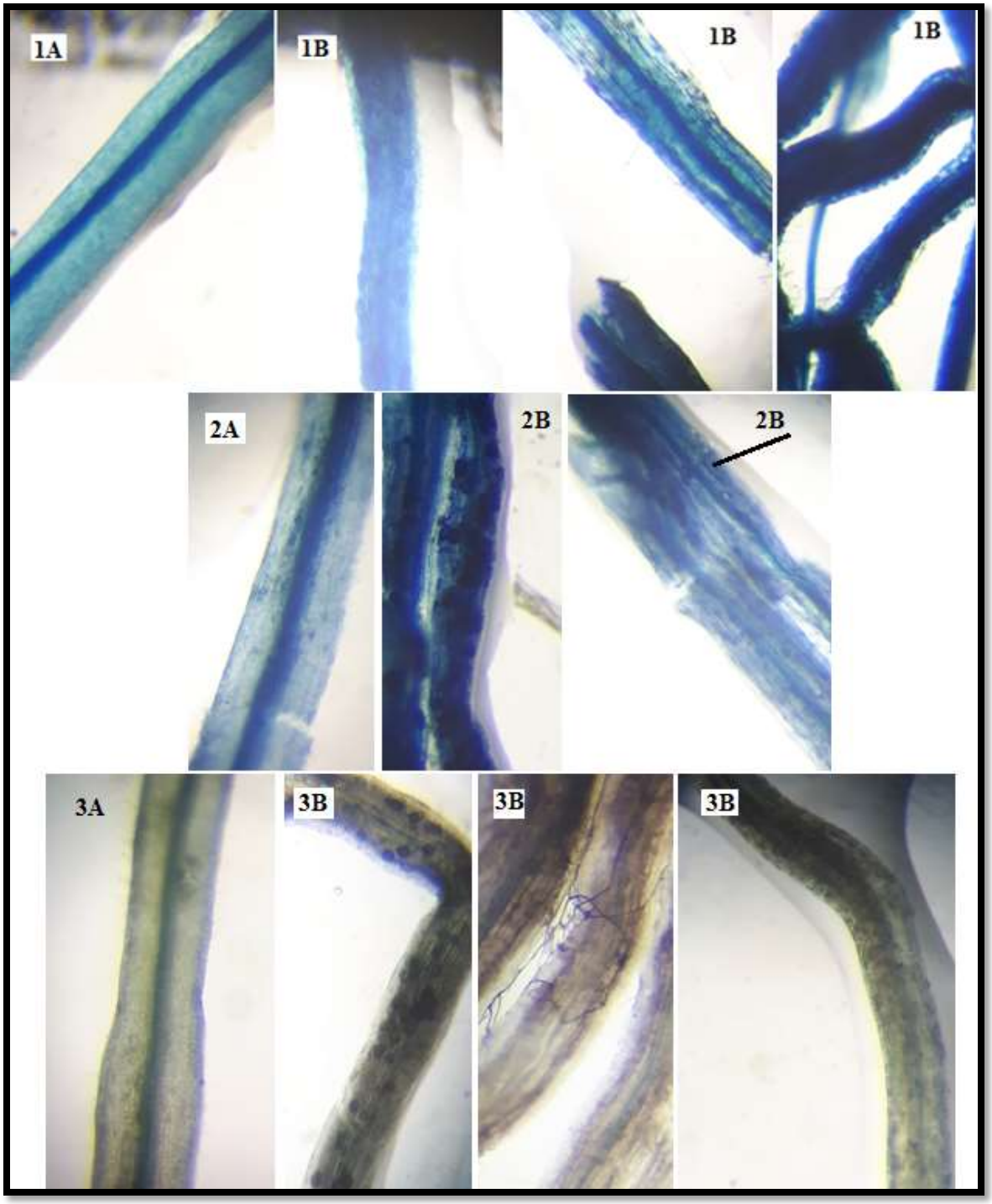

Figure 6: Roots of *Olea europaea* subsp. *cuspidata* with arbuscular mycorrhizal fungi structures stained using 1) washable blue Hero ink, 2) black Parker-Quink ink, and 3) black Hero ink. Black Hero ink resulted in better contrast where a) indicates root not colonized with AMF and b) indicates root colonized with AMF. Note: all pictures have similar scales.

3.1.3. Spore abundance determination

The remaining eight seedlings per species were used for spore abundance (SA) determination. In the field, seedling shoots were cut and plastic pots removed, and the soil containing the roots was placed in plastic bags (eight seedlings per bag), gently broken, mixed and transported to Addis Ababa University (AAU). At AAU, the soil was allowed to air dry for a few days and then was thoroughly mixed to form one composite sample. SA was determined in three replicates by taking 50 g subsamples from each composite soil sample. Spores were extracted from soil by wet-sieving (1 mm, 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 90 mm plastic Petri dish according to INVAM protocol (https://invam.wvu.edu/methods/spores/enumeration-of-spores [accessed 10.01.19]) 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}).

3.2. The resilience of AMF to the degradation and deforestation of the Chilimo forest

The Chilimo-Gaji forest, commonly called Chilimo forest, is one of the few DAF in the central part of 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, the forest comprises of 12 forest patches that are managed through the participatory forest management (PFM) scheme (Annex 4). Geographically, Chilimo forest is located within 38.09° to 38.2°E longitude and 9.04° to 9.095°N latitude with the forest currently found within 2340-2960 elevation (Fig.7). The mean annual temperature ranges between 15 and 20°C, while the mean annual precipitation is 1264 mm (Mehari Tesfaye et al., 2016). Based on the available literature, the soil types of Chilimo are mainly Vertisols, Luvisols, and Cambisols (Teshome Soromessa and Ensermu Kelbessa, 2014).

Chilimo, to mean darkness, reflects the denseness of the forest at one time (Mohammed and Inoue, 2012). Currently, however, the forest is severely threatened by human activities and in only 42 years, around 54% of the forest has either been deforested and become settlement (1%), cultivated land (26%) or bare land including grazing lands, quarry and roads (9.8%) or has been degraded to be changed to shrubland (17.3%) (Terefe Tolessa et al., 2017). Hence, the remaining forest cover is estimated to be 1952ha 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; Mehari Tesfaye et al., 2016).

3.2.1. Soil sampling

In mid-February 2019, a reconnaissance survey was carried and sampling design determined. Hence, in this study, owing to the fact that the four land uses were not adjoined and grazinglands were present in dispersed patches, stratified systematic sampling (Kershaw Jr. et al., 2017) was employed. Using the mobile phone application, google map, it was determined that the natural forest lays within the elevation range of 2340-2960 m and therefore, to caprure as much variation as possible, plots location in the natural forest was determined to be at; low (2400-2430 m), mid (2600-2630 m), and high (2800-2830 m) elevations. This was also applied for cropland. For shrub land and grazing land however, at the high and low elevations of this strata, shrub land and grazing land respectively, were not found. Therefore, for shrub land, plots location were at 2400-2430 m, 2500-2530 m and 2600-2630 m to represent the low, mid and high elevations while for grazing land, two grazing lands at the low elevation (2400-2430 m) with a different location and cover vegetation and another at 2800-2830 m were selected for sampling (Table 3; Fig. 8; Annex 5).

Data collection took place from late-February to early-March 2019, during the dry season. From each stratum of the four land-use gradients, composite soil samples were collected from five (1 m x 1 m) plots with 50 m spacing 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 (0-10 cm), 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 determination while 700g subsamples were used for the greenhouse bioassay and the remaining 225g subsamples were used for soil physicochemical property determination.

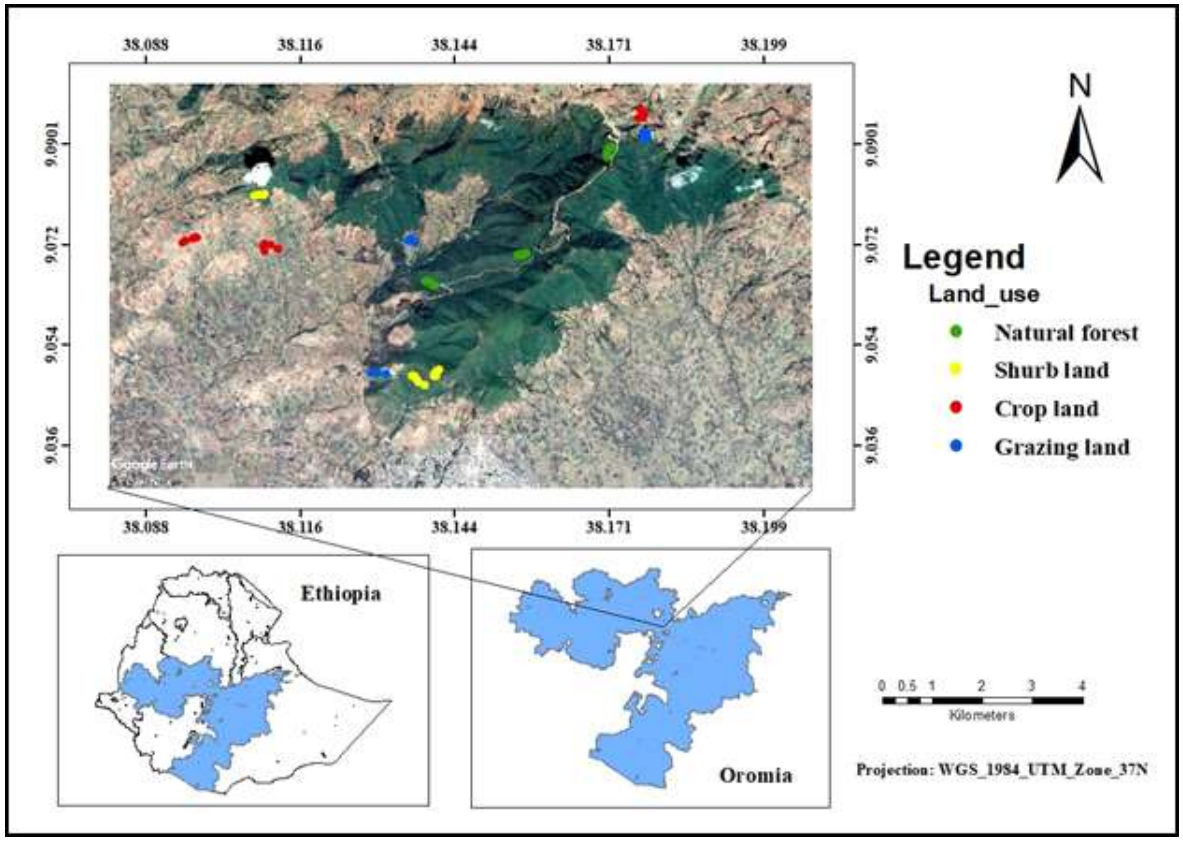

Figure 7: Map showing land use features of Chilimo and location of the sample plots

Table 3: The characteristics of the four land-use gradients studied. NF=Natural forest, ShL=Shrubland, CrL=Crop land, and GrL=Grazing land

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 farm lands or grass lands open for grazing that are dominantly covered with *Pennisetum clandestinum* (a) or *Pennisetum sphacelatum* (b)	Overgrazing

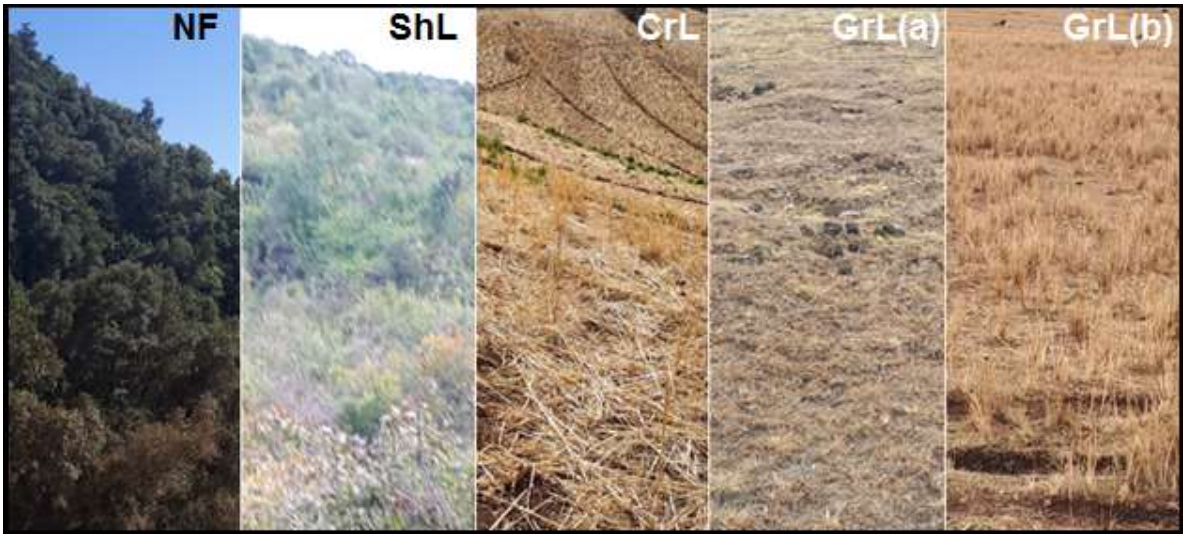

Figure 8: Land use gradients studied. NF=Natural forest, ShL=Shrubland, CrL=Cropland, and GrL=Grazing land covered with *Pennisetum clandestinum* (a) or *Pennisetum sphacelatum* (b)

3.2.2. Soil bulk density, texture, pH, EC, total nitrogen, available phosphorus, organic matter, cation exchange capacity, P:N, and C:N determination

Bulk density, soil texture, pH, and EC were determined at ecology and ecophysiology laboratory at the Addis Ababa University, Ethiopia. Bulk density was determined using a modified clod method (Blake, 1965). Since intact core soil sample collection was not possible, *in-situ* owing to the dryness and fragility of soil, sub-samples of the collected, sieved (≤2.5 mm) soil were used to form a clod by putting the equal volume of soil subsamples (130 ml) in bottom perforated plastic pots and watering them to field capacity for a week. After a week of watering, the new soil surface height of soil in each pot was marked and then the pots were brought to the lab and allowed to air dry for two weeks, and finally, the soil was dumped and oven-dried for 24 hours at 105°C in two cycles. The volume of the clod was determined by pouring water up to the marked soil surface height upon emptying of the pots for oven drying (the bottom perforation was plastered before pouring water). Hence, bulk density was determined by dividing the oven-dry weight of the clod by its volume. 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 20 g of sieved soil subsamples with 50 ml distilled water (1:2.5 (w:v)) following Cottenie (1980). The soil mixture was initially shacken 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).

Total nitrogen, available phosphorus, organic matter, cation exchange capacity were determined at Debrezeit Agricultural Research Center, Ethiopia. Total nitrogen (N) was determined on 2 g air-dried soil subsamples by digestion, distillation into boric acid, and titration of the resulting solution with hydrogen chloride (Bremner, 1965). Plant available phosphorus (P) was determined on 1g air-dried soil subsamples after Bray-II extraction (Bray and Kurtz, 1945) and spectral absorbance measurement (882 nm) of the resulting supernatant solution by using a spectrophotometer. Organic carbon (OC) was determined by oxidizing 1 g 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 conversion factor (Anderson and Ingram, 1993). Cation exchange capacity (CEC) was determined on 10 g air-dried soil subsamples according to Chapman (1965). The 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.

3.2.3. Spore abundance and Greenhouse bioassay

Spore abundance was determined by taking 50g air-dried soil subsample of each composite soil sample and following the procedures outlined in section 3.1.3. AMF infectivity was determined by the greenhouse bioassay method (Moorman and Reeves, 1979; Abbott and Robson, 1991) using the INVAM recommended host plant, *Zea mays* L. (https://invam.wvu.edu/methods/infectivity-assays/mean-infection-percentage-mip). *Zea mays* (Melkassa-4 variety) seeds were carefully disinfected with 5% household bleach for 10 minutes and allowed to germinate on filter paper. Then, one germinated seed was sawn on a 700g soil subsample collected from each plot (Fig. 9). After growing the *Zea mays* for 6 weeks, the shoot was cut off and the soil carefully washed off the roots to prepare them for AMF root colonization determination. AMF colonization was determined after roots were cleared with 10% KOH and following the ink and vinegar technique as described above in section 3.1.2.

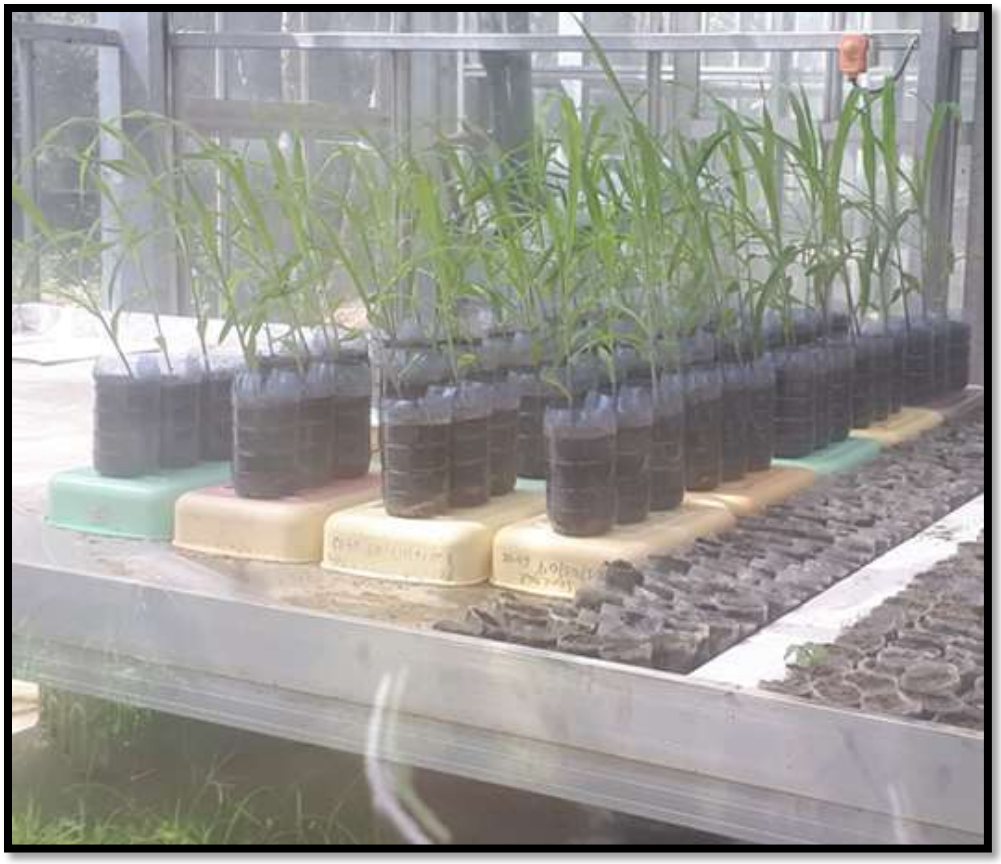

Figure 9: The greenhouse bioassay setup

3.3. Mesh-house AMF inoculation experiment

3.3.1. The study species

The mesh-house experiment was carried out on three of the economically and ecologically important Ethiopian native tree species namely, *Cordia africana, Juniperus procera*, and *Podocarpus falcatus* (Legesse Negash, 2010). Moreover, *J. procera* and *P. falcatus* were selected since they are DAF characteristic tree species and *C. africana* was selected because it is among the most widely raised native tree species in the DAF nurseries. Another characteristic DAF tree species selected for this experiment, *Olea europaea* subsp. *cuspidata*, did not germinate successfully in 2 cycles (3 months each) and hence, was, unfortunately, not included in this study.

3.3.1.1. *Cordia africana* Lam.

Cordia aficana is an early-successional tree species (Friis, 1992; Eshetu Yirdaw *et al*., 2002). It is an animal and bird pollinated, broad-leaved deciduous tree species that, depending on locality, grows from less than 9m to 30 m in height (Legesse Negash, 2010). It has a widespread distribution in Ethiopia growing within the altitudinal ranges of 550-2600m and a mean annual rainfall of 700-2000 mm (Friis, 1992). *Cordia aficana* grows well under moderate conditions but soil nutrient deficiency mainly P & N limits its growth potential. It has a very significant ecological (e.g nutrient cycling, bee forage, erosion control, and watershed management) and economical (e.g. high-quality furniture) benefits, and hence, it is considered to be one of the most highly valued tree species in Ethiopia (Legesse Negash, 2010). *Cordia africana* is known to be arbuscular mycorrhizal (Emiru Birhane et al., 2010; Tadesse Chanie and Fassil Assefa, 2013).

3.3.1.2. *Juniperus procera* (Hoechst.ex Endl.)

Juniperus procera has been described as a mid-successional tree species (Girma Abebe et al., 2010; DemelTeketay, 1997). It is an evergreen, wind-pollinated, dioecious coniferous (a gymnosperm) tree species. The juvenile individuals have conical crowns while the adult individuals have irregular and spearing crowns (Legesse Negash, 2010). It is a typical DAF characteristic tree species growing within the altitudinal ranges of 1100-3500 m and a mean annual rainfall of 500-1000 mm (Friis, 1992). It is a relatively slow-growing species and seedlings grow very slowly and establish hardly on N&P deficient soils and moreover, it is recognized as being severely degraded tree species which the food and agriculture organization (FAO) has considered it to be a conservation priority

tree species in Africa (Legesse Negash, 2010). Seed germination of *J. procera* was reported to range 23-60% in the laboratory and 12-30% on the nursery beds (Negash Mamo et al, 2006). Therfore, its asexual propagation from rooted cuttings could be considered an alternative way of propagation (Legesse Negash, 2010). *Juniperus procera* is known to be arbuscular mycorrhizal (Tesfaye Wubet *et al*., 2003a).

3.3.1.3. *Podocarpus falcatus* (thumb.) Mirb.

Podocarpus falcatus has been described as late-successional tree species (Girma Abebe et al., 2010; Demel Teketay, 1997). It is an evergreen, dioecious, wind-pollinated coniferous (a gymnosperm) tree species, and is the only representative of the family Podocarpaceae found in Ethiopia. The male cones are axillary; rod-shaped while the female cones are terminal and spherical (Legesse Negash, 2010). *Podocarpus falcatus* is heterorhizic, having two distinct root forms, i.e., long indeterminate roots and determinate nodules; modified root hair (Fig. 10; Dickie and Holdaway, 2010). According to the authors, root nodule formation in podocarps is thought to be an adaptation for arbuscular mycorrhization and efficient nutrient acquisition but little conclusive evidence is there.

Podocarpus falcatus is a characteristic DAF tree species but also grows well in the moist Afromontane forests (MAF) of Ethiopia (Friis et al., 1992). It is a very good timber tree, its seeds produce oil that is edible and medicinal (to treat Gonorrhea) (Demel Teketay, 2011). *Podocarpus falcatus* is a very important tree for microclimate amelioration and watershed management and beneath it, cold and clean spring water is produced; since it is

a very important feed for the Colobus monkey (*Colobus guereza*), its ecological benefit is thus significant (Legesse Negash, 1995 & 2010). Due to illicit cutting the genetic resource of *Podocarpus falcatus* is severely degraded and less than 1% of its previous populations are now remaining making it a conservation priority in Ethiopia (Legesse Negash, 1995). The species has a very irregular and slow germination rate and, due mainly to photoinhibition, its seedlings have a difficulty of establishing in degraded sites (Legesse Negash, 2010). Very promising researches have been conducted by this author and, now, its biology is very well understood. Hence, efficient ways of germinating the seed, producing rooted cuttings, and establishing it on degraded sites are made possible. *Podocarpus falcatus* is known to be arbuscular mycorrhizal (Tesfaye Wubet *et al*., 2003a).

Figure 10: A microscopic view of *P. falcatus* fine root with the nodule (also a modified fine root) indicated by the arrow (Photo by Fisseha Asmelash).

3.3.2. Seedling preparation and inoculation

Cordia africana seeds were purchased from Eden seeds enterprise, Addis Ababa and *J. procera* and *P. falcatus*, respectively, were obtained from EBI forest gene bank, Addis Ababa, and International Livestock Research Institute (ILRI), Addis Ababa. Seeds were prepared, sown, and looked after by strictly following the procedures outlined by Legesse Negash (2010). Hence, before sowing, *P. falcatus* seeds, the sclerotesta (the outer harder coat) was carefuly broken by using basalt stone. Seeds with the sclerotesta removed were immediately soaked in 5% household bleach for 10 minutes and further soaked in household food-grade vinegar for another 5 minutes (to neutralize the base) and finally, they were thoroughly washed using tap water. Then, the cleaned seeds were soaked in distilled water for 20 hours to initiate imbibition. Finally, the water was decanted and seeds, with their mouth in the upside direction, were sown on plastic pots (7 cm diameter and 8 cm height) filled with sterile sand. Seeds were sown by inserting not deeper but just below the sand surface. The seeds of *C. africana* and *J. procera* were also cleaned similarly to that of *P. falcatus* and were also sown by drilling them in the sterile sand. For *P. falcatus* and *J. procera* respectively, 2 and 3 seeds were sown per pot. The sand was sterilized by cooking for 30 minutes in two cycles of 24 hours interval according to Legesse Negash (2010).

When seedlings developed well enough, 24 individuals with comparable sizes (initial heights and collar diameters were checked statistically and were found to have no significant difference) were selected per species for the inoculation experiment. AMF inoculation was done when transplanting these seedlings to the 1-liter plastic pots. The

substrate soil used in this experiment was collected from within the degraded gaps of the Ankorcha forest, Addis Ababa (Fig. 11). Ankorcha forest was once covered with DAF but went through severe degradation and later, as part of the Addis-bah project, was rehabilitated with Eucalyptus (Horst, 2006). Currently, Ankorcha forest is mainly composed of Eucalyptus trees but it also has the native tree and shrub species including *J. procera*, *O. europaea*, and *Dovyalis abyssinica* and is severely degraded (Fig.11). Ankorcha forest is among the Addis Ababa city's Peri-urban forests and is protected by the city administration's Environmental Protection Authority (AAEPA). The substrate soil has pH= 6.245±0.015, EC= 32.35±0.35 dS/m, TN= 0.07%, P (Bray-II) = 4.74 ppm, OM= 5.36%, CEC= 19.04 mequi/100 g, and AMF spore abundance=13.95±1.6 g^{-1}.

Figure 11: Partial view of the Ankorcha state forest (Photo by Fisseha Asmelash).

According to the meta-analysis result by Hoeksema et al. (2010), whole-soil AMF inocula were found to be more effective than inocula produced by single or multiple AMF species alone. Therefore, in this study, AMF inoculation was done by using whole-soil AMF inocula. Hence, rhizospheric soils at a depth between 10 and 30 cm were collected from *C. africana* and *J. procera* adult trees at a distance of 1 m further away

from the trunks (Zarik et al., 2016) and were used as whole-soil AMF inocula. We wanted to make inocula to be the rhizospheric soils beneath conspecific tree species found in Ankorcha forest with comparable soil physicochemical property. However, this was not possible since there were no *C. africana* and *P. falcatus* individuals found in Ankorcha forest. Hence, *C. africana* seedlings were inoculated with *C. africana* rhizospheric soil collected from EBI while both *J. procera* and *P. falcatus* seedlings received rhizospheric soil of *J. procera* collected from Ankorch forest.

3.3.3. Experiment setup

In this experiment, there were two levels of AMF inoculation (+AMF and –AMF) and two types of substrate (sterile degraded DAF soil and non-sterile degraded DAF soil). Hence, the experiment was a two by two factorial, i.e., two AMF inoculation, two soil types, and six replication (2x2x6), design. Hence, the total number of seedlings used was 72, i.e., 24 per species. Seedlings with "+AMF" received 45 g whole-soil AMF inoculum while those with "–AMF" received 40g heat sterilized whole-soil AMF inoculum and 40ml microbial filtrate (Onguene and Kuyper, 2005). The microbial filtrate was obtained following the procedure by Pánková et al. (2014). Hence, the inoculum was soaked in distilled water (10% (w:v)) for 30 minutes and further shaken for another 30 minutes and finally, the soil suspension was double filtered by using Watman #1 filter paper to obtain the microbial filtrate in an Erlenmeyer flask. Both substrate soil and "–AMF" sterilizations were done by heating in oven for 30 minutes at 120°C in two cycles with 24 hours interval (Hart and Reader, 2004). The experiment was carried out in the Ethiopian Biodiversity Institute (EBI) mesh-house. Mesh-house experiment rather than greenhouse

or field experiment was preferred because it has both the advantage of mimicking the filed condition (DAF ecosystem condition) better than greenhouse while it in the meantime enables the much-needed control of the experiment better than field experiments which can be chaotic. Pots were arranged in a completely randomized design (CRD) (Fig. 12) and the experiment lasted from November 14, 2019- April 14, 2020 (for five months) for *C. africana*, November 14, 2019- April 28, 2020 (for five and half months) for *J. procera*, and September 26, 2019-March 25, 2010 (for six months) for *P. falcatus*. Seedling watering was done every other day to field capacity, especially for the first three months. Afterwards, *P. falcatus* seedlings were watered to field capacity every week and both *C. africana* and *J. procera* were watered every 3rd or sometimes 4th day. This was also done partly, to mimic the rainfall condition at the field in the DAF ecosystem.

Figure 12: Transplanted seedlings of *J. procera*, *C. africana*, and *P. flacatus* (from left to right) in the mesh-house at EBI.

3.3.4. AMF root colonization, mycorrhizal responsiveness, and seedlings growth and quality variables determination

AMF root colonization (RC) was determined by using the ink and vinegar technique followed by the gridline intersects method as described earlier (section 3.1.2). In order not to reduce the root weight, only five ~1cm long fine roots per seedlings or 30 cm long fine root samples per treatment were sampled for RC determination. However, since the root system of *J. procera* was very small (Fig.13), RC was not determined.

Figure 13: Root system of *J. procera* after 22 weeks of growth. Some of the roots have developed very poorly and fine root sampling for RC determination was not possible.

AMF responsiveness (MR) was determined according to Rowe et al. (2007). Janos (2007) has evaluated several ways in which MR has been determined and recommended the two of the equations to be suitable. Hence, Janos (2007) recommended MR to be determined alternatively as; MR= ([+AMF]-[-AMF]/[-AMF])*100 or MR= ([+AMF]-[-AMF]/[+AMF])*100, where [+AMF] and [-AMF] represent average dry mass of inoculated and noninoculated seedlings respectively. Rowe et al. (2007) on the other hand, determined MR based on randomly paired dry masses and using the equation; MR=*ln*([+AMF]/[-AMF]), where *ln* is the natural logarithm. MR determination based on paired dry masses is suitable for statistical computations while the ones based on the average dry mass per treatment are not. Hence, in this study, we adopted the Rowe et al. (2007) equation and determined MR based on the ranked, paired dry masses of inoculated and non-inoculated seedlings per species and soil type. Since the non-sterile-non-inoculated potting soil has native Ankorcha AMF inoculum; it could be considered as an existing whole-soil AMF inoculum and it could influence seedlings' growth and quality. Therefore, in this study we determined MR; 1) for the effect of whole-soil inoculation on sterile pot soil and 2) non-sterile pot soil, and 3) for the existing native Ankorcha AMF inoculum of the non-sterile potting soil. MR due to the existing native Ankorcha AMF inoculum was determined as; MR=*ln* ([nSnI]/[SnI]), where "nSnI" refers to the dry mass of seedlings grown on the non-sterile-non-inoculated potting soil and "SnI" refers to the dry mass of seedlings grown on the sterile-non-inoculated potting soil.

Important seedling growth and quality variables were also determined. When initial measurements were available, growth variables were determined as relative growth rates.

Hence, relative height growth rate (RGR-H), relative collar diameter growth rate (RGR-CD), and relative leaf number growth rate (RGR-LN) were determined. For those variables with no initial data measurement, i.e., initial leaf number, initial leaf area, initial seedlings total fresh mass, initial shoot fresh mass, initial root fresh mass, initial total dry mass, initial shoot dry mass, and initial root dry mass, relative growth rates were not determined. Root dry mass to shoot dry mass ratio (R:S), root dry mass to total seedling dry mass ratio (R:T), and rooting depth (RD) were also determined as seedling quality variables. Considering leaf number as one of the important growth variables along with leaf area in this study was justified since both of these factors are similarly affected by soil resource limitations and mainly moisture (Taiz and Zeiger, 2002) which is a reality of DAF soils. Seedling height and rooting depth were measured by using a ruler and collar diameter was determined by using a digital caliper. Seedlings' fresh and dry mass was measured by using an analytical balance. Root and shoot dry masses were determined respectively after oven drying at 70°C and 65°C to constant mass (Ouahmane et al., 2006) which in our case took 24 hours. Leaf area was measured by the direct method of graph-paper tracing which is considered the most accurate way of determining leaf area (Pandey and Singh, 2011), especially when the sample size is small and detaching leaves do not affect the experiment.

Relative growth rate (RGR) expresses growth in a rate of increase in weight or size per unit of initial weight or size (Hunt, 1990; Poorter and Garnier, 2007). The rate can be determined per day or week and RGR can also be expressed as percent per day or per week (Hunt, 1990). RGR is believed to be one of the most ecologically significant and

useful indices of plant growth, especially when comparing seedlings of different initial weights or sizes (Rees et al., 2010; Pommerening and Muszta, 2015). RGR is mainly determined for dry mass but also can be computed for leaf area, stem diameter/ volume, or basal area (Pommerening and Muszta, 2015). It can also be determined for fresh mass, only shoot mass, or leaf number (Poorter and Garnier, 2007).

The two standard and also alternative equations used to determine RGR (Hunt, 1990; Poorter and Garnier, 2007) are shown below for RGR in dry mass as an example. Hence,

$$RGR = 1/M_i\ (\Delta M/\Delta T) \quad \textit{equation 1}$$

$$RGR = (ln M_f - ln M_i)/\Delta T \quad \textit{equation 2}$$

Where ΔM is is the change in dry mass (M_f-M_i), M_f is the final dry mass, Mi is initial dry mass, ΔT is the time for the change in dry mass (T_f-T_i), and *ln* is the natural logarithm. In this study, we adopted the first formula to determine RGR-H, RGR-CD, and RGR-LN. We measured ΔT in weeks and it was 20, 22, and 24 weeks respectively for *C. africana*, *J. procera*, and *P. falcatus*.

3.4. Statistical analysis

The AMF status of DAF nurseries: One-way ANOVA was computed to determine the effect of tree species and nursery location (a proxy variable to nursery management) on root AMF colonization (RC) and pot soil spore abundance (SA). This was done by considering all the tree species and considering the commonly raised tree species; *C. africana, J. procera*, and *O. europaea* separately. Data were checked for the normality of residuals and equality of variances and parametric ANOVA was computed when

residuals were normal and variances equal. Parametric ANOVA was also computed when data rectification by log10 transformation was possible. In other cases, the Kruskal-Wallis test was computed. When a significant (p<0.05) effect was present, Post-hoc tests; Tukey-Kramer (p<0.05), Tukey-HSD (p<0.05) and Dunn-Bonferroni (p<0.05), were carried out after, parametric ANOVA with unequal sample sizes, parametric ANOVA with equal sample sizes and Kruskal-Wallis test, respectively. To evaluate the correlations between RC, SA, seedling age, pot height (PHI), pot diameter (PD), pot volume (PV), seedling height (SHI), seedling collar diameter (CD), SHI per age (RSHI) and CD per age (RCD) Spearman's rank correlation was computed for all tree species and *C. africana*, *J. procera*, and *O. europaea* separately.

The resilience of soil physicochemical property to forest degradation and deforestation: Nonmetric multidimensional scaling (NMDS) using similarity ratio and ward method was plotted to determine the soil physicochemical property resilience. NMDS was also plotted to explore which physicochemical variables discriminated the land uses. Moreover, one-way ANOVA was computed to know if forest degradation and deforestation had significant effect on each of the soil physicochemical variables (%sand/silt/clay, bulk density, 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 non-parametric ANOVA respectively.

The resilience of SA and infectivity to forest degradation and deforestation: Nonparametric Kruskal Wallis tests were carried out to know the impact of forest degradation and deforestation on SA and infectivity. When significant impact was

present, Dunn-Bonferroni ($p \leq 0.05$) pairwise mean comparisons were made between land uses. Similarly, nonparametric Kruskal Wallis test and Dunn-Bonferroni ($p \leq 0.05$) pairwise mean comparisons were carried out to know the effect of elevation and location on SA and infectivity within each land use. Spearman's rank correlation was analyzed to know the correlations between SA, infectivity, and the various soil physicochemical variables.

Mycorrhizal responsiveness, AMF root colonization, and growth and quality of C. africana, J. procera, and P. falcatus: One-way ANOVA and Tukey-HSD tests were computed to compare the mycorrhizal responsiveness of *C. africana*, *J. procera*, and *P. falcatus*. Two-way ANOVA was computed to know the effects of AMF inoculation, soil type, and AMF x soil type interaction on the RC, growth, and quality of *C. africana*, *J. procera*, and *P. falcatus* seedlings grown on the degraded DAF ecosystem potting soil.

Software application: NMDS ordination was plotted using R version 3.6.1. SPSS version 20 was used to compute parametric and non-parametric one-way ANOVA, two-ANOVA, mean pairwise comparisons, and Spearman's rank pairwise correlations. Bar graphs were plotted by SYSTAT version 13.1.

4. RESULTS

4.1. Nursery survey results

4.1.1. Overview of the DAF nurseries

Almost all of the nurseries surveyed were owned by GOs (Table 2) and except N3, they were comparatively well staffed. Based on the discussions made with relevant forestry officials of the various districts of the study area, it was learned that most of the DAF nurseries do not raise native trees anymore. Similarly, the surveyed nurseries also were found to raise more exotic tree species with the proportion of native tree species, being around 40% (Annex 1). Compared with all the tree and shrub species raised in the surveyed nurseries, the native trees *Cordia africana*, *Juniperus procera*, and *Olea europaea* subsp. *cuspidata*, were among the most widely raised tree species while *Acacia abyssinica*, *Millettia ferruginea*, *Podocarpus falcatus* and *Prunus africana* were the least ones (Annex 1).

4.1.2. Root AMF colonization and pot soil spore abundance

Average RC levels ranged from 8.00-97.67% while average SA levels ranged from 1.30-24.63 spores g^{-1} (Table 4). Based on the RC rating adopted by Michelsen (1992), (i.e., low =0-20%, medium=21-50% and high = 51-100%), 53.8, 30.7 and 8.3 % of the samples had high, medium and low rates of RC, respectively, while based on our SA rating (i.e., low=0-5, medium=6-10, high=11-20, and very high= above 20 spores g^{-1}), 3.8, 30.8, 46.1 and 19.3% of the samples had very high, high, medium and low SA rates, respectively (Table 4).

Table 4: Root AMF colonization (RC) and spore abundance (SA) of the surveyed tree seedlings across nurseries with the corresponding ratings.

Code	Species	Nursery	RC (%)		SA (g^{-1})	
			Mean RC (±SE)	Rate	Mean SA (±SE)	Rate
1c	*Olea europaea* subsp. *cuspidata* (Wall. ex G. Don) Cif.	N5	63.50±2.02	High	12.93±0.89	High
2c	*O. europaea*	N6	93.17±1.30	High	10.92±1.37	High
3b	*O. europaea*	N2	91.17±2.68	High	15.93±1.26	High
4a	*O. europaea*	N9	65.33±1.30	High	2.37±0.43	Low
5a	*O. europaea*	N10	75.50±1.04	High	2.12±0.16	Low
6b	*O. europaea*	N8	85.03±0.58	High	1.30±0.30	Low
1d	*Cordia africana* Lam.	N5	80.97±0.97	High	7.17±0.37	Medium
3d	*C. africana*	N2	25.43±1.37	Medium	10.35±2.04	High
5b	*C. africana*	N10	50.00±1.44	Medium	2.27±0.22	Low
6a	*C. africana*	N8	70.67±0.33	High	3.57±0.83	Low
9b	*C. africana*	N4	60.10±1.65	High	10.25±1.44	High
10c	*C. africana*	N1	71.83±2.89	High	15.07±1.82	High
1b	*Juniperus procera* Hochst. ex Endl.	N5	29.67±0.88	Medium	7.97±0.66	Medium
2a	*J. procera*	N1	39.67±1.45	Medium	11.32±1.62	High
3a	*J. procera*	N2	41.67±2.03	Medium	7.43±0.57	Medium
5c	*J. procera*	N10	11.67±0.33	Low	5.93±0.93	Medium
7a	*J. procera*	N7	8.00±0.58	Low	6.07±1.39	Medium
1a	*Dovyalis abyssinica* (A. Rich.) Warb.	N5	95.67±0.33	High	7.60±0.22	Medium
7b	*D. abyssinica*	N7	99.67±0.33	High	6.80±0.68	Medium
8a	*Podocarpus falcatus* (Thunb.) R. Br. ex Mirb.	N3	12.20±1.33	Low	8.92±1.21	Medium
10a	*P. falcatus*	N1	34.67±0.67	Medium	9.27±0.32	Medium
2d	*Hagenia abyssinica* J.F. Gmel.	N6	32.67±1.33	Medium	7.73±0.25	Medium
9a	*H. abyssinica*	N4	46.50±6.50	Medium	15.10±2.40	High
10b	*Acacia abyssinica* Hochst. ex Benth.	N1	96.00±0.58	High	24.63±1.92	Very High
2b	*Millettia ferruginea* (Hochst.) Baker	N6	97.33±0.67	High	6.17±0.93	Medium
3c	*Prunus africana*	N2	18.67±2.33	Low	7.30±1.23	Medium

	(Hook. f.) Kalkman					

N1=Susuni, N2=Entoto, N3=Teferi & Mulugeta, N4=Sokondo, N5=Jello, N6=Qebenewa, N7=Debre birhan teachers college, N8=Mush, N9=Tis aba lima, and N10=Hizba tekelehaimanot

Across nurseries, *O. europaea* seedlings had high rates of RC regardless of the SA level. *Cordia africana* seedlings also mostly had high RC regardless of the SA levels. On the other hand, *J. procera* seedlings had medium to low RC levels. *Acacia abyssinica* was found to have high RC and exceptionally, very high SA. In general, none of the seedlings had low RC and SA rates simultaneously. However, *J. procera* in N7 and N10, *P. falcatus* in N3, and *P. africana* in N2 (collected only in that nursery) had comparatively the least RC and SA rates (Fig. 14). Moreover, from the general trend observed, RC and SA rates did not necessarily have a one-to-one correspondence (Fig. 14).

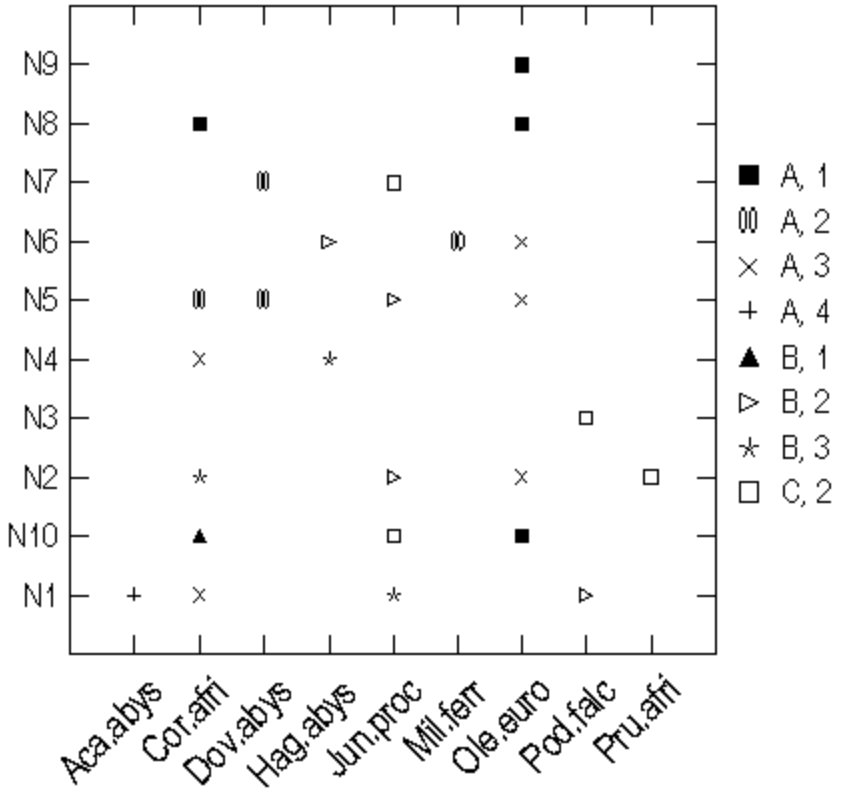

Figure 14: Rates of AMF root colonization (RC) and spore abundance (SA) for all tree species and across the nurseries. Aca.abys=*Acacia abyssinica*, Cor.afri=*Cordia africana*,

Dov.abys=*Dovyalis abyssinica*, Hag.abys=*Hagenia abyssinica*, Jun.proc=*Juniperus procera*, Mil.ferr=*Millettia ferruginea*, Ole.euro=*Olea europaea* subsp *Cuspidata*, Pod.falc=*Podocarpus falcatus*, and Pru.afri=*Prunus africana*. N1=Susuni, N2=Entoto, N3=Teferi & Mulugeta, N4=Sokondo, N5=Jello, N6=Qebenewa, N7=Debre birhan teachers college, N8=Mush, N9=Tis aba lima, and N10=Hizba teklehaimanot. A=High RC rate, B=Medium RC rate, C=Low RC rate, 1=Low SA rate, 2=Medium SA rate, 3=High SA rate, 4=Very high SA rate. None of the seedlings fall under the (C, 1) category indicating none of them had both low RC and SA rates.

4.1.3. Tree species and location effect on RC and SA

Six of the nine tree species and eight of the ten nurseries were suitable for the ANOVA test. Those species collected only once and those nurseries from which a single species was collected were not suitable for statistical analysis. Due to this data mismatch, two-way ANOVA was not computed and thus, interaction effects were not determined. Parametric and non-parametric one-way ANOVA tests computed showed that RC was affected by tree species but not by nursery location (Table 5). On the contrary, SA was found to be affected by nursery location and not by tree species (Table 5).

Table 5: Parametric one-way ANOVA and Kruskal-Wallis test results for the effect of tree species and nursery location on seedlings root AMF colonization (RC) and AMF spore abundance (SA).

	RC			SA		
Variables	F-ratio	Chi square	P-value	F-ratio	Chi square	P-value
Tree species	11.354	-	0.000***	-	1.801	0.876
Nursery location	0.430	-	0.870	**7.114**	-	**0.001****

"**" significant at $P<0.01$ and "***" significant at $P<0.001$. Values in bold indicate analysis after log10 transformation of the data

The mean RC of *D. abyssinica* was the highest compared to the mean RC of the six tree species analyzed (Fig. 15). Moreover, it was significantly ($P<0.05$) 146.72, 273.78, and 316.7% higher than the mean RC of *H. abyssinca*, *J. procera*, and *P. falcatus*

respectively. On the other hand, the highest mean SA form those nurseries considered for analysis was found in N1. Mean SA in N1 was also significantly (P<0.05) 338 and 517.62% higher than the mean SA in N10 and N8 respectively (Fig. 15).

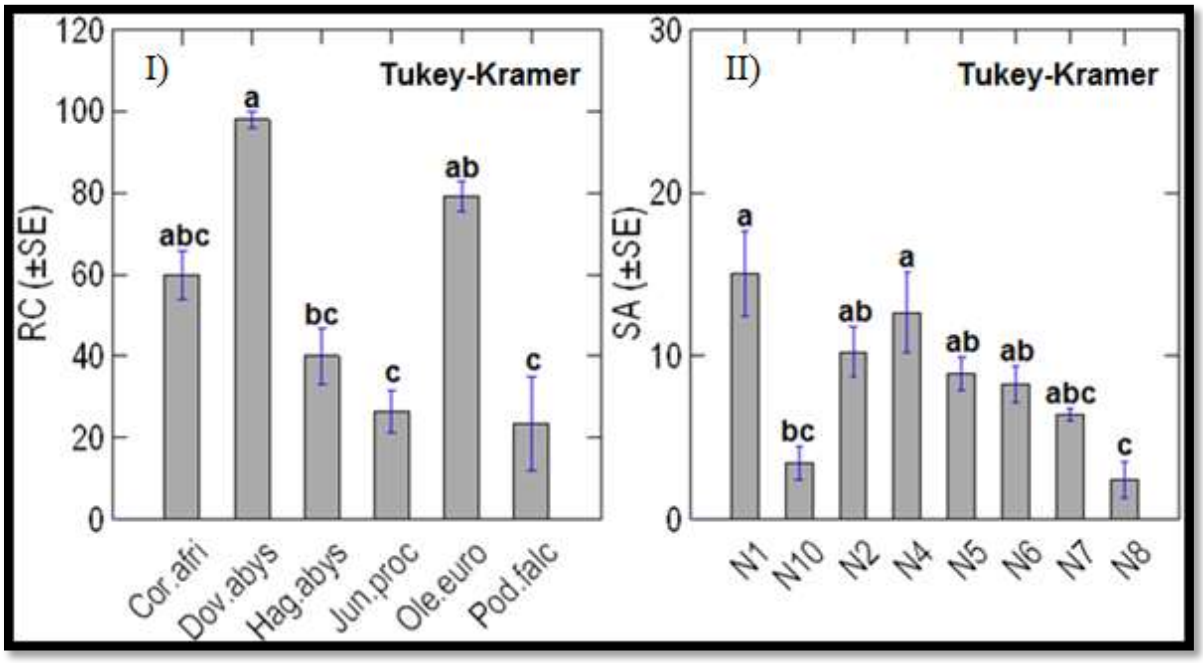

Figure 15: Mean percent root colonization (RC) (I) of *Cordia africana* (Cor.afri), *Dovyalis abyssinica* (Dov.Abys), *Hagenia abyssinica* (Hag.Abys), *Juniperus procera* (Jun.procera), *Olea europaea* subsp. *cuspidata* (Ole.euro), *Podocarpus falcatus* (Pod.afri) in percent and mean spore abundance (SA) in spore number g^{-1} (II) across nurseries (N1=Susuni, N10=Hizba teklehaimanot, N2=Entoto, N4=Sokondo, N5=Jello, N6=Qebenewa, N7=Debre birhan teachers college, N8=Mush). Different letters indicate significant differences after Post-hoc Tukey tests (p< 0.05).

To determine the effect of nursery management, we evaluated the effect of location on RC and SA of the three most commonly raised native tree species; *C. africana*, *J. procera*, and *O. europaea*. Nursery location had a significant (p<0.05) effect on the three species analyzed (Table 6). The mean RC of *C. africana* seedlings was the highest in N5 and it was significantly (p<0.05), 218% higher than the mean RC in N2. The highest mean SA of *C. africana* seedlings was recorded in N1 which was significantly (p<0.05)

and 110, 322, and 564.69% greater than the mean RC in N5, N8, and N10, respectively. In the case of *J. procera*, the highest mean RC was found in N2, and with 420.87% more colonization, it was significantly (p<0.05) higher than the mean RC in N7 only. On the other hand, the mean SA of *J. procera* seedlings was the highest in N1 (Fig. 16). Moreover, the mean SA in N1 was significantly (p<0.05) higher than the mean values in N7 and N10 with the corresponding abundance increases of 86.5 and 90.7%. *Olea europaea* seedlings had the highest mean RC in N6. The mean RC in N6 was also significantly (P<0.05), 9.57, 23, 42.6, and 46.46% greater than the mean RC in N8, N10, N9, and N5, respectively. Hence, mean RC of *O. europaea* seedlings varied markedly even in the very closely located nurseries, N5 and N6. The highest mean SA of *O. europaea* seedlings was found in N2 and also significantly (p<0.05) higher than mean SA in N6, N9, N10, and N8 with the corresponding increases of 45.95, 573, 652.74, and 1123.28%, respectively (Fig. 16).

Table 6: Parametric ANOVA and Kruskal-Wallis test results for the effect of location (proxy to nursery management) on root AMF colonization (RC) and AMF spore abundance (SA) of seedlings within a species.

Species	RC			SA		
	F-ratio	Chi square	P-value	F-ratio	Chi square	P-value
Olea europaea L. subsp. *cuspidata* (Wall. ex G. Don) Cif.	61.504	-	0.000***	54.219	-	0.000***
Cordia africana Lam.	-	16.179	0.006**	13.105	-	0.000***
Juniperus procera Hochst. ex Endl.	-	13.005	0.011*	3.855	-	0.038*

"*" significant at p<0.05, "**" significance at p<0.01 and "***" significance at p<0.001

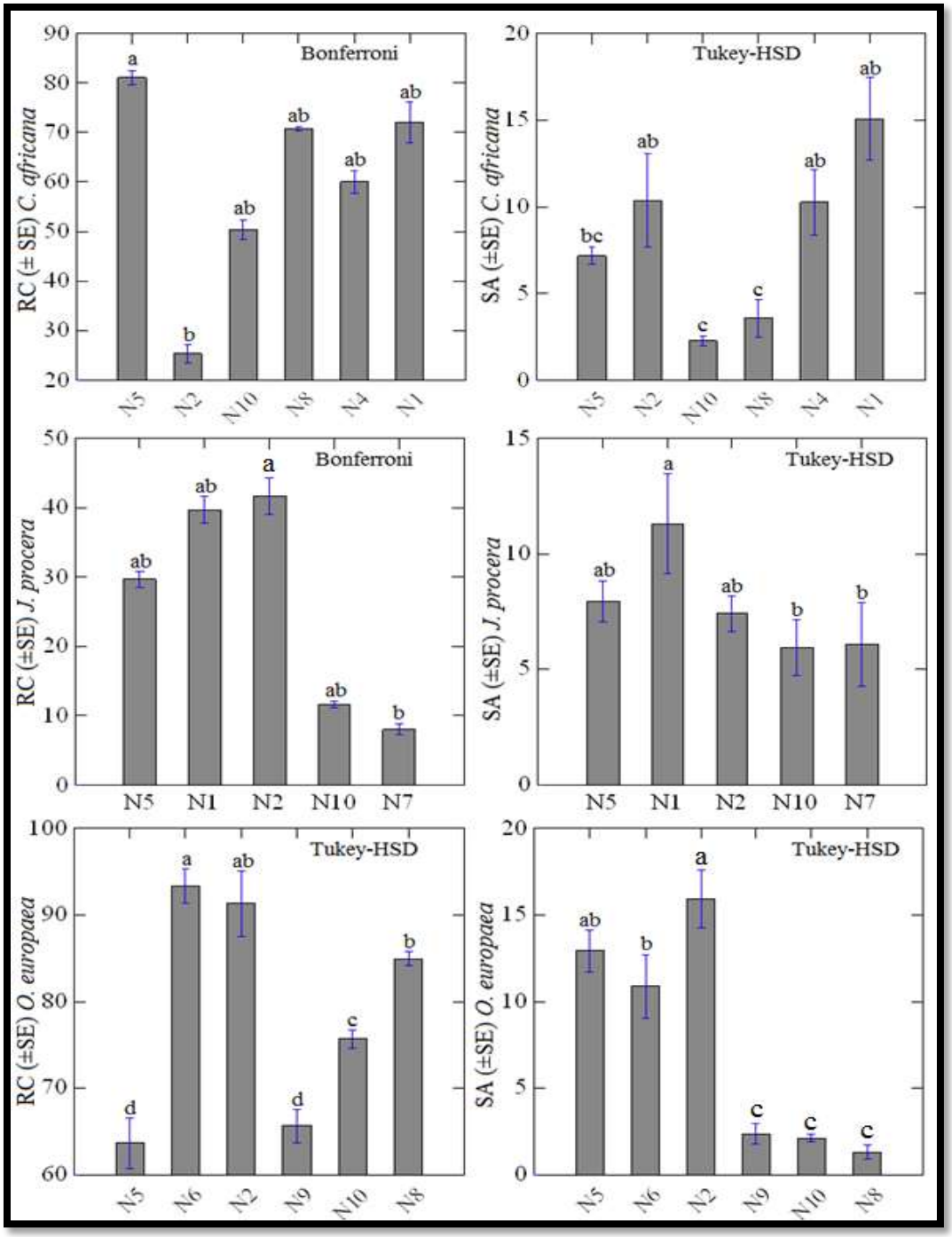

Figure 16: Mean root colonization (RC) in percent and spore abundance (SA) in spore number g^{-1} of the three common tree species *Cordia africana* (C. africana), *Juniperus procera* (J. procera) and *Olea europaea* subsp. *cuspidata* (O.europaea) across nurseries (N1=Susuni, N2=Entoto, N4=Sokondo, N5=Jello, N6=Qebenewa, N7=Debre birhan teachers college, N8=Mush, N9=Tis aba lima, N10=Hizba teklehaimanot). Different letters indicate significant differences after Post-hoc Tukey and Dunn-Bonferroni tests ($p< 0.05$) for the effect of location.

4.1.4. Correlation between AMF spore abundance and AMF root colonization with seedling growth and nursery management variable

Spearman's rank correlation results indicated that, when all the tree species were considered, there was a very weak correlation between RC and SA (r_s =0.028, p =0.891) and between RC and age (r_s =-0.050, p =0.807). The results also indicated that RC and SA correlations with pot and seedling growth-related variables were weak and not statistically significant (Table 7). Analyzing data of *C. africana*, *J. procera*, and *O.europaea* separately, however, most of the correlation results showed a moderate-high strength. Significant (p<0.05) correlations were those between RC and pot diameter (PD) (r_s =0.820, P =0.046), between RC and pot volume (PV) (r_s =0.928, p =0.008), between SA and age (r_s =0.943, P =0.005), and between SA and root collar diameter per age (RCD) (r_s =-0.812, P =0.050) for *C. africana*, and between RC and age (r_s =0.829, p =0.042) for *O. europaea* (Table 7).

Table 7: Spearman's rank correlation [*P* (2-tailed)] of root AMF colonization (RC), AMF spore abundance (SA), and various pot and seedlings growth-related variables

Variables	For all species		*Cordia africana* Lam.		*Juniperus procera* Hochst. ex Endl.		*Olea europaea* L. subsp. *cuspidata* (Wall. ex G. Don) Cif.	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
RC-SA	0.028	0.891	0.086	0.872	0.600	0.285	0.086	0.872
RC-Age	-0.050	0.807	-0.143	0.787	0.600	0.285	0.829*	0.042
RC-PHI	0.358	0.072	0.676	0.140	0.707	0.182	-0.393	0.441
RC-PD	0.158	0.441	0.820*	0.046	0.000	1.000	0.393	0.441
RC-PV	0.172	0.401	0.928**	0.008	0.224	0.718	0.101	0.848
RC-SHI	0.184	0.368	0.200	0.704	0.600	0.285	0.429	0.397
RC-CD	0.127	0.536	-0.371	0.468	0.500	0.391	0.600	0.208
RC-RSHI	0.253	0.212	0.257	0.623	-0.200	0.747	-0.600	0.208
RC-RCD	0.286	0.157	0.319	0.538	-0.154	0.805	-0.600	0.208
SA-Age	0.294	0.144	0.943**	0.005	0.200	0.747	0.371	0.468
SA-PHI	0.135	0.510	0.169	0.749	0.354	0.559	-0.131	0.805
SA-PD	0.224	0.272	0.516	0.295	-0.289	0.638	0.655	0.158
SA-PV	0.192	0.348	0.377	0.461	-0.224	0.718	0.507	0.305
SA-SHI	0.364	0.067	0.429	0.397	0.700	0.188	0.543	0.266
SA-CD	0.221	0.277	0.371	0.468	0.400	0.505	0.657	0.156
SA-RSHI	0.030	0.885	-0.543	0.266	0.600	0.285	0.029	0.957
SA-RCD	0.044	0.831	-0.812*	0.050	0.564	0.322	0.029	0.957

PHI=Pot height, PD=Pot diameter, PV=Pot volume, SHI=Seedling height, CD=Seedling collar diameter, RSHI=Seedling height per age, RCD=Seedling collar diameter per age. * significant at $P<0.05$, and ** at $P<0.01$

4.2. Land use survey results

4.2.1. The soil physicochemical property across land-use gradients

The NMDS ordination depicted that soil physicochemical property was resilient to forest degradation but not to deforestation (Fig. 17a). It also indicated that within the grazing land, the soil physicochemical property had low similarity between GrL(a) and GrL(b) (Fig. 17a). 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

(Fig. 17b). Available phosphorus and P:N had no role in the discrimination of NF and ShL from CrL and GrL but were responsible for the discrimination of GrL(a) and Grl(b) (Fig. 17b).

Except P, P:N and C:N, all the other soil physicochemical variables were significantly (p<0.05) affected by the land-use change (Table 8). 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) decreased by degradation and deforestation. EC although not significantly (p>0.05), decreased by degradation while it significantly (p<0.05) decreased by deforestation. Total nitrogen increased due to degradation but not significantly (p>0.05), while it significantly (p<0.05) decreased due to deforestation. Available phosphorus showed a variable trend but there were no significant (p>0.05) degradation and deforestation effects. P:N increased both by degradation and deforestation but not significantly (p>0.05). Organic carbon or organic matte were not affected by degradation but decreased significantly (P<0.05) by deforestation. C:N, although not significantly (P>0.05), decreased by degradation while it, in contrary, increased by deforestation. CEC significantly (P<0.05) increased due to degradation but it more or less remained similar despite deforestation (Table 8).

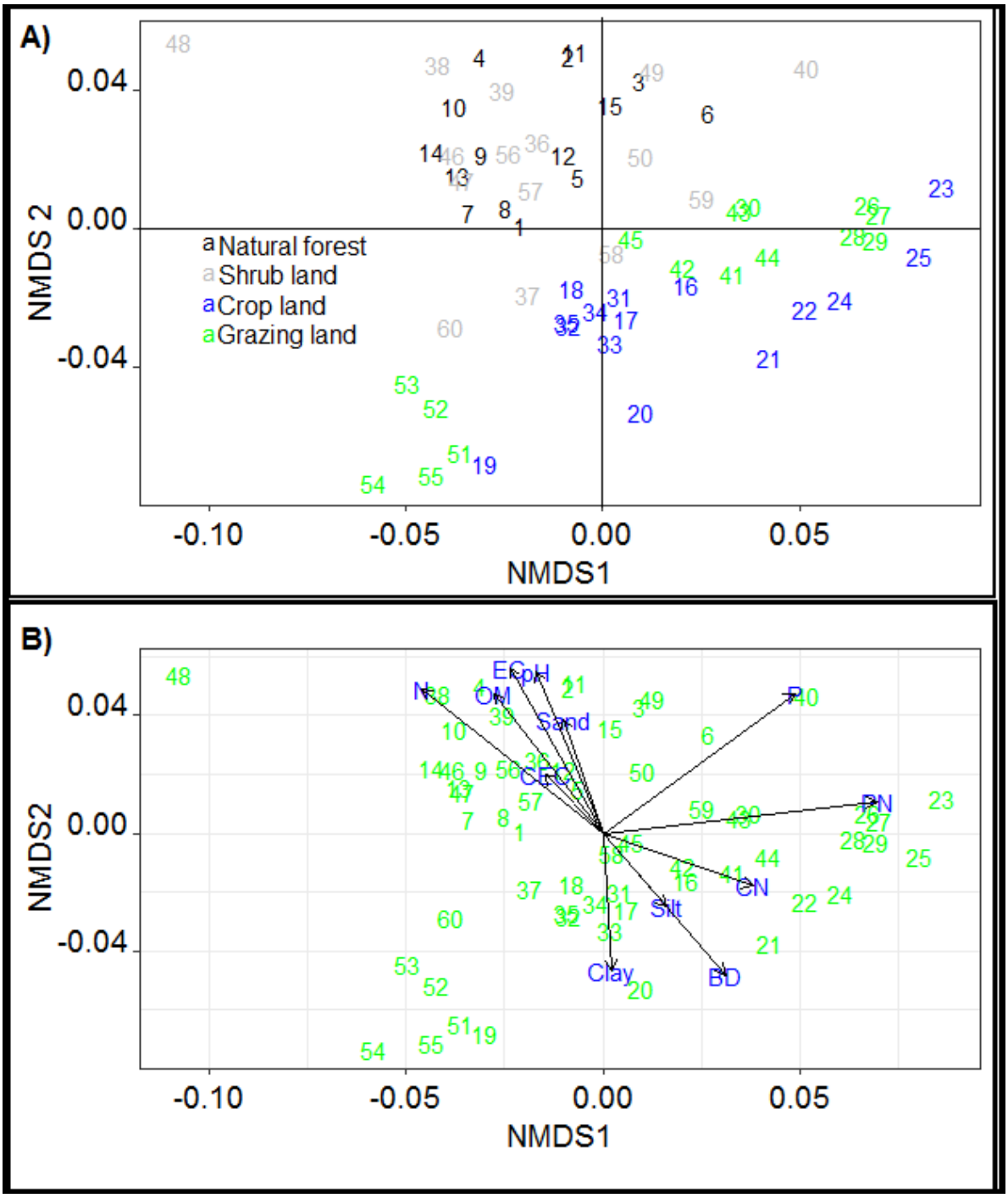

Figure 17: NMDS ordination plots showing the resilience of the soil physicochemical property to Chilimo forest degradation and deforestation. The soil physicochemical property of the natural forest and shrub land was similar, while it was distinct between natural forest and the crop and grazing lands. The soil physicochemical property within the grazing land was variable with Gr3L(b), i.e., plots 51-55, discriminated by P and P:N.

Table 8: Mean (±SE) soil physicochemical variables comparison across the Chilimo forest degradation gradient.

Variables	Land uses				ANOVA	
	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}	-	33.86***2
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^{ns}	17.18±3.61^{ns}	15.10±3.77^{ns}	19.96±3.68^{ns}	0.434	-
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 (across the row) are statistically ($p \leq 0.05$) significant after Tukey or Dunn Bonferroni tests. "ns" indicates there was no land use effect. *** significant at $P \leq 0.001$.

4.2.2. Spore abundance (SA) and infectivity across land uses

Spore abundance and infectivity ranged from 3.4-25.3g^{-1} and from 12-82.5% respectively (Annex 5) and land-use change had a significant effect on both SA and infectivity (Table 9). However, the effect of forest degradation and deforestation was not necessarily similar to both SA and infectivity. Hence, both forest degradation and deforestation significantly increased SA while they resulted in the reduction in infectivity (Fig. 18).

infectiSA while deforestation had no effect when the forest was changed to cropland (Fig. 18). The highest SA was in the GrL and the lowest in NF. SA in the ShL, CrL, and GrL were significantly, 92.71%, 105.33%, and 148.85%, respectively greater than the SA in the NF. The highest infectivity, contrary to SA, was obtained from the NF, while the lowest was from the ShL. Infectivity of the NF was significantly, 56.43% and 52.23% higher than the infectivity of the ShL and GrL, respectively (Fig. 18). Infectivity of the NF, however not significant, was also 10.98% higher than the infectivity of CrL (Fig. 18).

Table 9: Effect of Chilimo forest degradation and deforestation on AMF spore abundance (SA) and and infectivity.

Variable	F	Chi-square	P-value
SA	-	35.403	0.000***
Infectivity	-	23.245	0.000***

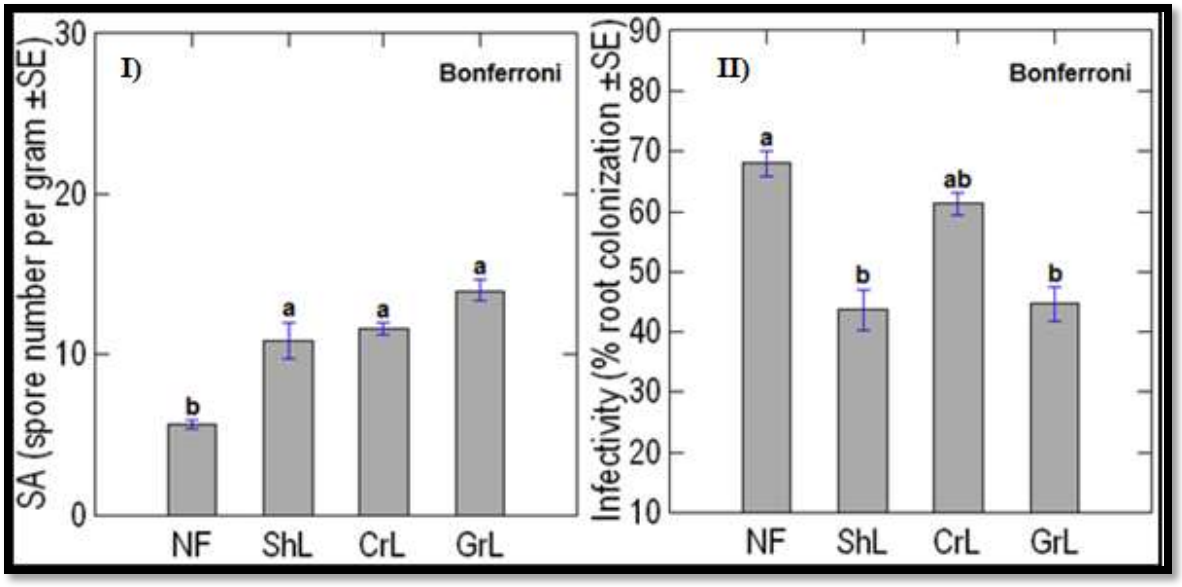

Figure 18: Mean SA (I) and infectivity (I) across land uses (NF=natural forest, ShL=shrubland, CrL=cropland, GrL=grazing land). Means with significant ($p \leq 0.05$) differences are indicated with different letters.

Elevation and/or location did not have significant effects on SA across land uses but were found to have an impact on the infectivity of ShL and GrL soils (Table 10). In the shrubland, the highest infectivity was found for ShL1 at the low elevation, while the lowest was found for ShL2 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 (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. 19-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 (123.35%) higher than the infectivity of GrL3b soil. Although not significantly, it was also 12.77% higher than the infectivity of GrL2a soil (Fig.19-II).

Table 10: Effect of elevation and location on AMF spore abundance and infectivity across the land-use gradients

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 $p<0.05$

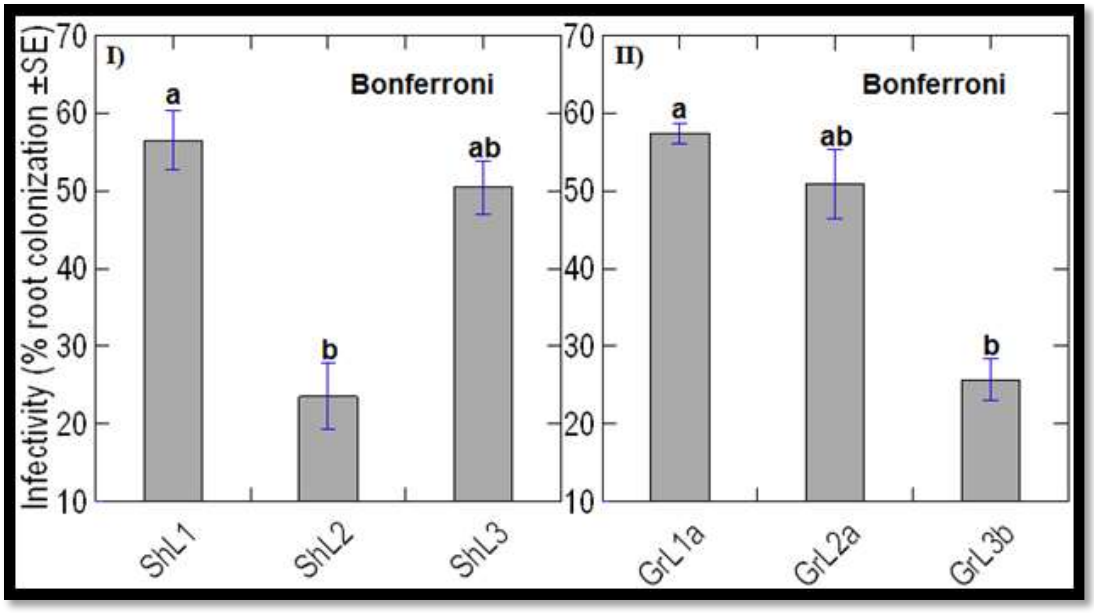

Figure 19: Mean infectivity across altitude and location in shrubland (I) and Grazing land (II). ShL1=Shrub land at 2400m altitude, ShL2 at 2500m and ShL3 at 2600m; GrL1a=Grazing land at 2800m altitude and with *Pennisetum clandestinum* the dominant cover, GrL2a at 2400m with grass *Pennisetum clandestinum* the dominant cover, and GrL3b at 2400 m but with *Pennisetum sphacelatum* being the dominant cover.

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

AMF spore abundance and infectivity were 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 positively correlated with both SA and infectivity. Moreover, SA correlated with OC (OM) and C:N differently (Table 11).

Table 11: Spearman's rank pairwise correlations [P (2-tailed)] between SA, infectivity, and the various soil physicochemical variables

	SA	Inf	BD	pH	EC	N	P	OM	P:N	C:N	ECE	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.* significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, and *** significant at $P \leq 0.001$.

4.3. Whole-soil AMF inoculation experiment results

4.3.1. Mycorrhizal responsiveness (MR)

The mean mycorrhizal responsiveness due to inoculation of *C. africana*, *J. procera*, and *P. falcatus*, was found to be 1.05±0.17, 0.4±0.09, and -0.02±0.09, respectively on the sterile potting soil, while on the nonsterile potting soil, it was 0.83±0.12, 0.26±0.28, and 0.002±0.07 (Fig. 20). The mycorrhizal responsiveness due to the existing AMF inocula of the nonsterile potting soil was also found to be 0.23±0.03, -0.31±0.17, and -0.08±0.008 respectively for *C. africana J. procera*, and *P. falcatus* (Fig. 20). Tree species was an important factor determining MR (Table 12). Moreover, MR of *C. africana* on the sterile pot soil to be, significantly and 167.64% greater than the MR of *J. procera*, while the MR of *P. falcatus* was also significantly and 102% lower than the MR of *C. africana*. On the nonsterile pot soil, the MR of *C. africana* was not significantly different from the MR of *J. procera* but MR of *P. falcatus* was significantly, 99.76% lower than the MR of *C. africana*. Similarly, MR of *J. procera* and *P. falcatus* due to the existing AMF inoculum were significantly and respectively, 234.65% and 169.79% lower than the MR of *C. africana* (Fig. 20).

Table 12: One-way ANOVA results for tree species-inoculum effect on mycorrhizal responsiveness.

		Sum of Squares	df	Mean Square	F	*P*
MRS	Between Groups	3.510	2	1.755	18.922	0.0001***
	Within Groups	1.391	15	0.093		
MRnS	Between Groups	2.174	2	1.087	5.560	0.016*
	Within Groups	2.933	15	0.196		
MRExi	Between Groups	0.909	2	0.454	6.915	0.007**
	Within Groups	0.986	15	0.066		

MRS=Mycorrhizal responsiveness on sterile potting soil, MRnS=Mycorrhizal responsiveness on nonsterile potting soil, MRExi=Mycorrhizal responsiveness due to the existing native Ankorcha AMF inoculum in the nonsterile potting soil.

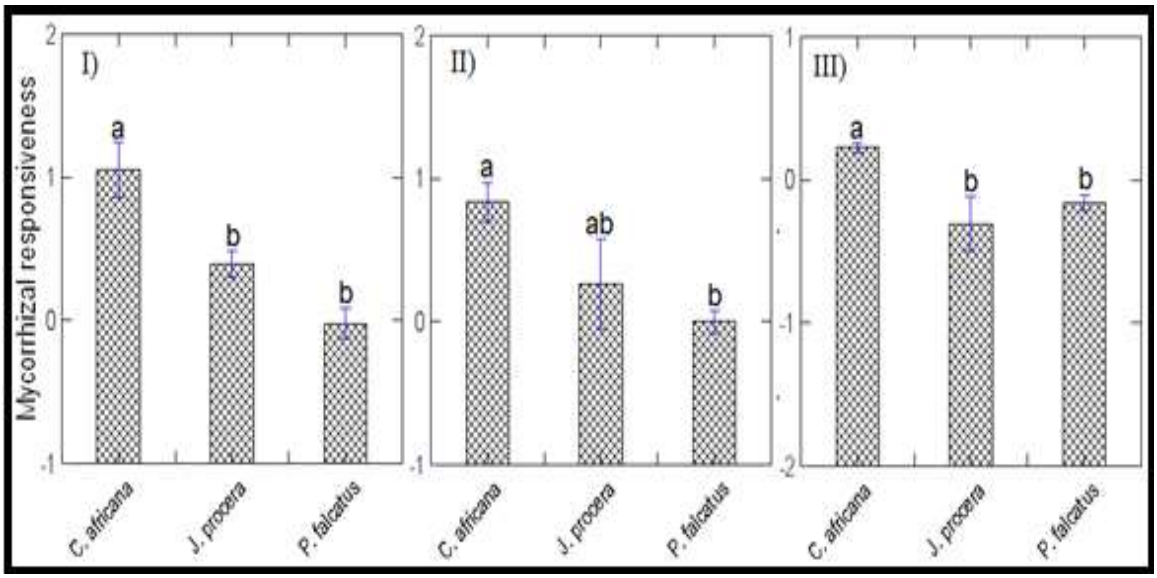

Figure 20: Comparative mycorrhizal responsiveness (MR) of *C. africana*, *J. procera*, and *P. falcatus* on sterile pot soil (I), non-sterile pot soil (II), and due to the existing native AMF inoculum of the non-sterile potting soil (III). Means with different letters indicate statistically significant differences after Tukey HSD ($p<0.05$).

4.3.2. The effects of whole-soil AMF inoculation and soil type on seedlings' root colonization (RC)

AMF root colonization of *C. africana* was significantly (p<0.05) influenced by the whole-soil AMF inoculation (Table 12). It was also significantly (p<0.05) influenced by the soil type and by the inoculation-soil interaction. However, neither inoculation, soil type, nor their interaction had a significant effect on the RC of *P. falcatus* (Table 13). As would be expected, noninoculated seedlings on the sterile potting soil were not colonized by AMF. Whereas the mean RC of whole-soil AMF inoculated *C. africana* seedlings were moderate-high (36.2-62.6%), it remained very low (4.17-6.67%) in the case of *P. falcatus* (Table 14).

4.3.3. The effects of whole-soil AMF inoculation and soil type on seedlings' growth and quality

Whole-soil AMF inoculation, except the significant (p< 0.05) effect it had on the relative collar diameter growth rate of *P. falcatus*, it was not found to have a significant effect on almost all the growth and quality variables of both *J. procera* and *P. falcatus* seedlings (Tale 13). However, soil type was found to have comparatively more effects and hence, significant soil type effects were found for leaf number and root fresh mass of *J. procera* and *P. falcatus*. Moreover, significant soil type x inoculation interaction effects were found for rooting depth in the case of *J. procera* and leaf number in the case of *P. falcatus* (Table 13). On the contrary, whole-soil AMF inoculation was found to have a significant (p< 0.05) effect on the growth of *C. africana* with significant effects on all of the growth variables except relative height growth rate. However, inoculation was not

found to have a significant effect on the quality of *C. africana* with no significant effects found for the measured seedling quality variables, viz., rooting depth, Root: shoot, and Root: plant dry mass ratios (Table 13).

Table 13: Two-way ANOVA results for the effect of AMF inoculation, soil type, and AMF inoculation x soil type interaction on RC and seedling growth and quality.

Variables	*C. africana*			*J. procera*			*P. falcatus*		
	Inoculation	Soil	Inoculation x Soil	Inoculation	Soil	Inoculation x Soil	Inoculation	Soil	Inoculation x Soil
	F	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*
RC (%)	*42.959****	*148.691****	*18.859****	n.d	n.d	n.d	0.269	0.269	1.301
LN	n.d	n.d	n.d	2.879	5.583*	2.642	2.428	6.159*	7.998**
LA	142.598***	1.000	0.407	n.d	n.d	n.d	n.d	n.d	n.d
RGR-LN	23.203***	0.698	2.608	n.d	n.d	n.d.	n.d	n.d.	n.d.
RGR-H	0.022	0.257	0.010	0.191	0.413	0.009	0.273	0.761	0.794
RGR-CD	6.931*	7.955*	0.944	0.703	0.637	0.229	4.821*	1.772	1.207
SfM	**96.571*** **	**0.036**	**1.345**	1.839	3.968	1.617	1.811	0.805	0.013
SdM	**120.902*** **	**8.479** **	**4.913***	1.062	2.569	1.887	0.530	1.993	0.161
RfM	25.511***	0.087	0.196	0.827	4.44*0	1.382	0.961	5.723*	0.186
RdM	26.821***	0.020	0.082	0.948	2.475	0.275	**0.248**	**3.246**	**0.032**
TfM	43.027***	0.413	0.410	4.629	1.429*	1.655	1.576	3.891	0.111
TdM	59.198***	0.012	0.410	1.081	2.701	1.081	0.012	3.881	0.068
RD	1.642	0.425	1.314	0.016	3.176	8.573**	n.d.	n.d.	n.d.
R:S	0.043	2.709	0.797	1.411	1.547	2.049	1.600	0.867	0.050
R:P	0.080	2.898	1.427	1.949	1.846	2.303	1.283	0.712	0.000

RC=AMF root colonization (%), LN=Leaf number, LA=Leaf area (cm^2), RGR-LN=Relative leaf number growth rate (per week), RGR-H=Relative height growth rate (cm per week), RGR-CD=Relative collar diameter growth rate (mm per week), SfM=Shoot fresh mass (g), SdM=Shoot dry mass (g), RfM=Root fresh mass (g), RdM=Root dry mass (g), TfM=Total plant fresh mass (g), TdM= Total plant dry mass (g), RD=Rooting depth (cm), R:S=Root to shoot dry mass ratio, R:P=Root to plant dry mass ratio, and "n.d" not determined. * Significant at $p \leq 0.05$, **significant at $p \leq 0.01$, *** significant at $p \leq 0.001$. Values in bold indicate analysis after log10 transformation of the data and values in italic indicate Levene's statistics for homogeneity was significant.

Whole-soil AMF inoculation resulted in a significant 376.17% increase in leaf area of *C. africana* seedlings grown on the sterile soil and a significant 379.03% increase in leaf area when grown on the non-sterile degraded DAF ecosystem potting soil (Fig. 21; Table 14a). Moreover, inoculation resulted in a significant and 311.11% and 320% increases in relative leaf number growth rate of *C. africana* grown on the sterile and non-sterile soil respectively. Whereas inoculation has resulted in a significant 255.56% increase in relative collar diameter growth rate, on the non-sterile soil as well, it resulted in a significant 123.33% increase. *C. africana* seedlings grown on the sterile soil had also a significant and 202.11% more shoot fresh mass after 5 months due to whole-soil AMF inoculation while those grown on the non-sterile soil had a significant and 143.15% more shoot fresh mass. On the other hand, inoculation increased dry shoot mass of *C. africana* seedlings significantly by 207.96% when grown on the sterile pot soil and by a significant 116.76% when grown on the non-sterile soil. Inoculation also increased total seedling fresh and dry masses of *C. africana* by a significant 96.63% and 198.8% respectively when growth on the sterile soil and by 121.86% % and 133.13% respectively when grown on the nonsterile potting soil (Table 14a).

Juniperus procera seedlings grown on the nonsterile potting soil grew a significant 42% and 36.62% lower root and seedling fresh masses respectively compared to those seedlings grown on the sterile soil. Rooting depth was greater for the inoculated seedlings on the nonsterile soil while it was lower on the sterile soil (Table 14b). On the other hand, *P. falcatus* seedlings also grew a significant 9.3% more number of leaves and 42.6% more root fresh mass on sterile soil compared to seedlings grown on nonsterile soil. Due to the inoculation x soil type interaction effect, while inoculated *P. falcatus* seedlings soil grew 16.24% more number of leaves on the sterile soil compared to the non-inoculated ones, on the non-sterile soil, inoculated ones rather grew 4.7% less number of leaves compared to the non-inoculated ones. Whole-soil AMF inoculation of *P. falcatus* seedlings also resulted in a significant 100% increase in relative collar diameter growth rate (Table 14c).

Table 14: Mean (±S.E) seedling growth and quality comparison for the effects of whole-soil AMF inoculation, soil type, and inoculation x soil type interaction for,

a) *C. africana*

Measured Variables	Treatments							
	Inoculation		Soil type		Inoculation x Soil type			
	+AMF	-AMF	S	nS	S		nS	
					+AMF	-AMF	+AMF	-AMF
RC (%)	49.2±2.3 [a]	27.4±2.3 [b]	18.1±2.3 [b]	58.5±2.3 [a]	***36.2±3.3*** [a]	***0.0*** [b]	62.2±3.3 [a]	54.8±3.3 [b]
LA	24.83±1.16 [a]	5.2±1.16 [b]	1.84±1.16	14.19±1.16	26.18±1.6 [a]	5.5±1.6 [b]	23.4±1.6 [a]	4.9±1.6 [b]
RGR-LN	0.007±0.004 [a]	-0.017±0.004 [b]	-0.007±0.004 [ns]	-0.003±0.004 [ns]	0.009±0.005 [ns]	-0.023±0.005 [ns]	0.005±0.005 [n.s]	-0.011±0.005 [ns]
RGR-H	0.02±0.006 [n.s]	0.019±0.006 [n.s]	0.018±0.006 [n.s]	0.022±0.006 [n.s]	0.018±0.008 [n.s]	0.017±0.008 [n.s]	0.023±0.008 [n.s]	0.021±0.008 [n.s]
RGR-CD	0.038±0.004 [a]	0.021±0.004 [b]	0.021±0.004 [b]	0.038±0.004 [a]	0.032±0.006 [a]	0.009±0.006 [b]	0.044±0.006 [a]	0.033±0.006 [b]
SfM	1.23±0.07 [a]	0.45±0.069 [b]	0.86±0.07 [n.s]	0.83±0.07 [n.s]	1.29±0.097 [a]	0.43±0.097 [b]	1.1±0.097 [a]	0.48±0.097 [b]
SdM	0.36±0.02 [a]	0.14±0.02	0.23±0.02 [b]	0.27±0.02 [a]	0.35±0.03 [a]	0.11±0.03 [b]	0.38±0.03 [a]	0.17±0.03 [b]
RfM	1.9±0.15 [a]	0.82±0.15 [b]	1.33±0.15 [n.s]	1.39±0.15 [n.s]	1.92±0.2 [n.s]	0.74±0.2 [n.s]	1.9±0.2 [n.s]	0.9±0.2 [n.s]
RdM	0.39±0.03 [a]	0.15±0.03 [b]	0.27±0.03 [n.s]	0.27±0.03 [n.s]	0.4±0.05 [n.s]	0.14±0.05 [n.s]	0.38±0.05 [n.s]	0.15±0.05 [n.s]
PfM	3.1±0.2 [a]	1.3±0.2 [b]	2.22±0.2 [n.s]	2.2±0.2 [n.s]	3.2±0.29 [n.s]	1.2±0.29 [n.s]	3.1±0.29 [n.s]	1.4±0.29 [n.s]
PdM	0.75±0.04 [a]	0.3±0.04 [b]	0.5±0.04 [n.s]	0.54±0.04 [n.s]	0.75±0.06 [a]	0.26±0.06 [b]	0.75±0.06 [a]	0.3±0.06 [b]
R:S	1.11±0.117 [ns]	1.076±0.117 [ns]	1.229±0.117 [ns]	0.958±0.117 [ns]	1.173±0.165 [ns]	1.286±0.165 [ns]	1.049±0.165 [ns]	0.867±0.165 [ns]
R:P	0.511±0.025 [ns]	0.501±0.025 [ns]	0.536±0.025 [ns]	0.476±0.025 [ns]	0.520±0.035 [ns]	0.552±0.035 [ns]	0.502±0.035 [ns]	0.450±0.035 [ns]

b) *J. procera*

Measured Variables	Treatments							
	Inoculation		Soil type		Inoculation x Soil type			
	+AMF	-AMF	S	nS	S		nS	
					+AMF	-AMF	+AMF	-AMF
LN	138.75 ±13.3 n.s	106.92±13 .3 n.s	145±13 .3 a	100.7± 13.3 b	176.2± 18.8 n.s	113.8± 18.8 n.s	101.3± 18.8 n.s	100±1 8.8 n.s
RGR -H	0.06±0. 008 n.s	0.054±0.0 08 n.s	0.060± 0.008 n.s	0.053± 0.008 n.s	0.06±.0 11 n.s	0.058±. 011 n.s	0.056± .011 n.s	0.05±. 011 n.s
RGR -CD	0.034±. 004 n.s	0.028±.00 4 n.s	0.033±. 004 n.s	0.028±. 004 n.s	0.035±. 006 n.s	0.033±. 006 n.s	0.032± .006 n.s	0.024± .006 n.s
SfM	0.224± 0.028 n.s	0.171±0.0 28 n.s	0.237± 0.028 n.s	0.158± 0.028 n.s	0.288± 0.04 n.s	0.185± 0.04 n.s	0.160± 0.04 n.s	0.157± 0.04 n.s
SdM	0.080± 0.01 n.s	0.065±0.0 1 n.s	0.084± 0.01 n.s	0.061±. 01 n.s	0.102±. 015 n.s	0.067±. 015 n.s	0.058± .015 n.s	0.063± .015 n.s
RfM	0.167± 0.03 n.s	0.132±0.0 3 n.s	0.19±0. 03 a	0.11±0. 023 b	0.23±0. 04 n.s	0.15±0. 04 n.s	0.105± 0.04 n.s	0.115± 0.04 n.s
RdM	0.053± 0.008 n.s	0.043±0.0 08 n.s	0.057± 0.008 n.s	0.039± 0.008 n.s	0.065± 0.011 n.s	0.048± 0.011 n.s	0.042± 0.011 n.s	0.037± 0.011
PfM	0.391± 0.05	0.303±0.0 5	0.426± 0.05 a	0.27±0. 052 b	0.517± 0.073 n.s	0.335± 0.073 n.s	0.265± 0.073 n.s	0.272± 0.073 n.s
PdM	0.133± 0.018 n.s	0.107±.01 8±0.018 n.s	0.141± 0.018 n.s	0.1±0.0 18 n.s	0.167± 0.025 n.s	0.115± 0.025 n.s	0.1±0. 025 n.s	0.1±0. 025 n.s
RD	29.733 n.s	30.067±1. 872 n.s	32.258 n.s	27.542 ±1.872 n.s	28.217 ±2.65 b	36.3±2. 65 a	31.25± 2.65 a	23.833 ±2.65 b
R:S	0.702± 0.07 n.s	0.59±0.07 n.s	0.705± 0.07 n.s	0.587± 0.07 n.s	0.693± 0.094 n.s	0.716± 0.094 n.s n.s	0.711± 0.094 n.s	0.464± 0.094 n.s
R:P	0.407± 0.03 n.s	0.35±0.03 n.s	0.406± 0.03 n.s	0.35±0. 03 n.s	0.403± 0.04 n.s	0.408± 0.04 n.s	0.41±0 .04 n.s	0.29±0 .04 n.s

c) *P. falcatus*

Measured Variables	Treatments							
	Inoculation		Soil type		Inoculation x Soil type			
	+AMF	-AMF	S	nS	S		nS	
					+AMF	-AMF	+AMF	-AMF
RC (%)	5.24±2.8 n.s	3.33±2.8 n.s	3.33±2.8 n.s	5.24±2.8 n.s	6.67±4 n.s	0.0 n.s	4.17±4 n.s	6.67±4 n.s
LN	41.5±1.02 n.s	39.25±1.02 n.s	42.167±1.02[a]	38.58±1.02[b]	45.33±1.44[a]	39±1.44[b]	37.67±1.44[b]	39.5±1.44[a]
RGR-H	0.029±0.003 n.s	0.031±0.003 n.s	0.032±0.003 n.s	0.028±.003 n.s	0.032±0.004 n.s	0.031±0.004 n.s	0.026±0.004 n.s	0.031±0.004 n.s
RGR-CD	0.012±0.002[a]	0.006±.002±0.002[b]	0.011±0.002	0.007±0.002	0.016±0.003[a]	0.006±0.003[b]	0.009±0.003[a]	0.005±0.003[b]
SfM	1.908±0.105 n.s	2.108±0.105 n.s	2.075±0.105 n.s	1.942±0.105 n.s	1.967±0.149 n.s	2.183±0.149 n.s	1.850±0.149 n.s	2.033±0.149 n.s
SdM	0.677±0.04 n.s	0.718±0.04 n.s	0.737±0.04 n.s	0.658±0.04 n.s	0.705±.056 n.s	0.768±.056 n.s	0.648±.056 n.s	0.667±.056 n.s
RfM	1.342±0.15 n.s	1.55±0.15 n.s	1.7±0.15 a	1.192±0.15[b]	1.55±0.212 n.s	1.85±0.212 n.s	1.133±0.212 n.s	1.25±0.212 n.s
RdM	0.4630.036 n.s	0.433±0.036 0.036 n.s	0.4970.036 n.s	0.399±0.036 n.s	0.512±0.05 n.s	0.482±0.05 n.s	0.415±0.05 n.s	0.383±0.05 n.s
PfM	3.25±0.23 n.s	3.658±0.23 n.s	3.775±0.230 n.s	3.133±0.23 n.s	3.517±0.325 n.s	4.033±0.325 n.s	2.983±0.325 n.s	3.283±0.325 n.s
PdM	1.14±0.063 n.s	1.15±0.063 n.s	1.233±0.063 n.s	1.057±0.063 n.s	1.217±0.09 n.s	1.25±0.09 n.s	1.063±0.09 n.s	1.05±0.09 n.s
R:S	0.7±0.053 n.s	0.605±0.053 n.s	0.687±0.053 n.s	0.618±0.053 n.s	0.743±0.075 n.s	0.632±0.075 n.s	0.657±0.075 n.s	0.579±0.075 n.s
R:P	0.403±0.018 n.s	0.374±0.018 n.s	0.399±0.018 n.s	0.378±0.018 n.s	0.414±0.025 n.s	0.385±0.025 n.s	0.392±0.025 n.s	0.364±0.025 n.s

+AMF=whole-soil AMF inoculated, -AMF=whole-soil AMF not inoculated, S=sterile potting soil, nS=nonsterile potting soil, RC=root AMF colonization, LN=Leaf number, LA Leaf area (cm^2),=RGR-LN=Relative leaf number growth rate (LN per week), RGR-H=Relative height growth rate (cm per week), RGR-CD=Relative collar diameter growth rate (mm per week), TfM=Total plant fresh mass (g), SfM=Shoot fresh mass (g), SdM=Shoot dry mass (g), RfM=Root fresh weight (g), RdM Root dry mass (g), RD=Rooting depth (cm), R:S=Root to shoot dry mass ratio, and R:T=Root to plant dry mass ratio. Means with different letters (across the row) are statistically significantly different after *t-test* ($p<0.05$).

Considering the existing Ankorrcha forest AMF inoculum, it was found that, similar to the MR results, all growth variables except R:S and R:P showed a positive percentile increase in mean values due to the existing Ankorcha AMF inoculum in the case of *C. africana*. However, in the case of *J. procera* and *P. falcatus*, those seedlings grown on nonsterile-noninoculated potting soil had percentile decreases in the mean growth values compared to those grown on the sterile-non-inoculated soil with the exeption of LN of *P. falcatus* which had a slight percentile increase (Table 15).

Table 15: Growth responses of *C. africana*, *J. procera* and *P. falcatus* due to native Ankorcha AMF inoculum. Growth response is expressed as percent difference in growth of seedlings grown on the non-sterile non-inoculated soil compared to those grown on sterile non-inoculated Ankorcha soil.

Growth and quality variables	Tree species		
	C. africana	*J. procera*	*P. falcatus*
LN	+163.9	-11.5	+1.2
RGR-H	+23.53	-13.79	0
RGR-CD	+266.67	-27.27	-16.67
SfM	+11.63	-15.14	-6.87
SdM	+54.55	-5.97	-13.15
RfM	+21.62	-23.33	-32.43
RdM	+7.14	-29.92	-20.54
PfM	+16.67	-18.8	-18.6
PdM	+15.4	-13	-16
R:S	-32.58	-35.2	-8.4
R:P	-18.48	-28.9	-5.45

Figure 21: *C. africana* seedlings after 5 months of growth in the mesh-house. The difference in leaf number and area between treatments is visible. S&nI=sterile potting soil and seedlings not inoculated, S&I=sterile soil and seedlings inoculated, nS&nI=non-sterile soil and seedlings not inoculated, nS&I=non-sterile soil and seedlings inoculated with whole-soil AMF.

5. DISCUSSION

5.1. The AMF status of the DAF nurseries surveyed

The production of resilient native tree/shrub seedlings is an integral component of DAF restoration as passive restoration, i.e., restoration without tree/shrub planting, is hardly possible (Aerts et al., 2007; Mulugeta Lemenih and Demel Teketay, 2004). Therefore, our survey about AMF status in DAF nurseries is very relevant. Our results indicate that similar to a report from a tropical forest in Brazil (Zangaro et al, 2000), RC levels were variable between tree functional groups. Hence, RC levels of the early-mid successional tree species; *A. abyssinica*, *C. africana*, *D. abyssinica*, and *O. europaea* had high RC rates while the mid-late successional tree species; *J. procera*, *P. falcatus*, and *P. africana* had medium or low RC rates. Compared with another study from other DAF nurseries in Ethiopia (Michelsen 1992), RC levels found in our study were similar in the case of *P. falcatus*, but were higher in the case of *A. abssynica*, *C. africana*, *J. procera*, and *O. europaea*.

Previously, Tesfaye Wubet et al (2009) have found that the AMF species composition of *O. europaea*, *P. falcatus* and *P. africana* seedlings and their conspecific adult trees varied significantly. Similarly, our results indicated that RC levels could also vary between seedlings and conspecific adult trees of mid-late successional tree species. Hence, the RC levels we found for the mid-late successional tree species *J. procera*, *P. falcatus*, and *P. africana*, were lower than the RC levels previously reported for their conspecific adult trees in DAF (Emiru Birhane et al., 2017; Tesfaye Wubet et al., 2003a). However, in the case of the early-mid successional tree species; *C. africana*, *D. abyssinica*, and *O.*

europaea, RC levels we found were comparable with the RC levels reported for their conspecific adult trees (Emiru Birhane et al., 2017, 2018; Tesfaye Wubet et al., 2003a). Hence, mid-late successional tree species could also be less responsive to AMF inoculation compared to early-mid successional tree species at the seedling stage (Zangaro et al., 2003). Carrillo-Garcial et al., (1999) also argued that seedlings of late-successional tree species could remain with low RC level for the first year and become highly infected afterwards. If proven true, this may indicate that AMF inoculation of mid-late successional tree species would be more relevant not in the nursery but during field plantation after seedlings grew for more than a year.

The SA levels we found in DAF nurseries were comparable with the SA levels we found across land uses in the Chilimo forests (section 4.2.2) and with that reported for the DAF ecosstem of north Ethiopia (Emiru Birhane et al., 2018, 2020) but were still very lower than the SA levels reported for another similar ecosystem (Yoseph Tewodros et al., 2017). Seedlings raised in N1 and N4 had significantly (p<0.05) high SA compared to those raised in N8 and N10. This difference could less likely be due to pot substrate ratio or climate difference between these nurseries as both of these variables did not have directional differences along with the high-to-low SA nurseries. Therefore, this difference could most likely be related to the type of potting substrate the nurseries used. It was observed that the potting soil in N1 and N4 was red clay soil while in N8 and N10 it was black loam soil. It is reported that SA could be significantly influenced by clay content (Silva-Flores et al., 2019). The difference may as well be related to other nursery management variables not considered in this study. It could also, most probably, be

related to soil physicochemical factors (Apple et al, 2005; Emiru Birhane et al., 2018; Oliveira and Oliveira, 2010) not considered in this study.

Generally, none of the seedlings were found to have both low RC and SA at the same time, hence, inoculation of DAF seedlings in the nursery or during field planting may be less important than thought. However, comparatively, *J. procera*, *P. falcatus*, and *P. africana* had the lowest levels of both RC and SA and these could still be considered for inoculation. We found RC to be mainly affected by host species, while SA was by nursery location. This is in perfect agreement with findings published by John (1980), Schüßler et al. (2016), and Silva-Flores (2019). John (1980) has indicated that RC levels within a host species may be within a constant range. Silva-Flores (2019) has on the other hand, found that SA was affected by soil factors, but not by host species. A nursery AMF inoculation experiment by Schüßler et al. (2016) has also shown that RC was more significantly influenced by host and AMF species than soil factors. The fact that *A. abyssinica* and *M. ferruginea* (Fabaceae) had both high RC rates, while *H. abyssinica* and *P. africana* (Rosaceae) had variable RC rates, also agrees with the assertion by John (1980), who stated that; RC levels at the plant genus and family levels could show variable trends.

Root AMF colonization and SA of *C. africana*, *J. procera*, and *O. europaea* significantly ($p<0.05$) varied across nurseries indicating that nursery management had potentially influenced both RC and SA. For *J. procera* and *O. europaea*, nurseries that resulted in low RC and high RC levels corresponded to those raising young and older seedlings,

respectively. For *O. europaea* in particular, the RC level in N6 was significantly (p<0.05) the highest, and in N5, it was the lowest. These nurseries were very closely located with similar climate and maybe, comparable soil properties but they differed in pot substrate ratio and age of seedlings, viz., in N6, they were 11 months in age while in N5, they were 5 months. This may indicate that age had a significant effect on RC of *O. europaea* seedlings. The correlation results also indicated that the RC-age correlation for *O. europaea*, was significant, very strong, and positive (r_s=0.829, p=0.042). Similarly, according to Michelsen's observation, nursery seedlings of smaller age tended to have low RC than seedlings of bigger age (Michelsen, 1992). According to our results, the effect of age was, however, not similar for *C. africana*, *J. procera*, and *O. europaea*. Moreover, RC-age correlation with all tree species considered, was very weak and not significant (rs=-0.050, P=0.807) indicating RC-age correlation was most probably host-dependent. Similarly, Abbott and Robson (1991) have compiled three different works that showed age and RC correlations could be quite variable between different host species.

The significant (p<0.05) very strong positive RC-pot diameter (PD) and RC-pot volume (PV) correlations we found for *C. africana* are also in agreement with previous reports although not true for other plant species (Audet and Charest, 2010; Zangaro et al., 2015). The strong negative SA-RSHI and the significant (p<0.05), very strong negative SA-RCD correlations for *C. africana,* and the strong negative RC-RSHI and RC-RCD correlations for *O. europaea*, could be related to AMF parasitism as indicated by Johnson et al (1997). However, the most probable reason for the observed negative correlations

could be pseudo negative growth rates due to the small pot size. Due to smaller pot height and diameter, *C. africana* and *O. europaea* seedlings may not grow to their physiological potential. Hence, seedlings could remain with the same SHI and CD despite the increase in age (i.e., negative growth rates) while SA and RC on the other hand, increased with age.

The very weak correlation we found between RC and SA is supported by previous studies (Abbott and Robson, 1982; Douds and Schenck, 1990). The fact that AMF community composition of roots, spores, and extraradical mycelia have been shown to significantly vary (Varela-Cervero et al., 2015) also indicates that RC and SA could be independent. Moreover, the RC-SA correlation could depend on the AMF species composition in the rhizosphere with different AMF species requiring different critical RC levels to reach maximum sporulation (Gazey et al., 1992).

Despite its importance in planning AMF inoculation (Verbruggen et al., 2013), in this study, we did not determine the AMF composition of seedlings. Therefore, future nursery researches should consider the AMF composition of seedlings to better inform AMF inoculation projects. We did not also determine soil physicochemical properties because these variables may less likely be modified by nursery managers. However, future researches could consider these variables as they potentially have significant effects on RC and SA. In the previous DAF nursery AMF survey, SA was not determined for each tree species and across nurseries. Considering both RC and SA in AMF nursery surveys is important since the root, spores, and external hyphae are the source of different AMF

inoculum (Varela-Cervero et al., 2015). It is also important because different AMF species have a different mode of infection (Chagnon et al., 2013; Hart and Reader, 2004), and AMF species favored by trees may differ at various growth stages (Husband et al., 2002). In this study, we determined both RC and SA per individual seedlings across nurseries. Future nursery surveys could further consider soil hyphae abundance such that all AMF inoculum pools are evaluated.

To our knowledge, this is the first large scale AMF study conducted by using black Hero ink for AMF staining. The suitability of black hero ink that we have found is partly in agreement with Vierheilig et al., (2005) who stated that; almost any black ink could be suitable for AMF staining. However, it is against the report by Cao et al. (2013) which indicated that washable blue hero ink was more suitable for AMF staining than black hero ink. *Acacia abyssinica*, *M. ferruginea,* and most importantly, *D. abyssinica* seedlings' roots were very highly infected with AMF and hence, these species could be potential trap-tree species to prepare root AMF inoculums in the future. Since root inoculum could be produced in a short period and since it can infect seedlings' root much faster, root inoculum is considered to be a better alternative to conventional crude inoculum (Mitiku Habte and Osorio, 2001).

5.2. The resilence of AMF to the degradation and deforestation of the Chilimo forest

5.2.1. The resilience of soil physicochemical property and AMF communities composition 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 resilience study we carried out for the highly threatened Chilimo forest (Terefe Tolessa et al., 2017) was very timely. Previously, Terefe Tolessa and Feyera Senbeta (2018), and Siraj Mammo et al. (2019) have studied the physicochemical property of the natural forest (NF) while Mehari Tesfaye, et al. (2016) have 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 Chilimo and other DAF (Table 16). 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 Hillette 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 (Emiru 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 (Tekalegn Mamo et al., 2002). Moreover, Hillette Hailu et al. (2015) have sampled vertisol, but only a few of our samples were vertic. The difference in the BD values compared to Siraj 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 Siraj 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, Mehari Tesfaye et al. (2016) have observed that OC and N reduced due to the deforestation of the Chlimo forest. Mulugeta Lemenih et al (2005), Yoseph Tewodros et al. (2017), and Emiru 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 physicochemical functional resilience is evaluated using DAF characteristic tree species. Mulugeta Lemenih et al (2005) evaluated DAF soil physicochemical functional resilience to deforestation by considering maize 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 in relation to maize yield.

The other and the main reason why we evaluated soil physicochemical property resilience was to use it as a proxy to estimate AMF community composition resilience. Hence, our

results indicated that AMF community composition may be resilient to degradation but not deforestation of the Chilimo forest. However, considering pH and P the important soil chemical variables determining AMF community composition (Garcia de Leon et al., 2018), the fact that no significant difference in P was found either due to degradation or deforestation may indicate AMF community composition could be resilient to deforestation as well. Moreover, Schechter and Bruns (2012) have remarked that soil physicochemical property resilience could be an important proxy to AMF community composition if there is a wider difference in a physicochemical property. Hence, it may not be possible to conclude whether the AMF community composition was or was not resilient to the deforestation of the Chilimo forest based on the soil physicochemical property alone. Furthermore, AMF community composition resilience could be greatly dependent on the AMF species pool. In areas where the species pool is dominated by generalists, AMF community composition could potentially be resilient despite a significant soil physical and chemical property changes (Hawkes and Keitt, 2015). However, our results have indicated that degradation and deforestation (change to grazing land) of Chilimo forest, have resulted in a significant AMF functional changes (infectivity) and this could indicate that there were significant changes in AMF community composition; possibly fast colonizer species dominated the NF and CrL and the slow colonizers dominated the ShL and GrL (Oehl et al., 2003). Moreover, from the factors which could determine infectivity, viz., host species, climate, edaphic factors, and soil AMF community (Moreira et al., 2006), since in our case infectivity was determined from trap culture of single species and a similar greenhouse microclimate, infectivity may be most likely related to the soil factors and AMF communities.

Table 16: The values of soil physicochemical variables previously reported comparative to the values found in this study.

Variables	NF of CF			CrL of CF	NF of other DAF		
	Mehari Tesfaye et al (2016)[1]	Siraj Mammo et al (2019)[3]	Terefe Tolessa and Feyera Senbeta (2018)[4]	Hillette Hailu et al. (2015)[2]	Mulugeta Lemenih et al. (2005)[1]	Yoseph Tewodros et al. (2017)[1]	Emiru Birhane et al. (2018)[5]
BD	**+14.75 %**	+26.23%			**+8.2%**		
pH		**+1.96%**	**-4.52%**	+36.84 %	**-6.63%**	**+3.31%**	+25%
EC				+214.3 %			-28.6%
N	+89.14%	+182.86 %		-26.27%	+125.71 %	+88.6%	**-2.86**%
P		+272.93 %		-47.68	-60.1%		-65.74%
P:N							
OC	+46.34%	**-3.3**%		-61.5%	**+17.94%**	**+24.5%**	-54.11%
C:N					+53.12		
CEC					+43.56%		
Sand					**-17.62%**		
Silt					+45.45%		
Clay					+123.3%		

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=cropland, DAF=dry evergreen Afromontane forests. [1]1-10 cm sample, [2]1-15 cm sample, [3]1-20 cm sample, [4]1-30 cm sample, [5]rhizospheric soil

5.2.2. The resilience of soil AMF infectivity and spore abundance (SA) to Chilimo forest degradation and deforestation

The degradation and deforestation of the Chilimo forest have reduced the canopy cover to expose the soil to elevated temperature that poses moisture and heat stresses. We have also found substantial reductions in soil nutrients due to the degradation and

deforestation. Hence, the resilience (increase in SA) we found despite forest degradation or deforestation could be related to the AM fungi stress avoidance strategy. When fungi are stressed due to scarcity of carbohydrates, soil nutrients, and moisture among others, AMF sporulation increases to avoid the stress period at a resting phase, the spore (Violi et al., 2008; Silva-Flores et al., 2019). On the contrary, AMF infectivity was not resilient to the degradation and the conversion of natural forest to grazing lands. Hence, generally, our results are in perfect agreement with Abbott and Robson (1982) who, in their review, had demonstrated that while SA could be considerably lower in virgin soils compared to disturbed soils, infectivity could, on the contrary, be higher in virgin soils. Previously, Emiru Birhane et al. (2018) have also reported a similar increase and reduction of SA and infectivity, respectively due to DAF degradation in north Ethiopia. Hence, the fact that we found SA increase along with the increase in forest degradation and soil nutrient stress gradient is corroborated. However, Yoseph Tewodros et al (2017) have reported a reduction of SA due to DAF deforestation and degradation in northern Ethiopia.

The change in infectivity, in the case of NF-GrL conversion, most probably, has 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 Zn and Cu not considered in this study may have not been resilient to NF-ShL conversion and hence resulted in AMF composition change (Xu et al., 2017) and therefore 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 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 big role. However, in the case of GrL, the significantly low infectivity in GrL3b coincides with lower P and P:N values (Fig. 17b; Fig. 19) and 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* in return could have significantly modified AMF composition and thereby infectivity. The predominance of ruderal plants for a long 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 composition shift (Faggioli et al., 2019). The fact that we found *Pennisetum sphacelatum* dominated grazing land 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 (3.64-9.89 spores g-1) for the two of the remnant DAF of north Ethiopia (Emiru Birhane et al., 2018, 2020) and it is also comparable with the SA levels we found for DAF nurseries of central and northern Ethiopia (section 4.1.2). However, the SA found was much lower than the SA level reported (41-129 g-1) by Yoseph Tewodros et al. (2017) across land uses in the DAF ecosystem of northern Ethiopia. This difference could, to some extent, be related to the difference in the lowest sieve size used (53 µm used in this study vs. 38 µm) to separate spores. 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) the soil physicochemical variables, while infectivity significantly correlated to a few of (+pH, +P:N, and -Clay) 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, Emiru Birhane et al. (2018) also reported a slightly negative but statistically non-significant correlation (Pearson) of SA with pH, EC, and soil nutrients (N, P, OC). However, Yoseph Tewodros et al. (2017) have 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, and therefore; moisture could less likely be the reason for the observed SA, infectivity, and clay relationships. Hence, the main reason could be the clay content relationship with BD, CEC, OM, and other soil factors.

5.3. Mycorrhizal responsiveness and whole-soil AMF inoculation effects on the growth and quality of *C. africana, J. procera,* and *P. falcatus* seedlings planted on a degraded DAF ecosystem soil

Similar to the RC levels, we found in the nursery survey (section 4.1.2), *C. africana* seedlings were found to have moderate-high RC, while *P. falcatus* had very low RC including on the non-sterile potting soil and also despite receiving whole-soil AMF inoculum. Moreover, similar to the MR trend reported for tropical tree species (Kiers et al., 2000; Zangaro et al., 2003, 2007), we found a general trend of high, medium, and low MR of early successional, mid successional, and late-successional tree species, viz., *C. africana*, *J. Procera*, and *P. falcatus*, respectively. Hence, it could possibly be the fact that early-successional tree species' success on degraded sites is partly related to the fact that they evolved to be highly arbuscular mycorrhizal. Late-successional tree species, on the other hand, recruit under the shade and on forest soils with better soil attributes, and

hence, they may have evolved to be less dependent on AMF at the seedling stage. Hence, in accordance with the optimal partitioning theory which suggests that plants' photoassimilate allocation to organs is proportioned in such a way that whole-plant growth is optimized (Comas et al., 2013), late-successional tree species may allocate more carbohydrate to the shoot/leaf rather than to the root and mycorrhiza so that they can better compete for light. Similarly, the R:S ratio we recorded for *C. africana* was generally much higher than the R:S ratios of both *J. procera* and *P. falcatus* (Table 13). Our observation is, however, against the famous assertion by Janos (1980) who stated that late-successional tropical tree species are obligately mycotrophic and therefore, necessarily require AMF for their growth. Our results are also against the report by Huante et al. (2012) which indicated that early successional tropical lowland forest tree species could be less responsive to AMF compared to the mid and late successional counterparts. However, it could also be the case that mycorrhizal responsiveness of late-successional tropical tree species increases once the trees have grown well enough and start to partition more carbon to the below-ground biomass (Carrillo-Garcial et al., 1999).

Our results clearly show that whole-soil AMF inoculation of *C. africana* had significant effects on the growth of this tree species, while it had little or no effects on *J. procera* and *P. falcatus* growth. Similarly, Beyene Dobo et al. (2016) have reported a significant growth response of *C. africana* to AMF inoculation. Moreover, the fact that no inoculation x soil type interaction effects were found on *C. africana* growth, showed that AMF inoculation of *C. africana* seedlings increased growth equally both on the sterile and non-sterile potting soil and hence this indicates that AMF inoculation of *C. africana*

could be relevant in most field conditions. The only inoculation x soil type interaction effect found was on the SfM of *C. africana*, and this shows that there was more growth due to inoculation on the sterile soil compared to the non-sterile potting soil.

From the previous field experiment (Fisseha Asmelash et al., 2019), it was also found that *J. procera* and *P. falcatus* did not benefit from whole-soil AMF inoculation, although on fertile soil. Similarly, in this study, *J. procera* and *P. falcatus* grown on degraded soil were also found to be less responsive to AMF inoculation. These may indicate that instead of going for *J. procera* and *P. falcatus* AMF biotechnology, considering after planting care as recommended by Legesse Negash (2010) may be more beneficial. Moreover, the co-plantation of these tree species with AMF inoculated-highly mycorrhizal responsive nurse shrub as demonstrated by Barea et al. (2011) and Duponnois et al. (2011) could be the other option. However, it should be noted that we inoculated *P. falcatus* with *J. procera* rhizospheric soil and the low or no inoculation effects may have resulted due to lack of *P. falcatus* specific fungi in the inoculum. Tesfaye Wubet et al (2006) have earlier found that co-occurring *J. procera* and *P. falcatus* were colonized by distinct AMF communities. However, the fact that *J. procera* did not benefit despite receiving conspecific inoculum may indicate that low AMF responsiveness of *J. procera* and *P. falcatus* could be inherent. Moreover, it has been shown that the seedlings of tropical tree species could respond positively to a wide range of AMF inoculum (Schüßler et al., 2016). Hence, in our case, host AMF inoculum specificity could be less important to the observed low response of the two conifers.

The fact that *C. africana* was also highly responsive to the existing Ankorcha AMF inoculum and the fact it had an increase in growth due to the existing AMF inoculum compared to *J. procera* and *P. falcatus* shows that *C. africana* could respond positively to a wide range of AMF inocula, while *J. procera* and *P. falcatus* could not. Similarly, Gavito et al. (2008) have found that at least at a seedling stage, plant species was a more important determinant of mycorrhizal response than inoculum type. The other interesting result found in this study was the negative R:S or R:P of *C. africana* seedlings to both inoculation and existing AMF inoculum depite positive responses for most of the growth variables (Fig. 20, Table 15). A similar result has been reported for tropical early-successional tree species by Zangaro et al. (2007). Moreover, in his review, Diriba Muleta (2017) has indicated that AMF inoculation could, in most cases, decrease the R:S of legume plants.

In both of the nursery survey and the mesh-house experiment, we observed *P. falcatus* roots to bear abundant nodules. These nodules, are considered to significantly improve root volume, and hence, mycorrhization and due to their high turnover could be important in the maintenance of effective mycorrhization (Dickie and Holdaway, 2010). However, both our nursery and mesh-house results and that of the previous nursery report results by Michelsen (1992) indicate that *P. falcatus* seedlings have a very low or low AMF root colonization. Therefore, although based on these observations it could be hard to associate the abundant root nodules with mycorrhization, it could be possible that these nodules are important for soil resource acquisition by Podo seedlings and this could be an interesting area of future studies.

6. CONCLUSION

This Ph.D. research was carried out to answer five of the most important research hypotheses that are crucial for DAF restoration. Accordingly, we have sufficiently answered these hypotheses. We hypothesized that DAF native tree species seedlings in DAF nurseries could be low with infective AMF inoculum and hence, we considered this could be one of the reasons why low rates of DAF native tree seedlings field survival and establishment is commonly observed. However, our results did not validate this hypothesis. Rather our results, contrary to previous reports, indicated that DAF native tree species were not deficient with infective AMF inoculum, i.e., SA and RC were not low simultaneously. However, we have observed that RC was species-dependent and the mid and late-successional tree species *Juniperus procera*, *Podocarpus falcatus*, and *Prunus africana* were with low RC levels while it was the vise-versa in the case of the early-successional tree species.

Our results indicate that none of the nursery management variables considered affected RC and SA when all trees are considered. However, when individual tree species are considered, age (seedlings nursery stay time) and pot volume were found to have significant effects for *O. europaea* and *C. africana*, respectively. Therefore, nursery managers could be advised not to take *O. europaea* seedlings out for plantations at a very young age despite height attainment. Nursery managers could also use comparatively larger volume pots in order to improve AMF inoculum of *C. africana* seedlings.

The second research hypothesis we had was that we thought AMF spore abundance and infectivity may not be resilient to the degradation and deforestation of the Chilimo forest and hence, AMF inoculation could be relevant in DAF restoration. According to our results, it was found that SA was resilient to DAF degradation and deforestation but infectivity was resilient only to NF conversation to CrL. Hence, based on our result, it could be safely concluded that since both SA and infectivity were resilient to NF to CrL conversation, it could be less likely that AMF inoculation would serve a purpose when reforestation takes place on CrL in Chilimo forest.

Thirdly, we hypothesized that soil physicochemical property of Chilimo forest is not resilient to the degradation and deforestation and hence, fourthly, we hypothesized, soil AMF community composition may not be resilient as the result and therefore, AMF inoculation of forest adapted communities would be beneficial in Chilimo forest (DAF) restoration. These hypotheses were also proved to hold partly. Hence, soil physicochemical property of the Chilimo forest was only resilient to degradation (NF conversion to ShL) but not to deforestation (Conversion into either CrL or GrL). Therfore, we may conclude that AMF inoculation could be important if plantation sites are either CrL or GrL. However, since the CrL was resilient to SA and infectivity, we may safely conclude it is the GrL that needs inoculation. An important conclusion to draw is that when planting sites are dominated with non-palatable grass species like *Pennisetum sphacelatum*, inoculating tree seedlings with forest adapted inoculum may be more beneficial.

Our last hypothesis was that we considered native whole-soil AMF inoculation could significantly improve the growth and quality of *Cordia africana*, *Juniperus procera,* and *Podocarpus falcatus* when planted on a degraded DAF ecosystem soil. Our results once again proved to hold partly. Accordingly, we found inoculation was effective only to the early-successional tree species *C. africana* and not to the mid and late-successional tree species *J. procera* or *P. falcatus*. Therefore, future DAF restoration projects should consider AMF inoculation to the early-successional tree species, and for the mid and late-successional tree species, after planting care should be the best way of ensuring seedlings field survival and establishment.

In this study, we have, for the first time, demonstrated that the locally available black Hero ink was very much suitable for AMF root colonization (RC) determination. Hence, future nursery or field level AMF surveys could consider black Hero ink as an alternative stain. Although, the AMF study of DAF ecosystem is crucial owing to the fact that DAF restoration is the most important environmental agenda, there has been a few of AMF studies of the DAF ecosystem. Hence, our study is crucial as it tried to address some of the understudied topics. Therefore, this Ph.D. work could potentially be useful to guide future AMF inoculation plans in DAF restoration while it could certainly be more useful to guide future research needs. Based on our results we can conclude that inoculating early-successional tree species and *C. africana* in particular could be beneficial in a wide range of soil conditions but would be more effective when planting sites are the non-palatable Pennisetum grazing lands of the DAF ecosystem.

7. RECOMMENDATIONS

- In this Ph.D. work, the AMF species composition across land uses in the Chilimo forest and the AMF composition of nursery samples was not determined using the collected soils, or trap culture and morphologically and/or molecularly. Therefore, in the future, the effect of forest degradation and deforestation on AMF community composition should be investigated comprehensively by using field-collected soils and trap culture and by employing morphological and molecular taxonomy. The AMF status of DAF nursery seedlings in relation to the AMF community composition of seedlings should also be investigated.
- We have found some nursery management practices could significantly affect tree seedlings AMF status, in the future, the effect of pot volume and age on the SA and RC of seedlings should be further investigated using tree species of various tree functional groups so that nursery managers could better be advised.
- Considering the fact that AMF study results from adult trees and forests do not necessarily translate to seedlings, AMF studies at the nursery level should receive sufficient attention.
- We have found *C. africana* (early-successional) tree species to be responsive to whole-soil AMF inoculation, while the mid (*J. procera*) and late (*P. falcatus*) successional tree species were not. Therefore, to prove if this observation is generally true, massive AMF inoculation experiments in which several DAF tree species of various successional stages are included should be carried out in the near future.

- Moreover, the effect of age on the AMF responsiveness of various tree species of different successional groups has to be investigated.
- We have observed that most of the DAF nurseries of northern and central Ethiopia do not raise native trees as was the case some years ago. Therefore, relevant government and non-governmental actors should work to promote and technically support the propagation of native trees in the DAF nurseries.

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9. ANNEX

Annex 1. Tree and shrub species being raised in the surveyed nurseries and the proportion of native tree and shrub species.

Species	Nurseries										Frequency		Remark
	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	Tally	Rank	
Acacia abyssinica	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**9**	**Native**
Acacia albida	0	0	0	0	0	0	0	0	1	1	2	8	Native
Acacia decurrens	1	1	0	0	0	0	1	1	0	0	4	6	Exotic
Acacia melanoxylon	1	1	0	0	0	0	0	0	0	0	2	8	Exotic
Acacia saligna	1	0	0	0	0	1	1	0	0	0	3	7	Exotic
Albizia gummifera	1	1	0	0	0	0	0	0	0	0	2	8	Native
Bersama abyssinica	1	0	0	0	0	0	0	0	0	0	1	9	Native
Borassus aethiopum	1	0	0	0	0	0	0	0	0	0	1	9	Native
Callistemon citrinus	1	0	1	0	0	0	0	0	0	0	2	8	Exotic
Carica papaya	0	0	0	0	1	0	0	0	1	0	2	8	Exotic
Casuarina equisetifolia	1	0	1	0	1	1	1	0	0	1	6	4	Exotic
Citrus sinensis	0	0	0	0	0	0	0	0	1	0	1	9	Exotic
Coffae arabica	0	0	1	0	1	0	0	0	1	0	3	7	Native

Cordia africana	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**0**	**1**	**7**	**3**	**Native**
Cupressus lusitanica	1	1	0	1	0	1	1	1	1	1	8	2	Exotic
Cytisus proliferus	0	0	0	0	0	0	1	1	0	1	3	7	Exotic
Delonix regia	0	0	0	0	0	0	0	0	1	0	1	9	Exotic
Dovyalis abyssinica	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**3**	**7**	**Native**
Dovyalis caffra	1	1	0	0	0	0	0	0	0	0	2	8	Exotic
Dracaena steudneri	0	0	1	0	0	0	0	0	0	0	1	9	Native
Duranta sp.	0	0	1	0	0	0	0	0	0	0	1	9	Exotic
Erythrina brucei	0	1	0	0	0	0	0	0	0	0	1	9	Native
Eucalyptus camaldulensis	0	0	0	0	1	0	0	0	0	1	2	8	Exotic
Eucalyptus globulus	0	0	1	1	0	0	1	1	0	1	5	5	Exotic
Grevillea robusta	1	0	1	1	1	1	1	1	1	1	9	1	Exotic
Hagenia abyssinica	**0**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**3**	**7**	**Native**
Jacaranda mimosifolia	1	0	0	0	1	0	0	0	0	0	2	8	Exotic
Juniperus procera	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**6**	**4**	**Native**
Mangifera indica	0	0	1	0	0	0	0	0	1	0	2	8	Exotic
Melia azedarach	1	0	1	0	0	1	0	0	0	0	3	7	Exotic
Millettia ferruginea	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**8**	**Native**
Moringa oleifera	0	0	0	0	0	0	0	0	1	0	1	9	Exotic
Musa x paradisiaca	0	0	0	0	0	0	0	0	1	0	1	9	Exotic
Olea europaea* subsp. *cuspidata	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**1**	**7**	**3**	**Native**
Persea americana	0	0	1	0	1	0	0	0	0	0	2	8	Exotic
Phoenix reclinata	1	1	0	0	0	0	0	0	1	0	3	7	Native

Pinus patula	1	0	0	0	0	0	0	0	0	0	1	9	Exotic
Podocarpus falcatus	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**8**	**Native**
Populus tremuloides	0	0	0	0	0	0	1	1	0	0	2	8	Exotic
Prunus africana	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**9**	**Native**
Psidium guajava	0	0	0	0	1	0	0	0	0	0	1	9	Exotic
Rhamnus prinoides	0	0	0	0	0	0	1	0	0	0	1	9	Native
Schinus molle	1	0	0	0	1	0	0	0	0	0	2	8	Exotic
Sesbania sesban	0	0	0	0	0	0	1	0	0	0	1	9	Native
Terminalia mantaly	0	0	1	0	0	0	0	0	0	0	1	9	Exotic
Number of species raised	**22**	**11**	**13**	**5**	**13**	**10**	**12**	**8**	**13**	**10**	**-**	**-**	**-12**
Native proportion	**45 %**	**64 %**	**31 %**	**40 %**	**38 %**	**50 %**	**33 %**	**25 %**	**38 %**	**40 %**	**-**	**-**	**-40%**

N1 Susuni, N2 Entoto, N3 Teferi & Mulugeta, N4 Sokondo, N5 Jello, N6 Qebenewa, N7 Debre birhan teachers college, N8 Mush, N9 Tis aba lima, N10 Hizba tekelehaimanot

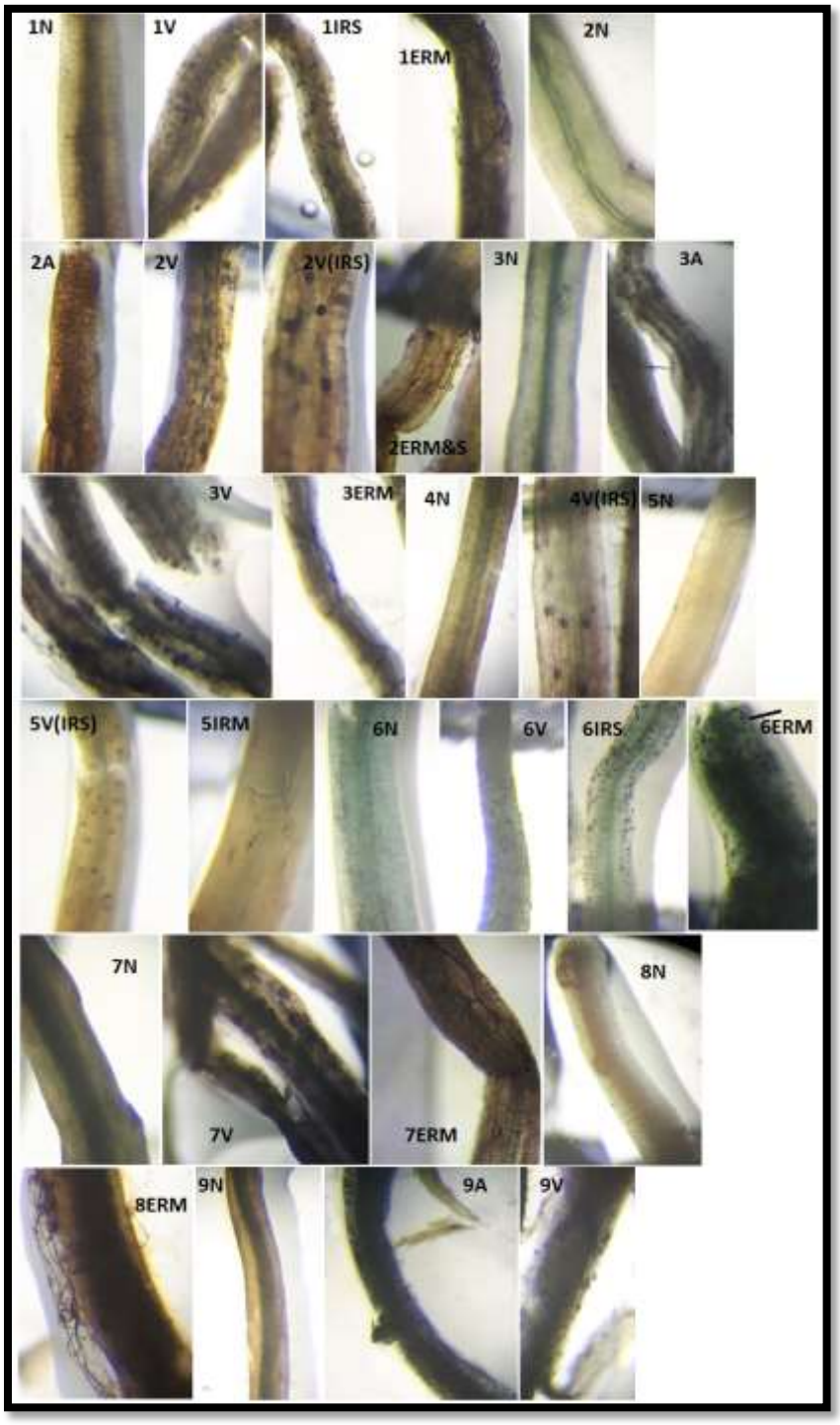
Annex 2. Collected seedlings associated data.

Code	Species	Nursery	Age (months)	PHI (cm)	PD (cm)	PV (cm^3)	SHI (cm)	CD (mm)	RSHI (cm $month^{-1}$)	RCD (mm $month^{-1}$)
1c	*Olea europaea* subsp. *cuspidata*.	N5	5	13	5	255.13	26.57	3.38	5.31	0.68
2c	*O. europaea*	N6	11	13	5	255.13	27.15	5.24	2.47	0.48
3b	*O. europaea*	N2	16	13	6	367.38	23.46	4.48	1.47	0.28
4a	*O. europaea*	N9	2	14	5	274.75	11.14	1.75	3.71	0.58
5a	*O. europaea*	N10	9	13	5	255.13	17.69	2.95	1.97	0.33
6b	*O. europaea*	N8	8	13	5	255.13	19.54	2.91	2.44	0.36
1d	*Cordia africana*	N5	5	13	8	653.12	11.37	4.07	3.79	1.36
3d	*C. africana*	N2	16	10	6	282.6	8.99	6.43	0.56	0.4
5b	*C. africana*	N10	3	13	5	255.13	20.28	6.53	6.76	2.18
6a	*C. africana*	N8	4	13	6	367.38	8.77	5.44	2.19	1.36
9b	*C. africana*	N4	6	13	6	367.38	21.11	5.85	3.52	0.97
10c	*C. africana*	N1	9	14	7	538.51	31.18	9	3.46	1
1b	*Juniperus procera*	N5	5	13	5	255.13	14.29	2.24	2.86	0.45
2a	*J. procera*	N1	14	13	5	255.13	25.66	5.4	1.83	0.39
3a	*J. procera*	N2	16	13	6	367.38	22.26	4.13	1.39	0.26

5c	*J. procera*	N10	9	13	5	255.13	13.71	2.78	1.52	0.31
7a	*J. procera*	N7	10	12	6	339.12	18.24	3.14	1.82	0.31
1a	*Dovyalis abyssinica*	N5	5	13	5	255.13	14.1	3.57	2.82	0.71
7b	*D. abyssinica*	N7	11	12	6	339.12	22.94	4.48	2.09	0.41
8a	*Podocarpus falcatus*	N3	10	8	4	100.48	11.92	2.48	1.19	0.25
10a	*P. falcatus*	N1	4	13	6	367.38	32.97	5.87	4.12	0.73
2d	*Hagenia abyssinica*	N6	11	13	5	255.13	9.34	5.07	0.85	0.46
9a	*H. abyssinica*	N4	5	13	5	255.13	23.62	4.07	4.72	0.81
10b	*Acacia abyssinica*	N1	8	14	7	538.51	10.13	2.86	2.53	0.72
2b	*Millettia ferruginea*	N6	7	13	5	255.13	21.74	5.54	3.11	0.79
3c	*Prunus africana*	N2	5	13	6	367.38	14.75	3.64	2.95	0.73

PHI pot height, PD pot diameter, PV pot volume, SHI seedling height, CD seedling collar diameter, RSHI seedling height per age, RCD seedling collar diameter per age, N1 Susuni, N2 Entoto, N3 Teferi & Mulugeta, N4 Sokondo, N5 Jello, N6 Qebenewa, N7 Debre birhan teachers college, N8 Mush, N9 Tis aba lima, N10 Hizba tekelehaimanot.

Annex 3. Sample of AMF root colonization structures observed:



1 Acacia abyssinica, **2** Cordia africana, **3** Dovyalis abyssinica, **4** Hagenia abyssinica, **5** Juniperus procera, **6** Millettia ferruginea, **7** Olea europaea subsp. cuspidata, **8** Podocarpus falcatus, **9** Prunus africana, **N** Root not colonized with AMF, **A** Arbuscule, **ERM** Extraradical mycelium, **IRM** Intraradical mycelium, **IRS** Intraradical spore, **S** Spore, **V** Vesicle, **()** or, **&** and. Note: all pictures are have similar scales.

Annex 4. Forest patches of Chilimo forest with their location and estimated forest cover (Data source: Oromia forest and wildlife organization, February 2019).

No	Name of forest patch	Location/ district	Area of natural forest & shrub land (ha)	Area of plantation forest (ha)
1	Chilimo	Dandi	681.688	81.422
2	Gaji	Dandi	777.498	19.126
3	Mesalemiya	Dandi	664	246
4	Werabo	Dandi	529.08	16.56
5	Yubdo Kashinna	Dandi	386.24	4.66
6	Galessa	Dandi	382.624	3.346
7	Danno	Dandi	326.74	13.92
8	Togicha	Ejersa lafo	207.124	0.686
9	Goben	Ejersa lafo	136.68	0.61
10	Jijiga	Dandi	135.69	6.06
11	Yubdo Gerarssa	Ejersa lafo	150.34	0.44
12	Tiyo	Dandi	73.18	3.65
TOTAL			4450.884	396.516

Annex 5. Measured soil physicochemical variables, SA, and infectivity across land uses, altitude and per plot

Code	Land use	Village	Altitude (m)	SA (g^{1})	Infectivity (%)	BD (g/cm3)	pH	EC (dS/m)	Texture			N (%)	P (ppm)	P:N	OC (%)	OM (%)	C:N	CEC (mequi/100g)
									% Sand	% Silt	% Clay							
1	Natural Forest	Chilimo	2630	5.1	57.5	0.60	6.29	0.11	84.51	8.89	6.60	0.25	8.84	35.36	4.74	8.16	18.94	18.32
2	Natural Forest	Chilimo	2622	6.6	76	0.53	6.84	0.32	83.51	12.28	4.21	0.34	18.65	54.85	7.74	13.34	22.77	25.92
3	Natural Forest	Chilimo	2626	6.1	80	0.50	6.53	0.22	85.76	5.73	8.52	0.32	25.01	78.16	8.11	13.98	25.36	32.64
4	Natural Forest	Chilimo	2611	6.6	50	0.49	7.19	0.34	86.38	9.41	4.21	0.42	13.01	30.98	7.31	12.59	17.40	26.56
5	Natural Forest	Chilimo	2619	3.6	82	0.68	6.7	0.18	81.14	10.34	8.52	0.26	13.17	50.65	5.37	9.26	20.67	25.68
6	Natural Forest	Chilimo	2425	6.65	73.5	0.62	6.7	0.16	77.65	13.84	8.52	0.33	36.08	109.33	5.65	9.73	17.11	14.72
7	Natural Forest	Chilimo	2401	5	73	0.60	6.3	0.27	66.42	15.02	18.56	0.36	10.09	28.03	5.90	10.17	16.40	18.40
8	Natural Forest	Chilimo	2409	3.4	73.5	0.66	6.44	0.16	76.40	12.21	11.39	0.34	9.57	28.15	6.07	10.46	17.86	17.04
9	Natural Forest	Chilimo	2419	6.65	81.5	0.64	6.97	0.22	80.14	10.86	8.99	0.39	10.01	25.67	6.80	11.72	17.44	25.44
10	Natural Forest	Chilimo	2424	3.7	72	0.61	7.02	0.33	81.39	12.01	6.60	0.39	9.65	24.74	8.48	14.61	21.74	18.80
11	Natural Forest	Galessa	2810	9	68	0.56	6.75	0.26	82.64	13.15	4.21	0.43	23.10	53.72	8.22	14.17	19.13	21.52
12	Natural	Galessa	2806	6.9	68.5	0.65	5.	0.08	80.	15.	4.2	0.	12.	41.2	6.	12.	23.	27.28

	Forest			5			99		14	65	1	30	36	0	99	04	29	
13	Natural Forest	Galessa	2829	5.75	66	0.71	6.52	0.12	80.14	15.65	4.21	0.32	8.39	26.22	5.44	9.37	16.99	26.56
14	Natural Forest	Galessa	2817	5.35	57	0.63	6.49	0.13	80.14	15.65	4.21	0.42	9.16	21.81	6.25	10.77	14.88	26.56
15	Natural Forest	Galessa	2800	4	41.5	0.60	6.81	0.20	82.64	10.76	6.60	0.38	24.56	64.63	7.21	12.43	18.98	22.64
16	Cropland/Teff	Jijiga	2411	13.55	67.5	0.88	6.13	0.10	66.90	12.23	20.86	0.17	13.74	80.82	3.92	6.76	23.08	13.12
17	Cropland/Wheat	Jijiga	2422	10.45	62.5	0.93	5.68	0.06	64.79	17.97	17.24	0.19	9.04	47.58	4.07	7.02	21.44	12.88
18	Cropland/Wheat	Jijiga	2420	9.35	56	0.82	5.79	0.10	66.90	15.86	17.24	0.21	8.07	38.43	3.89	6.71	18.54	22.40
19	Cropland/Bean	Jijiga	2425	10.7	50	0.93	5.4	0.07	59.50	12.40	28.11	0.15	2.51	16.73	3.67	6.33	24.49	18.72
20	Cropland/Lathyrus	Jijiga	2404	9.75	55	0.92	5.24	0.07	50.80	7.52	41.68	0.14	7.38	52.71	3.31	5.71	23.67	15.76
21	Cropland/Wheat	Tiyo	2804	12.8	82.5	0.87	5.86	0.05	64.26	19.59	16.15	0.09	10.94	121.56	1.73	2.98	19.22	19.44
22	Cropland/Wheat	Tiyo	2806	14.25	63	0.86	5.8	0.04	65.32	15.63	19.05	0.11	18.73	170.27	2.50	4.31	22.74	26.40
23	Cropland/Wheat	Tiyo	2802	9.9	68.5	0.93	5.73	0.06	66.37	18.20	15.43	0.11	49.41	449.18	7.67	13.22	69.75	20.72
24	Cropland/Wheat	Tiyo	2817	12.65	61.5	0.80	5.74	0.04	60.03	22.73	17.24	0.10	21.56	215.60	2.96	5.10	29.60	24.56
25	Cropland/Wheat	Tiyo	2829	13.15	52	0.88	5.28	0.05	64.26	16.69	19.05	0.12	48.60	405.00	2.75	4.74	22.93	21.92
26	Grazing land-1a	Tiyo	2830	16.55	62.5	0.92	5.71	0.07	68.49	19.70	11.81	0.14	43.82	313.00	3.18	5.48	22.72	24.64
27	Grazing	Tiyo	2823	15.	61	0.95	5.	0.08	62.	22.	15.	0.	38.	257.	3.	6.1	23.	8.88

	land-1a			15			62		14	43	43	15	67	80	59	9	95	
28	Grazing land-1a	Tiyo	2806	18.8	54.5	0.98	5.47	0.08	70.61	16.14	13.26	0.13	34.70	266.92	2.32	4.00	17.86	24.80
29	Grazing land-1a	Tiyo	2805	17.55	55	0.90	5.12	0.07	71.66	15.08	13.26	0.11	36.20	329.09	2.74	4.72	24.90	26.32
30	Grazing land-1a	Tiyo	2825	12.5	54	0.87	5.03	0.06	71.66	17.25	11.08	0.25	27.52	110.08	3.15	5.42	12.58	28.16
31	Cropland/Wheat	Arera Kurae	2605	8.3	69	0.79	5.72	0.07	65.85	17.64	16.52	0.17	8.63	50.76	4.08	7.03	24.00	25.68
32	Cropland/Wheat	Arera Kurae	2618	9.65	66	0.91	5.58	0.07	66.48	16.28	17.24	0.19	6.69	35.21	3.48	5.99	18.30	23.28
33	Cropland /Pea	Arera Kurae	2604	12.45	40	0.88	5.99	0.06	67.96	17.70	14.34	0.16	7.30	45.63	2.29	3.94	14.29	27.84
34	Cropland / Pea	Arera Kurae	2604	11.55	59	0.93	5.88	0.08	66.90	22.01	11.08	0.18	7.99	44.39	2.58	4.45	14.35	24.16
35	Cropland/Wheat	Arera Kurae	2606	14.95	66.5	0.81	5.62	0.08	67.96	18.42	13.62	0.16	6.00	37.50	3.21	5.53	20.06	28.40
36	Shrub land-1	Mesale miya	2403	8	68	0.63	6.27	0.23	74.39	14.52	11.09	0.41	15.40	37.56	6.07	10.46	14.81	29.20
37	Shrub land-1	Mesale miya	2412	7.55	52.5	0.77	5.9	0.09	69.44	17.26	13.29	0.26	7.86	30.23	2.85	4.91	10.96	30.48
38	Shrub land-1	Mesale miya	2416	6.8	57.5	0.59	6.63	0.53	80.77	10.24	8.99	0.44	11.23	25.52	6.09	10.50	13.85	33.52
39	Shrub land-1	Mesale miya	2408	9.35	39	0.64	6.33	0.32	77.06	14.65	8.29	0.50	15.61	31.22	7.91	13.63	15.82	30.88
40	Shrub land-1	Mesale miya	2412	17.6	65.5	0.79	6	0.27	74.39	16.85	8.76	0.26	60.48	232.62	6.64	11.44	25.54	26.00
41	Grazing land-2a	Mesale miya	2415	18.05	40.5	0.83	5.55	0.09	58.55	10.81	30.64	0.17	18.44	108.47	5.29	9.12	31.14	23.92
42	Grazing	Mesale	2423	10.	34	0.77	5.	0.08	59.	16.	23.	0.	15.	79.1	5.	8.9	27.	25.68

	land-2a	miya		85			59		82	79	40	19	04	6	20	6	37	
43	Grazing land-2a	Mesale miya	2422	12.85	62	0.79	5.45	0.16	60.87	12.11	27.02	0.20	24.81	124.05	5.36	9.23	26.78	35.92
44	Grazing land-2a	Mesale miya	2429	9.4	65.5	0.80	5.56	0.09	60.87	13.56	25.57	0.15	22.70	151.33	4.36	7.51	29.06	27.60
45	Grazing land-2a	Mesale miya	2430	8.35	52.5	0.77	5.51	0.10	64.05	12.56	23.40	0.39	19.01	48.74	4.20	7.24	10.77	22.08
46	Shrub land-3	Jijiga	2600	22.9	53.5	0.80	6.92	0.15	79.72	12.98	7.29	0.53	11.76	22.19	6.30	10.86	11.89	24.08
47	Shrub land-3	Jijiga	2613	7.95	63	0.74	6.45	0.15	77.53	16.87	5.61	0.32	7.74	24.19	7.12	12.26	22.24	28.88
48	Shrub land-3	Jijiga	2607	5.8	53	0.65	6.26	0.16	77.53	15.18	7.29	1.83	13.09	7.15	8.30	14.30	4.54	37.52
49	Shrub land-3	Jijiga	2618	8.9	47.5	0.63	6.27	0.21	72.03	19.83	8.13	0.46	33.77	73.41	9.51	16.38	20.67	37.60
50	Shrub land-3	Jijiga	2612	25.3	35	0.76	6.46	0.13	74.23	16.37	9.40	0.33	23.10	70.00	7.15	12.32	21.67	34.40
51	Grazing land-b	Chilimo	2400	14.45	20	0.73	5.33	0.06	60.56	4.09	35.35	0.22	4.34	19.73	5.22	8.99	23.72	23.04
52	Grazing land-b	Chilimo	2402	12	18.5	0.74	4.93	0.07	65.85	9.67	24.48	0.20	3.08	15.40	5.29	9.11	26.44	25.84
53	Grazing land-b	Chilimo	2407	12.45	22	0.75	5.05	0.08	66.90	8.61	24.48	0.27	3.61	13.37	5.51	9.49	20.40	23.44
54	Grazing land-b	Chilimo	2417	10.2	30	0.82	5.3	0.09	62.67	1.98	35.35	0.25	4.26	17.04	4.92	8.47	19.66	25.20
55	Grazing land-b	Chilimo	2405	21.05	38	0.74	5.14	0.06	60.56	4.09	35.35	0.19	3.16	16.63	4.65	8.01	24.47	23.36
56	Shrub land-2	Mesale miya	2500	9.2	22	0.62	6.41	0.27	70.60	17.26	12.14	0.35	10.70	30.57	7.17	12.35	20.48	39.20
57	Shrub land-	Mesale	2517	12.	44.5	0.72	6.	0.17	75.	14.	10.	0.	10.	33.6	6.	11.	21.	22.40

	2	miya		05			26		00	93	07	31	42	1	65	45	44	
58	Shrub land-2	Mesale miya	2510	7.95	12	0.74	6.39	0.06	71.70	16.16	12.14	0.23	11.80	51.30	5.36	9.23	23.29	35.36
59	Shrub land-2	Mesale miya	2411	6.95	25	0.73	5.95	0.09	77.20	13.19	9.61	0.19	20.47	107.74	5.76	9.93	30.33	23.52
60	Shrub land-2	Mesale miya	2516	6.45	14	0.81	5.32	0.07	77.20	13.19	9.61	0.22	4.22	19.18	3.59	6.19	16.33	28.16

BD=Bulk density, EC=Electirical conductivity, N=total nitrogen, P=plant available phosphorus, P:N=available phosphorus to total nitrogen ratio, OC=soil organic carbon, OM=organic matter, C:N=carbon to nitrogen ratio, and CEC=cation exchange capacity

Annex 6. The raw data of the mesh-house AMF inoculation expermiment
Annex 6.1. The raw data for *C. africana*

Treatment	RC	Hi	Hf	RGR-H	CDi	CDf	RGR-CD	LNi	LNf	RGR-LN	LA	SfM	RfM	TfM	SdM	RdM	TdM	RD	R:S	R:P
S&I	26	7.00	7.20	0.00	3.00	5.00	0.03	6	5.00	-0.01	21.05	1.17	1.33	2.50	0.36	0.30	0.66	21.00	0.83	0.45
S&I	50	6.80	7.50	0.01	3.80	6.00	0.03	5	4.00	-0.01	28.76	1.70	3.60	5.30	0.38	0.78	1.16	28.00	2.05	0.67
S&I	32	6.00	7.60	0.01	3.50	5.00	0.02	5	5.00	0.00	32.16	1.40	1.52	2.92	0.47	0.31	0.78	27.00	0.66	0.40
S&I	37	6.00	7.50	0.01	4.00	6.00	0.03	4	5.00	0.01	21.33	1.18	1.91	3.09	0.35	0.34	0.69	29.00	0.97	0.49
S&I	29	5.00	8.00	0.03	3.00	5.00	0.03	4	6.00	0.03	27.50	1.31	1.81	3.12	0.25	0.38	0.63	25.00	1.52	0.60
S&I	43	3.00	5.70	0.05	2.00	4.00	0.05	3	5.00	0.03	26.25	0.98	1.32	2.30	0.28	0.28	0.56	15.50	1.00	0.50
S&nI	0	6.50	8.20	0.01	4.00	4.00	0.00	3	2	-0.02	5.80	0.42	0.76	1.18	0.10	0.17	0.27	21.50	1.70	0.63
S&nI	0	6.50	6.60	0.00	3.00	3.90	0.02	4	2.00	-0.03	6.00	0.40	0.53	.93	0.10	0.09	0.19	26.50	0.90	0.47
S&nI	0	6.50	7.80	0.01	3.00	3.80	0.01	3	2.00	-0.02	6.25	0.47	0.85	1.32	0.11	0.13	0.24	19.50	1.18	0.54
S&nI	0	3.80	5.70	0.03	2.50	3.10	0.01	4	2.00	-0.03	5.70	0.42	0.66	1.08	0.11	0.13	0.24	23.00	1.18	0.54
S&nI	0	5.00	6.10	0.01	4.00	4.00	0.00	5	1.00	-0.04	4.23	0.40	0.71	1.11	0.15	0.14	0.29	30.00	0.93	0.48
S&nI	0	3.00	5.70	0.05	3.00	3.90	0.02	3	2.00	-0.02	5.00	0.45	0.91	1.36	0.11	0.20	0.31	23.50	1.82	0.65

nS&I	65	10.20	11.50	0.01	5.00	5.90	0.01	5	4.00	-0.01	18.78	1.43	1.82	3.25	0.42	0.37	0.79	22.00	0.88	0.47
nS&I	70	6.00	7.80	0.02	3.00	4.50	0.03	5	5.00	0.00	20.80	0.98	2.38	3.36	0.28	0.48	0.76	33.00	1.71	0.63
nS&I	54	4.50	6.30	0.02	2.50	5.00	0.05	5	6.00	0.01	35.70	1.71	2.05	3.76	0.53	0.43	0.96	28.00	0.81	0.45
nS&I	66	5.00	5.50	0.01	2.00	4.00	0.05	5	5.00	0.00	17.54	0.58	1.23	1.81	0.24	0.24	0.48	30.00	1.00	0.50
nS&I	70	4.20	5.70	0.02	2.80	5.00	0.04	4	5.00	0.01	24.50	1.13	1.86	2.99	0.34	0.27	0.61	28.50	0.79	0.44
nS&I	48	2.80	6.90	0.07	1.80	5.00	0.09	3	4.00	0.02	23.57	1.20	1.96	3.16	0.44	0.48	0.92	24.00	1.09	0.52
nS&nI	42	9.50	9.30	0.00	3.00	4.20	0.02	3	3.00	0.00	5.18	0.56	0.75	1.31	0.15	0.14	0.29	16.00	0.93	0.48
nS&nI	48	7.30	12.80	0.04	2.00	3.80	0.05	4	3.00	-0.01	3.10	0.38	0.81	1.19	0.16	0.13	0.29	23.00	0.81	0.45
nS&nI	52	5.00	5.10	0.00	2.50	4.00	0.03	4	2.00	-0.03	3.74	0.40	1.09	1.49	0.18	0.15	0.33	25.00	0.83	0.45
nS&nI	66	4.50	5.60	0.01	2.00	3.50	0.04	4	3.00	-0.01	4.50	0.41	0.40	0.81	0.18	0.07	0.25	22.00	0.39	0.28
nS&nI	67	5.00	7.00	0.02	3.00	5.00	0.03	3	2.00	-0.02	7.65	0.71	1.69	2.40	0.18	0.26	0.44	31.50	1.44	0.59
nS&nI	54	2.80	5.90	0.06	1.80	3.00	0.03	3	3.00	0.00	5.24	0.43	0.63	1.06	0.19	0.15	0.34	21.00	0.79	0.44

S&I=seedlings grown on sterile soil and inoculated with whole soil AMF inoculum, S&nI= seedlings grown on sterile soil and not inoculated with whole soil AMF inoculum, nS&I=seedlings grown on non-sterile soil and inoculated with whole soil AMF inoculum, nS&nI=seedlings grown on non-sterile soil and not inoculated with whole soil AMF inoculum, RC= root percent AMF inoculation, Hi=initial seedling height, Hf=final seedling height, CDi= initial sedling collar diameter, CDf= final seedling collar diameter, RGR-CD=relative collar diameter growth rate, LNf=final leaf number, RGR-LN= relative leaf number growth rate, LA=leaf area, SfM=seedling fresh shoot mass at harvest, RfM=seedling root fresh mass at harvest, TfM= seedling total fresh mass at harvest,

SdM=seedling shoot freash mass at harvest, RdM= seedling root dry mass at harvest, TdM= seedling total dry shoot mass at harvest, RD=rooting depth, R:S=root to shoot dry mass ratio, R:P=root to total plant drymass ratio.

Annex 6.2. The raw data for *J. procera*

Treatment	Hi	Hf	RGR-H	CDi	CDf	RGR-CD	LNf	SfM	RfM	TfM	SdM	RdM	TdM	RD	R:S	R:P
S&I	6.40	10.55	0.03	1.50	2	0.02	165	0.27	0.23	0.50	0.10	0.07	0.17	21.00	0.70	0.41
S&I	6.80	12	0.03	1.50	2	0.02	177	0.31	0.40	0.71	0.12	0.10	0.22	24.40	0.83	0.45
S&I	2.90	8	0.08	1.00	2	0.05	186	0.23	0.16	0.39	0.09	0.05	0.14	41.00	0.56	0.36
S&I	4.10	9.6	0.06	1.00	2.1	0.05	233	0.41	0.27	0.68	0.14	0.08	0.22	27.40	0.57	0.36
S&I	2.00	5.5	0.08	1.00	1.8	0.04	60	0.09	0.07	0.16	0.02	0.02	0.04	22.50	1.00	0.50
S&I	2.90	8.5	0.09	1.00	2	0.05	236	0.42	0.24	0.66	0.14	0.07	0.21	33.00	0.50	0.33
S&nI	7.00	10.8	0.02	1.50	2	0.02	137	0.24	0.26	0.50	0.10	0.09	0.19	47.80	0.90	0.47
S&nI	5.60	9.5	0.03	1.50	2	0.02	108	0.17	0.16	0.33	0.06	0.05	0.11	38.00	0.83	0.45
S&nI	3.30	8.2	0.07	1.00	2	0.05	102	0.19	0.10	0.29	0.07	0.03	0.10	35.50	0.43	0.30
S&nI	2.30	6.8	0.09	1.00	1.8	0.04	72	0.11	0.09	0.20	0.04	0.04	0.08	32.00	1.00	0.50
S&nI	2.90	9.9	0.11	1.00	2	0.05	181	0.33	0.24	0.57	0.11	0.07	0.18	38.50	0.64	0.39
S&nI	3.20	5.1	0.03	1.00	1.8	0.04	83	0.07	0.05	0.12	0.02	0.01	0.03	26.00	0.50	0.33
nS&I	6.50	10.5	0.03	1.50	1.9	0.01	118	0.23	0.19	0.42	0.08	0.07	0.15	32.00	0.88	0.47
nS&I	4.00	8.9	0.06	1.50	2	0.02	92	0.15	0.12	0.27	0.05	0.05	0.10	33.50	1.00	0.50
nS&I	2.80	6	0.05	1.00	1.8	0.04	62	0.08	0.05	0.13	0.03	0.02	0.05	36.50	0.67	0.40
nS&I	2.80	9.2	0.10	1.00	2	0.05	148	0.23	0.12	0.35	0.08	0.05	0.13	24.50	0.63	0.38
nS&I	3.40	7.2	0.05	1.00	2	0.05	99	0.14	0.09	0.23	0.06	0.03	0.09	39.50	0.50	0.33
nS&I	2.80	5.6	0.05	1.00	1.9	0.04	89	0.13	0.06	0.19	0.05	0.03	0.08	21.50	0.60	0.38
nS&nI	6.70	11.5	0.03	1.50	2.05	0.02	165	0.33	0.30	0.63	0.13	0.09	0.22	27.00	0.69	0.41
nS&nI	4.50	7.9	0.03	1.50	2	0.02	127	0.18	0.19	0.37	0.07	0.06	0.13	20.00	0.86	0.46
nS&nI	2.20	5.5	0.07	1.00	2	0.05	82	0.11	0.06	0.17	0.05	0.02	0.07	25.00	0.40	0.29
nS&nI	2.90	4.7	0.03	1.00	1	0.00	35	0.03	0.01	0.04	0.01	0.00	0.01	20.50	0.00	0.00
nS&nI	3.20	8	0.07	1.00	1.9	0.04	94.00	0.15	0.06	0.21	0.06	0.02	0.08	23.50	0.33	0.25

nS&nI	3.10	7.8	0.07	1.00	1.6	0.03	97.00	0.14	0.07	0.21	0.06	0.03	0.09	27.00	0.50	0.33

S&I=seedlings grown on sterile soil and inoculated with whole soil AMF inoculum, S&nI= seedlings grown on sterile soil and not inoculated with whole soil AMF inoculum, nS&I=seedlings grown on non-sterile soil and inoculated with whole soil AMF inoculum, nS&nI=seedlings grown on non-sterile soil and not inoculated with whole soil AMF inoculum, Hi=initial seedling height, Hf=final seedling height, CDi= initial sedling collar diameter, CDf= final seedling collar diameter, RGR-CD=relative collar diameter growth rate, LNf=final leaf number, SfM=seedling fresh shoot mass at harvest, RfM=seedling root fresh mass at harvest, TfM= seedling total fresh mass at harvest, SdM=seedling shoot freash mass at harvest, RdM= seedling root dry mass at harvest, TdM= seedling total dry shoot mass at harvest, RD=rooting depth, R:S=root to shoot dry mass ratio, R:P=root to total plant drymass ratio.

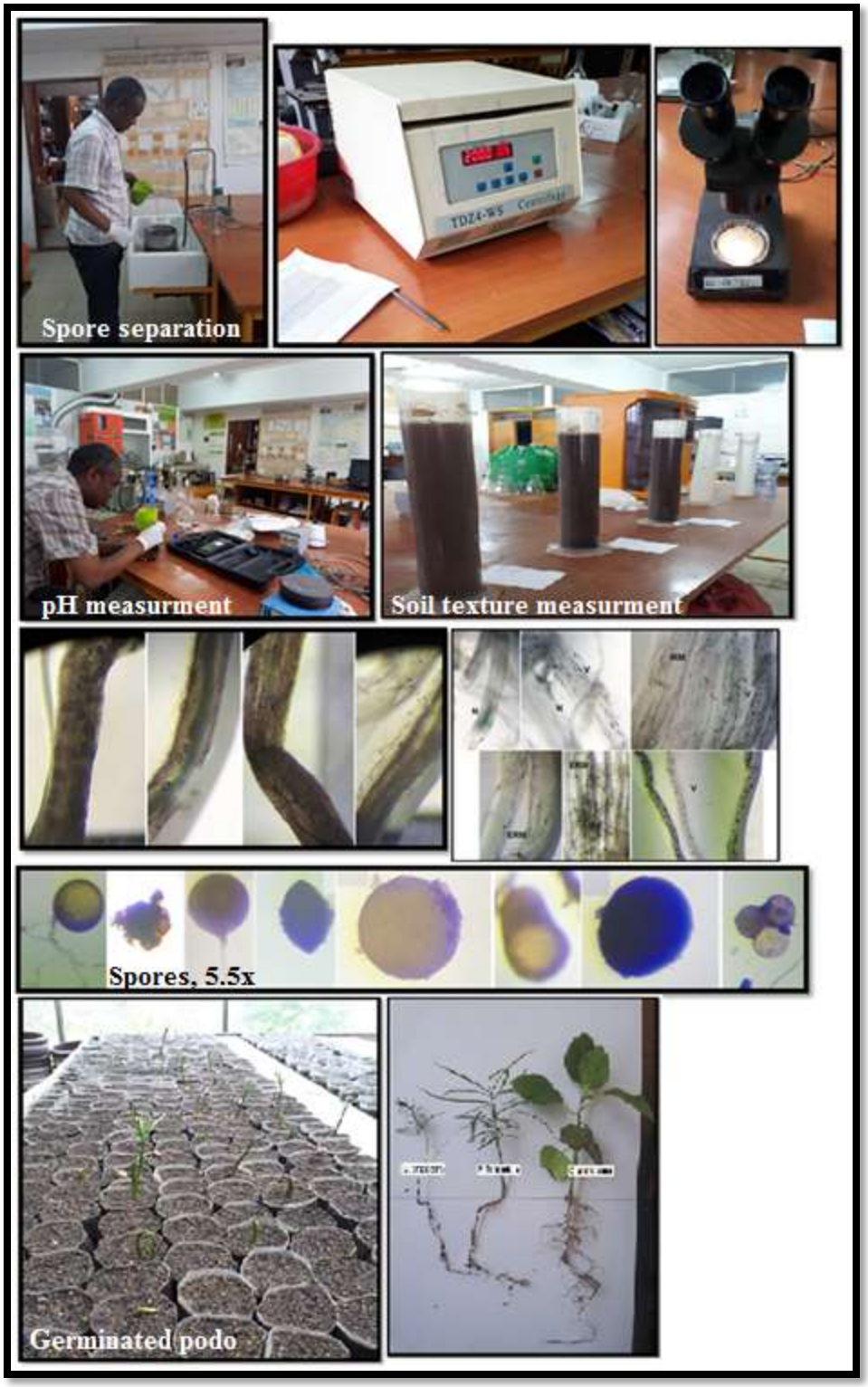
Annex 6.3. The raw data for *P. falcatus*

Treatment	RC	Hi	Hf	RGR-H	CDi	CDf	RGR-CD	LNf	SfW	RfW	TfW	SdW	RdW	TdW	R:S	R:P
S&I	40	7.40	15.50	0.00	2.70	3.90	0.03	48.00	2.60	2.20	4.80	0.90	0.58	1.48	0.64	0.39
S&I	0	8.50	13.00	0.01	2.10	3.80	0.03	45.00	1.70	1.50	3.20	0.60	0.73	1.33	1.22	0.55
S&I	0	6.50	11.30	0.01	2.10	2.70	0.02	38.00	1.70	1.00	2.70	0.64	0.33	0.97	0.52	0.34
S&I	0	7.30	15.30	0.01	2.00	2.70	0.03	55.00	2.20	2.30	4.50	0.75	0.70	1.45	0.93	0.48
S&I	0	9.50	11.50	0.03	2.00	2.60	0.03	42.00	1.60	1.50	3.10	0.54	0.39	0.93	0.72	0.42
S&I	0	7.00	12.60	0.05	2.80	2.90	0.05	44.00	2.00	.80	2.80	0.80	0.34	1.14	0.43	0.30
S&nI	0	11.20	16.20	0.01	2.50	2.90	0.00	38.00	2.50	2.80	5.30	0.90	0.66	1.56	0.73	0.42
S&nI	0	7.50	12.60	0.00	2.30	3.00	0.02	38.00	1.60	1.50	3.10	0.50	0.38	0.88	0.76	0.43
S&nI	0	6.00	11.50	0.01	3.10	2.90	0.01	40.00	1.90	1.40	3.30	0.64	0.35	0.99	0.55	0.35
S&nI	0	8.80	14.30	0.03	2.90	3.00	0.01	40.00	2.70	2.60	5.30	0.90	0.62	1.52	0.69	0.41
S&nI	0	6.70	12.50	0.01	2.60	3.40	0.00	37.00	2.10	1.70	3.80	0.77	0.44	1.21	0.57	0.36
S&nI	0	6.50	11.30	0.05	2.60	2.80	0.02	41.00	2.30	1.10	3.40	0.90	0.44	1.34	0.49	0.33
nS&I	0	8.80	11.80	0.01	2.20	2.90	0.01	37.00	2.10	1.30	3.40	0.67	0.48	1.15	0.72	0.42
nS&I	5	7.20	12.00	0.02	2.50	3.00	0.03	40.00	2.20	1.80	4.00	0.79	0.55	1.34	0.70	0.41
nS&I	20	8.90	14.80	0.02	2.40	2.50	0.05	39.00	1.60	.90	2.50	0.56	0.34	0.90	0.61	0.38
nS&I	0	8.00	11.50	0.01	2.40	2.90	0.05	38.00	2.30	1.10	3.40	0.85	0.40	1.25	0.47	0.32
nS&I	0	5.90	10.50	0.02	2.10	2.60	0.04	39.00	1.60	.70	2.30	0.56	0.30	0.86	0.54	0.35
nS&I	0	6.40	10.50	0.07	2.20	2.60	0.09	33.00	1.30	1.00	2.30	0.46	0.42	0.88	0.91	0.48

nS&nI	5	8.50	15.00	0.00	1.90	2.40	0.02	36.00	2.20	1.20	3.40	0.65	0.31	0.96	0.48	0.32
nS&nI	10	7.40	12.50	0.04	2.10	2.40	0.05	42.00	2.00	1.80	3.80	0.60	0.48	1.08	0.80	0.44
nS&nI	5	6.50	12.50	0.00	2.70	2.50	0.03	41.00	2.10	1.10	3.20	0.70	0.39	1.09	0.56	0.36
nS&nI	0	8.20	13.20	0.01	2.50	2.60	0.04	42.00	2.30	1.30	3.60	0.80	0.43	1.23	0.54	0.35
nS&nI	0	6.00	10.50	0.02	2.30	3.10	0.03	40.00	1.60	0.90	2.50	0.57	0.30	0.87	0.53	0.34
nS&nI	20	6.60	10.20	0.06	2.70	2.70	0.03	36.00	2.00	1.20	3.20	0.68	0.39	1.07	0.57	0.36

S&I=seedlings grown on sterile soil and inoculated with whole soil AMF inoculum, S&nI= seedlings grown on sterile soil and not inoculated with whole soil AMF inoculum, nS&I=seedlings grown on non-sterile soil and inoculated with whole soil AMF inoculum, nS&nI=seedlings grown on non-sterile soil and not inoculated with whole soil AMF inoculum, RC= root percent AMF inoculation, Hi=initial seedling height, Hf=final seedling height, CDi= initial sedling collar diameter, CDf= final seedling collar diameter, RGR-CD=relative collar diameter growth rate, LNf=final leaf number, SfM=seedling fresh shoot mass at harvest, RfM=seedling root fresh mass at harvest, TfM= seedling total fresh mass at harvest, SdM=seedling shoot freash mass at harvest, RdM= seedling root dry mass at harvest, TdM= seedling total dry shoot mass at harvest, RD=rooting depth, R:S=root to shoot dry mass ratio, R:P=root to total plant drymass ratio.

PHOTOS


Spore separation
pH measurment
Soil texture measurment
Spores, 5.5x
Germinated podo

DECLARATION

I, the undersigned declare that this Ph.D. Dissertation is my original work and it has not been presented to other universities, colleges or institutes for a degree or other purpose. All sources of the materials used have been duly acknowledged.

Name: Fisseha Asmelash Belay **Signature:** _____ **Date:** July 17, 2020