HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF BIOLOGY

SPECIALIZATION: APPLIED MICROBIOLOGY

IMPACT OF LAND USE TYPE ON THE DIVERSTY AND ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN LOKA ABYA, SIDAMA REGIONAL STATE, ETHIOPIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE IN BIOLOGY

(SPECIALIZATION: APPLIED MICROBIOLOGY)

BY: DILGASA TUSHURA

ADVISOR: BEYENE DOBO (PhD)

MARCH, 2025

HAWASSA, ETHIOPIA

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MSc. THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY, COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSTY

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SCHOOL OF GRADUATE STUDIES

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ADVISOR's APPROVAL SHEET

This is to certify that the Thesis entitled "*IMPACT OF LAND USE TYPE ON THE DIVERSTY AND ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN LOKA ABYA, SIDAMA REGIONAL STATE, ETHIOPIA***"** submitted in partial fulfillment of the requirements for the Master's with specialization in **Applied Microbiology,** the graduate program of **Department of Biology,** and has been carried out by **Dilgasa Tushura (GpApMiR/0001/15)** under my /our supervision. Therefore I recommend that the student has fulfilled the requirements and hence thereby can submit the thesis to the department.

Beyene Dobo (PhD)____	_____	_____
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We, the undersigned, members of the Board Examiners of the final open defense by **Dilgasa Tushura** have read and evaluated his thesis entitled "*IMPACT OF LAND USE TYPE ON THE DIVERSTY AND ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN LOKA ABYA, SIDAMA REGIONAL STATE, ETHIOPIA***, "**and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillments of the requirements for degree of Master of Science in Biology with specialization in Applied Microbiology.

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BIOGRAPHICAL SKETCH

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DECLARATION

I hereby declare that thisMSc thesisis my original work and has not been presented for the purpose of awarding any academic degree, diploma, or certificate in any other institution and all source of material used for this thesis have been duly acknowledged.

Name: **Dilgasa Tushura**

Signature: _____

Date of Submission: _____

ACKNOWLEDGMENTS

This work was made possible through the invaluable scientific and technical guidance of Dr. Beyene Dobo. I am deeply grateful for his unwavering support, which was instrumental in bringing this study to fruition. Dr. Beyene's commitment extended far beyond his role as a scientific advisor, as he facilitated access to laboratory facilities, reagents, and essential instruments for my research. His dedication to teaching me the skills of collecting and processing rhizosphere soil and root samples is greatly appreciated. For his exceptional mentorship and fatherly guidance, I offer my heartfelt thanks.

First and foremost, I would like to extend my sincere gratitude to Hawassa University and the NORAD project, College of Natural and Computational Sciences, and Department of Biology, for granting me the opportunity to undertake this research thesis.

I would also like to express my extraordinary appreciation to my elder brother, Melese Tushura, his wife, Tibletse Paulos, and Engineer Tariku Adola. Their motivational spirit and material support have been invaluable, empowering me to excel in my academic endeavors.

Additionally, I am grateful to the technical staff of the Molecular Biology Laboratory, Soil Science, and Plant Tissue Culture divisions at the College of Natural and Computational Sciences and the College of Agriculture at Hawassa University for their assistance and encouragement.

Lastly, I extend my sincere thanks to my friends, whose unwavering support and encouragement have been a source of strength throughout this research journey.

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LIST OF ABBREVIATIONS

AC	Arbuscular Colonization
AMF	Arbuscular mycorrhizal fungi
CSA	Central statistical agency
ECM	Ectomycorrhiza
ERM	Extraradical mycelium
INVAM	International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi
IRM	Intraradical mycelium
PGPR	Plant growth promoting rhizobacteria
PSB	Phosphate solubilizing bacteria
PVLG	Poly vinyl alcohol lacto glycerol
SD	Spore density
SOM	Soil organic matter
USAID	United states agency for international development
VAM	Vesicular arbuscular mycorrhiza
VC	Vesicular colonization

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ABSTRACT

Arbuscular mycorrhizal fungi play a crucial role in plant health by enhancing nutrient uptake, improving soil structure, and supporting ecosystem stability.This study investigates the role of AMF in the soil-plant interactions across different land-use types in Loka Abaya Woreda, Sidama Region, Ethiopia.The study examines the influence of soil physicochemical properties and plant species diversity on AMF distribution, spore density, and root colonization in agroforestry, natural forests, and cash crop systems.Soil characteristics, including pH, nitrogen, phosphorus, organic carbon, electrical conductivity, and texture, were analyzed across four land-use systems: agroforest, natural forest, cash crop, and grassland, revealing significant variation among different plant species. The soils were slightly acidic to neutral, with pH values ranging from 5.73 to 7.56 and phosphorus concentrations varying from 0.0100 mg/g to 0.3120 mg/g. Plant diversity was assessed using random sampling, resulting in the collection of 414 soil samples. Root colonization rates varied significantly, with Citrus sinensis exhibiting the highest colonization in field soil (94.5%), while Ehretia cymosa Thonn and Euphorbia abyssinica showed lower rates. In natural forests, Lennea schimper had 100% colonization, while Dodonaea angustifolia L.f. exhibited the lowest (84%). AMF colonization and spore densities were generally higher in trap cultures compared to field soils, with the highest spore densities in Psidium guajava (2251 spores/100 g) and Balanites aegyptiaca (1228 spores/100 g). Cash crops like Coffee arabica showed 100% AMF colonization and the highest spore density (838 spores/100 g in field soil). AMF analysis was conducted using Pearson's correlation, with data processed using SPSS software version 24.The study identified 30 AMF morphospecies from eight genera, with five genera Glomus, Acaulospora, Claroideoglomus, Enterophospora, and Rhizophagus intraradices showing significantly higher spore production, contributing 43.50%, 32.33%, 8.46%, 4.23%, and 3.62% of the total spore count, respectively. Notably, no single AMF species dominated across all land-use types, but four species Glomus sp.2, Claroideoglomus sp.1, Glomus mosseae, and Acaulospora sp.2 were commonly found, highlighting their ecological significance. The study found a positive correlation between spore density, root colonization, organic carbon, and nitrogen levels, while high phosphorus and soil pH negatively impacted AMF abundance. These findings emphasize the importance of agroforestry and natural forests in maintaining AMF diversity and enhancing soil health. The study recommends further research into AMF dynamics, particularly across different seasons and with molecular techniques, to enhance the understanding and application of AMF in agricultural systems.

Key words: Arbuscular Mycorrhizal Fungi, Root Colonization, Soil property, Spore Density, Spore diversity

1. INTRODUCTION

1.1. Background of the study

Land use change refers to the transformation of natural landscapes into urban, agricultural, or industrial areas, driven by socio-economic, political, and environmental factors. This phenomenon significantly impacts biodiversity, ecosystem services, and climate change. According to Turner *et al.* (2015), land use change is a critical component of global environmental change, influencing carbon storage and habitat loss. The rapid expansion of urban areas and agriculture often leads to deforestation and fragmentation of ecosystems, posing challenges for sustainable development (Foley *et al.,* 2005). Understanding the drivers and consequences of land use change is essential for effective land management and conservation strategies.

In Sidama, agriculture is the predominant land use, with coffee serving as the primary cash crop, supplemented by maize, sorghum, and various fruits and vegetables (Hirsch, 2018). Traditional farming methods are common, although there is an increasing adoption of modern agricultural techniques and improved seed varieties (Teshome, 2021). The hilly terrain necessitates terracing to prevent soil erosion and enhance water retention, which is vital for sustainable agricultural practices (Gashaw *et al.,* 2019). Loka Abaya is characterized by pastoralism, where communities depend on livestock, including cattle, sheep, and goats, for their livelihoods. Pastoralists often migrate in search of grazing lands, adapting to the region's climatic variability (Mekonnen, 2022). The area also has significant forest cover, which plays a crucial role in biodiversity and climate regulation; however, deforestation due to agricultural expansion poses a significant threat to these ecosystems (Kassa *et al.,* 2021).

Mycorrhizal association is a mutualistic relationship between fungi and plant roots, where the fungi colonize the roots and form a symbiotic partnership with the plants. This association is essential for the health and growth of many plant species, as it enhances nutrient uptake, improves resistance to pathogens, and helps in overall plant development (Smith and Read, 2008).

Mycorrhizal associations, according to Smith and Read (2008), are essential to the health of terrestrial ecosystems because they facilitate the cycling of nutrients and increase plant diversity. In order to increase the surface area available to the plant roots for nutrients, the fungi involved in this association create a network of hyphae that extend into the soil. In exchange, the fungi receive carbohydrates from the plants that are made through photosynthesis. Besides, mycorrhizal affiliation has been appeared to have a critical effect on the efficiency of rural crops and the rebuilding of debased biological systems. Understanding and tackling the benefits of this advantageous relationship can lead to more maintainable and strong rural hones. By and large, mycorrhizal affiliation is a critical biological marvel that has far-reaching suggestions for plant wellbeing, environment working, and maintainable horticulture (Smith and Read, 2008).

Arbuscular mycorrhizal fungi are common and symbiotic abundant members of the soil biota, and they are well recognized as essential to the sustainability and functioning of agricultural ecosystems (Mohamed *et al.,* 2023). Through improved establishment and growth, increased nutrient and water uptake, preservation of diversity through accelerated host plant competition for resources, effective nutrient recycling, and long-term soil stability, these symbiotically associated fungi play a crucial role for the plants in agroforestry practices (Smith and Read, 2010; Birhane *et al.,* 2023). Tropical soils are home to a large variety of plant species, including trees (Da Silva Sousa *et al.,* 2013) and unexpectedly high diversity of arbuscular mycorrhizal fungi in fertile Chernozem croplands (Baltruschat *et al.,* 2019).

They are keystone organisms and form an interface between soils and plant roots, and are sensitive to changes in soil physicochemical properties and plant conditions (Soka and Ritchie, 2014).

Under the insufficient concentration of nutrients in the root zone of plants, exploring further in the soil with its extended extra radical hyphae, AMF supply phosphorus, macro and micro soil nutrients to roots which has symbiosis with AMF. In these symbiotic associations both partners benefit from each other under certain conditions (Demir, 2008).

Some of the plant's organic materials and carbohydrates are taken by fungi. Nutrients like phosphorus (P), nitrogen (N), calcium (Ca), copper (Cu), manganese (Mn), sulfur (S), and zinc

(Zn) are provided by AMF in exchange (Ortas, 2002). Individual plant species and plant communities in natural and agricultural systems influence the distribution and diversity of AMF species. However, when the soil is rich in essential nutrients, plants might not waste more energy for the association with AMF and instead optimize for the least expensive source of energy (Jefwa *et al.,* 2006).

The physicochemical properties of the soil (Mahmood *et al.,* 2017) and the species richness and density of plants (Beyene *et al.,* 2018) are factors that influence the diversity and density of the AMF community. Though there are some research reports (Zebene, 2003; Beyene *et al*., 2016).Therefore, it is imperative to study the density and diversity of AMF in relation to soil physicochemical properties and plant species diversity and density in moisture prone Loka Abaya woreda of Sidama regional state to manage the environmental resilience and the diversity of soil microbial biota.

1.2. Statement of the problem

Arbuscular mycorrhizal fungi (AMF) are a particular kind of mycorrhizal fungi that are linked to plants penetrating the cortical cells of their roots and creating their structure. They form symbiotic relationships with over 80% of vascular plants on land (Davison *et al*., 2015; Yan *et al*., 2023). These fungi are widely dispersed and play a crucial role in a range of terrestrial ecosystems, supporting the upkeep and enhancement of vegetation communities, soil conditions, and ecosystem functions (Elhindi *et al.,* 2018; Qiang *et al*., 2019). According to research conducted by Cheng *et al*. (2012) and Veresoglou *et al*. (2012), AMF has the ability to control the composition of soil inter-root microbial communities, soil structure, and nutrient cycling. Additionally, when AMF forms a symbiotic relationship with plants, it can enhance the plants' ability to absorb N, P, and other nutrients, as well as increase their resistance to pests, diseases, drought, salinity and stimulate plant growth (Hodge and Storer, 2015).

Numerous environmental factors, including altitude, plant communities, soil physicochemical properties, and climatic factors, also have an impact on AMF communities. The diversity and abundance of AMF communities are primarily influenced by the species of the host plant and the C, N, and P contents of the soil (Kruger *et al*., 2017; Ezeokoli *et al*., 2019). Thus, studies

examining the interaction between AMF communities and surrounding conditions advance our knowledge of terrestrial ecosystems.

The possibility that the AMF community is equally affected and that their diversity and density may be affected accordingly. Diversity of the AMF community and vegetation are positively correlated, and their relationship is frequently close. However, human activities like intensive agriculture, habitations, and the local climate have a negative impact on the natural environment in this study area, which further affects the function of the environment.

The stability of various vegetation ecosystems in Loka Abaya low land may be maintained and protected in part by the rich AMF resources found in different elevations of Sidama low lands; however,. In order to provide a theoretical framework for preserving the stability and development of low-land rain-erratic ecosystems and investigating the function of AMF communities in terrestrial ecosystems, this research is intended to investigate the diversity of AMF communities in rhizosphere soil at Loka Abaya lowlands of Sidama regional state. It also analyzed the correlation between the physical and chemical properties of rhizosphere soil and the structural characteristics of AMF communities. On the basis of this fact this research is designed to achieve the following general and specific objectives.

1.3. Objective of the study

1.3.1. General Objective

The general objective of this study was to investigate AMF diversity and abundance across different land use systems in Loka Abaya lowlands of Sidama regional state

1.3.2. Specific Objectives

Specific objectives associated with the general objective, the specific objectives includes :

- To investigate how plant species diversity influences different land use systems
- To determine how soil properties (e.g. pH, nutrient availability) affect the abundance and diversity of mycorrhizal fungi
- To examine how land use types affect AMF root colonization, spore density and diversity

1. 4. Research questions

The current research project was designed to answer the following questions:-

1. How plant species diversity influence mycorrhizal-plant associations?
2. How does the nutrient content of soil (e.g., pH, nitrogen, phosphorus,organic carbon,electrical conductivity) influence mycorrhizal association spore density and diversity?
3. How does the different land use type affect AMF root colonization, spore density and diversity?

1.5. Significance of the study

Mycorrhizal association research is important for a number of reasons, including its relationship to soil characteristics and plant species diversity. First, knowing how different soil types' mycorrhizal connections differed helped us understand how we might improve plant growth and productivity in diverse agricultural and natural ecosystems. Sustainable land use methods and soil management techniques can benefit from this information. Second, the effects of pH and nutrient content on mycorrhizal association can have significant effects on the dynamics of plant communities and the functioning of ecosystems. We can gain a better understanding of how environmental conditions affect the distribution and abundance of plant species linked with mycorrhizal fungus by clarifying these interactions. Moreover, mycorrhizal connections play a critical role in supporting ecosystem resilience and plant species diversity, which is critical for conservation and restoration initiatives. Our understanding of the processes through which mycorrhizal linkages support the diversity of plant species helped us create more potent plans for protecting biodiversity and repairing damaged environments. Finally, the implication of mycorrhizal associations for ecosystem restoration and conservation efforts are significant, as they can help guide management practices aimed at enhancing the health and resilience of natural ecosystems. By understanding the relationships between mycorrhizal associations, soil properties, and plant species diversity, we can develop more targeted approaches to conservation and restoration that take into account the important role of mycorrhizal fungi in ecosystem functioning.

1.6. Scope of the Study

The scope of this study is to investigate the mycorrhizal association in relation to soil properties and plant species diversity only in Lokka Abaya woreda. The study is aimed to identify the types of mycorrhizal fungi present in the soil, in plant root structure,their abundance and diversity, and their relationship with different soil properties such as pH, organic matter content, nutrient availability, and texture.

2. LITERATURE REVIEW

2.1. Mycorrhizal association

The term mycorrhiza can be defined as fungus root. According to Bonfante and Genre (2010), fungi form many types of symbiotic association with the roots or other underground organs of plants. They are estimated to occur in at least 80% of all vascular plants, including angiosperms (the flowering plants), gymnosperms (the cone bearing plants), many pteridophytes (ferns and their allies), and some bryophytes especially liverworts (Kumari *et al.,* 2024). Many of these associations are thought to be mutualistic, because the fungus typically absorbs mineral nutrients from the soil and channels these to the plant, while the plant provides the fungus with sugars. However, there are several different types of mycorrhiza, with different properties and feature that fossils from the Rhynie Chert deposits in Scotland contain fungal structures similar to those of the most common mycorrhiza fungi today the arbuscular mycorrhiza fungi. So, it seems that some of the earliest land plants had already established mycorrhiza associations, and these might even have been a prerequisite for life on land (Gleason *et al.,* 2010).

Fungi are involved in a wide range of intimate symbiotic associations with other organisms. In several cases the fungi and their partners have become so intimately dependent on one another that they have lost the ability to live alone. In other cases the fungi can be cultured in laboratory media but they are, in effect, ecologically obligate symbiosis because they seldom if ever grow as free-living organisms in nature. The many thousands of species of lichen are classic examples of this. They grow in some of the most inhospitable environments on earth, where no other organisms can grow, including cooled lava flows and arid desert sands, where they literally hold the place in place (Ayana, 2020).

2.2. Symbiotic association between plant roots and fungi

Symbionts are part of the plant-associated microbial diversity, and they protect their hosts from a variety of threats. Soil toxic chemicals and soil-borne pathogens are buffered by mycorrhizal and rhizospheric microorganisms. Plant protection is provided by endophytic bacteria and fungi, some of which are vertically inherited through seeds, by acting directly on aggressive forces mostly pathogens and herbivores or by augmenting plant responses. Plants' ecological success is

determined by their protective microbial symbionts, which radically alter plant communities and trophic webs (Akiyama *et al.,* 2005).

Beneficial bacteria that protect their hosts from attack are found among the microbes associated with plants. Toxic chemicals and soil pathogens are thus protected by mycorrhizal and rhizospheric bacteria. Endophytes, bacteria, and fungi, which are occasionally transmitted by seeds, protect plants by acting directly on stressors or increasing plant defenses (Asghari and Cavagnaro, 2012). Plants' ecological success is determined by these protective symbioses, which alter plant communities and food webs.

Physiologists have often treated plants as if they were a single creature, ignoring the diversity of their microbial symbioses. As a result, the importance of the latter's contributions was occasionally missed. One of the reasons for this is that the presence and diversity of these microbial symbiosis may be masked due to their tiny size and incorporation into substrates. Similarly, the search for plant models that can be cultivated axenically under laboratory settings and are thus more practical for experimental purposes is bolstering this trend. Although more than 90% of terrestrial plants establish mycorrhizae with soil fungus, non-mycorrhizal models, such as Arabidopsis thaliana, ignore the role of fungal partners (Averill *et al.,* 2014).

Plants form symbiotic relationships with a variety of soil microbial symbionts that increase their nourishment. Mycorrhizal symbiosis, which involves soil fungus and plant roots, is the most common relationship, and it is thought to be ancestral, dating back to the colonization of terrestrial ecosystems. The plant receives water and mineral nutrients accumulated in the soil by the fungal companion in a mycorrhiza (Barto *et al.,* 2011). Some plants, such as legumes with Rhizobiaceae and some Rosids with Cyanobacteria and Actinomycetes, meet their nitrogen needs by forming partnerships with N_2-fixing prokaryotes. As a reward, host plants give carbon to their symbionts in every situation. However, because symbiosis is defined as a mutual enhancement of fitness, a bidirectional nutrition flux is not required to create a symbiotic relationship; any protective effect of one partner toward the other, boosting the latter's survival or reproduction, is adequate (Bascompte *et al.,* 2003).

2.3. Classification of mycorrhizal fungi based on function

2.3.1. Arbuscular Mycorrhiza Fungi

Mycorrhizal fungi are the most widespread and abundant fungi in nature and form a symbiosis relationship with many plants. The fungi belong to Ascomycota, Glomeromycota, Basidiomycota, and Mucoromycota (Smith & Read, 2008). Mycorrhizal associations based on the type of fungus, host plant lineages, and structures produced by the root-fungus combination are classified into seven groups: **endomycorrhiza (AM), ectomycorrhiza (EC), ectendomycorrhiza, ericoid, arbutoid, orchid and monotropoid** (Tao *et al.,* 2016) (Figure. 1). The movement of carbon from plant to fungus is common in all types of mycorrhizal association (Allen *et al.,* 2003). Most often, mycorrhizal fungi are beneficial for host plants by improving the nutritional status of the plant (Chen *et al.,* 2018).

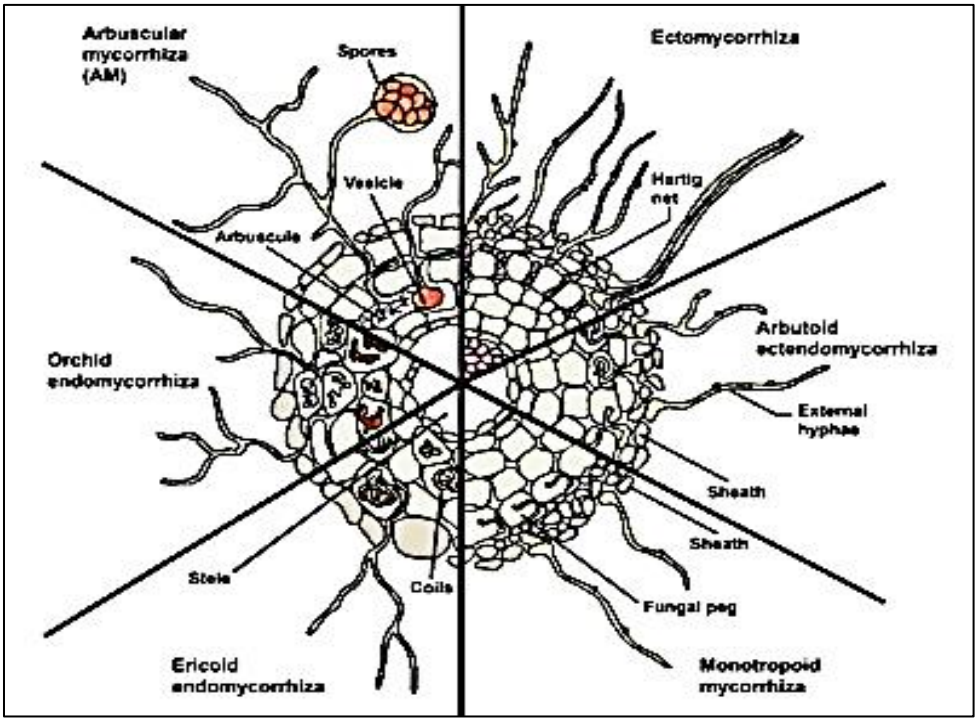

Figure 1. Schematic representation of various types of mycorrhizal fungi(Amballa and Bhumi, 2017)

The arbuscular mycorrhizal symbiosis is probably the most widely beneficial interaction between fungi belonging to the Glomeromycota phylum and the host plant (Giovannini *et al.,* 2020). AMF are obligate symbionts that able to establish a mutualistic symbiotic association with the majority (upwards of 80%) of vascular plants, including Bryophytes, Hepatics, Pteridophytes, Gymnosperms and Angiosperms, in terrestrial ecosystems (Zhang *et al.,* 2020).

AMF receives the carbohydrates and lipids necessary for the survival of the photosynthetically produced carbohydrates by the host plant; Such that 10 to 40 % of the carbohydrates can be absorbed by fungi (Begum *et al.,* 2019, Genre *et al.,* 2020). In return, AMF improve the acquisition of essential mineral nutrients (particularly phosphorus and nitrogen) and water supply. This improvement is done using development a branching mycelial network in the soil that can be expanded the host root surface area by 40 times (Liu *et al.,* 2019).

Fundamentally, these fungi provide other benefits such as carbon recycling, improvement soil aggregation, production of plant growth hormones, enhance the photosynthetic efficiency of the host, and helping the plant to tolerance abiotic (such as drought, salinity, and heavy metals contamination) and biotic stresses (pathogens and insects) through the implementation of various mechanisms (Yang *et al.,* 2021). As obligate biotrophs, AMF cannot complete their life stages without a compatible and living host plant (Smith & Read 2008). Under optimal conditions, an AMF spore (s) germinates, and after receiving signals released by the symbiotic partner, the hyphal germ tube navigates toward the host plant root (Tahat & Sijam , 2012). Once in contact with the surface of the host's root, fungal hyphae form attachment structures, called "hyphopodia or appressoria" (Bonfante & Genre 2015). Then, hyphae penetrate toward the inner cortex cell and produce intraradical tree-like branched hyphae structures called "arbuscules". Arbuscules are the inorganic minerals (especially phosphorus and carbon compounds) and water exchange sites among the symbionts (Ivanov *et al.,* 2019). Several AMF species, including Glomeraceae and Paraglomeraceae, produce vesicles the outer to middle cortical layers of root. Vesicles are storage structures contain lipids and glycogen and have a thin to thick wall layer (Smith & Read 2008). AMF structures in host plant roots are usually not observed without appropriate staining (Figure. 2).

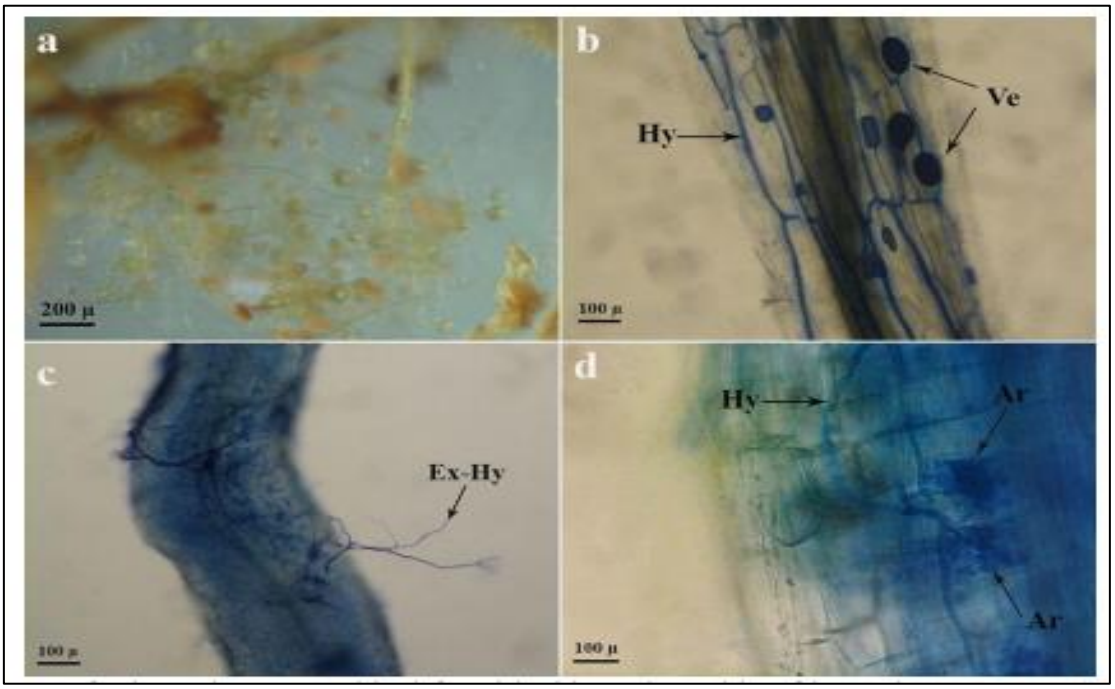

Figure 2. Structures of arbuscular mycorrhizal fungi inside and outside of host plant roots. a: Spores; b: Intraradical hyphae (Hy) and Vesicle (Ve); c: Extra-radical hyphae (Ex-Hy); d: Hyphae (Hy) and Arbuscule (Ar) (Sedaghat *et al.*, 2021)

Intra-radical and extra-radical hyphae of AMF are composed of aseptate multinuclear mycelia (Redecker *et al.*, 2013). AMF spores are produced singly, in loose clusters, tight clusters, or sporocarps in the soil or within host plant roots (Schüßler & Walker, 2010).

2.3.2. Ectotrophic Mycorrhizas

Ectotrophic(Ectomycorrhizas) are predominantly associated with woody Ethiopian trees such as *Podocarpus falcatus* and *Juniperus procera*, particularly in montane ecosystems, while tropical species like *Ficus* spp. mainly form arbuscular mycorrhizas. Some, like *Salix* spp., can form both types. These symbiotic relationships, involving fungi such as *Amanita* and *Boletus*, play key roles in nutrient cycling and are thought to have evolved independently over 130–180 million years (Weber & Webster, 2007).

The characteristic feature of ectomycorrhizas is the presence of a substantial sheath of fungal tissue that encases the terminal, nutrient-absorbing rootlets and the rootlets themselves are often short stumpy with no root hairs. An extensive network of individual hyphae or aggregated mycelia cords radiates from the surface of the root sheath, while beneath the sheath the fungus

invades between the root cortical cells to form a “Hartignet. Although the fungus is in close contact with the root cells in this region, there is no penetration of the host cells, hence the name of these mycorrhizas-ectotrophic (outside-feeding) mycorrhizas (Agrios, 2005). Because of the lack of root hairs and the encasement of the feeder roots by a fungal sheath, virtually all the mineral nutrients that enter the root must be channeled through the fungus. The uptake of mineral nutrients from soil is facilitated by the mass of fungal hyphae that radiate into the soil and transport nutrients back to the mycorrhiza sheath. The fungus benefits from these associations by obtaining sugars from the plant. Trees invest a considerable amount of photosynthate to support the fungal network conservatively estimated at 10% or more of the annual photosynthetic production of a tree (Bonfant and Genre, 2010).

2.3.3. Ericoid Mycorrhizas

The cold, nutrient-poor, acidic upland soils of the northern hemisphere are dominated by heathland plants of the Ericaceae family, such as *Calluna* (ling), *Erica* (bell heathers), and *Vaccinium* (bilberry, cranberry). In the southern hemisphere, similar soils support plants from the Epacridaceae family. These heathland plants form ericoid mycorrhizas with Ascomycota fungi, which create hyphal coils within the cells of thin lateral "hair roots," facilitating nutrient exchange. These fungi, such as *Hymenoscyphu ericae*, are unusual because they function as free-living saprotrophs in soil, displaying zigzag hyphal growth in culture. A key role of ericoid mycorrhizas is nitrogen supply; this was demonstrated by 15^{N}-labeled ammonium experiments where mycorrhizal plants accumulated more total nitrogen, despite reduced ammonium uptake. This nitrogen provision is attributed to fungal proteinase activity at p^{H} 3, which releases amino acids from soil organic matter, underscoring the crucial role of these mycorrhizas in mineral nutrient acquisition for Ericaceae plants (Bonfante & Genre, 2010).

2.3.4. Orchid Mycorrhizas

Orchid mycorrhizas are unique because orchids are parasitic on fungi during the early stages of their life cycle. Orchid seeds are extremely small, containing only an embryo and minimal nutrient reserves, and require fungal colonization soon after germination to survive (Ventre *et al.*, 2021). The fungi involved are typically species of *Rhizoctonia* (Basidiomycota) or closely related types that degrade soil organic matter, including cellulose and other polysaccharides. These fungi penetrate the orchid embryo, forming hyphal coils called pelotons within host cells.

The pelotons degenerate after a few days, only to be replaced by new coils, providing sugars such as trehalose to the developing orchid. Supporting this, orchid seedlings can be cultivated commercially with trehalose-enriched media. In natural settings, these fungi serve as the sole carbohydrate source for orchids during their early years, as most orchids do not produce chlorophyll or emerge above ground until 3–5 years of age, and about 200 species remain achlorophyllous throughout life (Ayana, 2020).

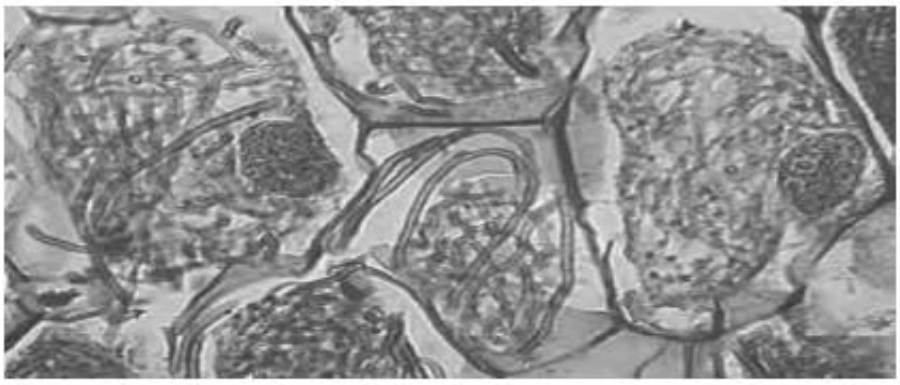

Figure 3. Section through part of the protocorm (basalstem region) of an orchid, Neottia, showing coils of hyphae (peletons) within the orchid cells. Source: (Ayana, 2020).

2.3.5. Monotropoid Mycorrhizas

Plants of the family Monotropaceae lack chlorophyll and rely entirely on mycorrhizal fungi for nutrients throughout their lives (Ali *et al.,* 2019). Found in deep forest shade under trees like oak, beech, pine, spruce, and fir, these plants parasitize ectomycorrhizal Basidiomycota fungi, such as *Boletus edulis*, which form mycelial networks connecting tree roots to *Monotropa* and related genera. The fungi, drawing sugars from the tree host, create a hyphal sheath around the *Monotropa* roots, typical of ectomycorrhizal sheaths with Hartig nets. This nutrient transfer, demonstrated through $^{14}CO_2$ labeling, moves sucrose from tree leaves to roots, converting into sugar alcohols or trehalose before reaching *Monotropa*. Additionally, fungal pegs projecting into plant root cells establish a unique three-way symbiosis between trees, fungi, and parasitic plants (Bonfante & Genre, 2010).

Table 1. Major Categories of mycorrhiza and their attributes. Source: (Barman *et al.,* 2016)

Mycorrhizal Type	Fungal taxa	Plant taxa	Intracellular colonization	Fungal Sheath	Vesicle
Arbuscular	Glomeromycota	Bryophyte Pteridophytes Gymnosperms Angiosperms	Present	Absent	Present/Absent
Ecto	Basidiomycota Ascomycota Zygomycota	Gymnosperms Angisperms	Absent	Present	Absent
Ectendo	Basidiomycota Ascomycota	Gymnosperm Angiosperms	Present	Present/ Absent	Absent
Arbutoid	Basidiomycota	Ericales	Present	Present/ Absent	Absent
Monotropoid	Basidiomycota	Monotropideae	Present	Present	Absent
Ericoid	Ascomycota	Ericales Gmnosperms	Present	Absent	Absent
Orchidaceous	Basidiomycota	Orchids	Present	Absent	Absent

2.4. Fungal Organs

The fungi morphology in symbiotic system includes chains outside of root and inside, support cells, vesicle inside the root between the cellular and intracellular Arbusculs (Rimington *et al.,* 2020). Considering that most of these organs can start a new colonization of plant roots are, a profile for each were described below.

2.4.1. Chain

Outside root chains that originated from germination spores present in the rhizosphere are of different morphology and functioning. Some participate in the colonization of roots, some are responsible for material absorption from the environment (soil), and some are spore producers. These chains have no lateral wall and where surface root cells contact, they are derived and from the end of each branch after forming aspersorium on the root surface, a thin chain penetrates inside the roots. Intracellular chains are always in plasma membrane surrounding the cell. In pot culture studies have shown that the outward root chain in Glomaceae family and

Acaulosporaceae have great ability for root colonization, but in the Gigosporaceae family the ability is very low (Bofante and Genre, 2010).

2.4.2. Arbuscule

This shrub looking organs from divergence of successive and bifurcate branches of inner root chains, after passing through the cellular wall and in an enclosed case in plasmatic membrane are formed within the root skin cells with a high level of contact with plant cells, Arbuscular plays the role of an exchange organ for limbs and nutrient exchange between fungus and host plants. Studies have shown that in the chain's core of an Arbuscul a lot of seed, mitochondria, glycogen particles, fat cells and dense granules made of poly-phosphate exist in vacuoles (Bofante and Genre, 2010). In the final fine branches, the number of vacuoles are high and the granules inside disappear.

2.4.3. Vesicle

Vesicle are spherical or oval bodies with a thin wall, and contain lipid cells which in terms of swollen chain ends or some of its' middle parts, are created inside or between roots' skin cells, and in the case of intercellular they are confined with plasma membrane similar to Arbuscul. These organs are only formed in fungal species belonging to the order of the Glomineae (Rimington*et al.*, 2020).

2.4.4. Support Cells

Various forms of bulged cells with thin walls, which are sourced from the outer root chains in funguses under the order of Gigasprineae, are called support cells. The level of these cells is in the barbed Gigasprineae genus, but in the Schatele spore they are seen as small bulges with an almost flat surface. Before mycorrhiza colonization begins the support cells appear on the Germination tube resulted by the spores. In pot culture, the number of support cells reaches its' peak point shortly after the start of spores, but after four months onwards, their number is reduced or they completely disappear (Davis *et al.*,2022).

2.4.5. Spore

Except the Gigapora genus which forms its' spores only in soil, all other spores are produced in the soil or in the roots. Although members of the order Glomal are categorized in Zygomycetes

category, but none of them produce Zygospore and their non-genus spores are in the form of Chlamydospore or Azygospore. Inner root sporisation are formed in some famous genus types of Glomus intraradices and Glomus diaghanum. The time for sporisation, often starts three to four weeks after the onset of root colonization, unless the growth context is of high absorbable phosphorus which in that case all the fungus growth stages will be limited. It is thought that spores of all arbuscular mycorrhiza fungi have the ability to colonize the root by forming a germinant pipe. However, if the Giga spora gigantea spores are healthy, they have more ability to colonize the root (Davis *et al.*,2022).

2.5. History of the taxonomy of AMF

Fossil records and molecular evidence suggest that AMF symbiotic relationship with host plants formed 400-480 million years ago (between the Ordovician and the Devonian) and would have had essential roles in the establishment of terrestrial plants on land (Sedaghat *et al.,* 2021). The symbiosis name of arbuscular mycorrhiza has changed over the years. First, the symbiosis was called "phycomycetous endomycorrhiza" to distinguish it from the endomycorrhizal symbioses that formed between members of the Orchidaceae or Ericaceae and a fungus of endotrophic mycorrhiza. After discovering fungal structures, vesicles and arbuscules, the name "vesicular-arbuscular mycorrhiza" was established and persisted until recently. With regard to not all fungi forming vesicles, this symbiosis was renamed "arbuscular mycorrhiza" (Bagyaraj *et al.,* 2021).

Before molecular techniques, the only way to identify AMF was by microscopic examination of fungal spores. As early as 2001, Morton & Redecker erected two new families, Archaeosporaceae and Paraglomaceae in the order Glomales, by the data from molecular, morphological, and biochemical.

According to the classification made by Redecker *et al.* (2013), more than 250 species of AMF were reported, which were divided into one class (Glomeromycetes), four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales), 11 families and 25 genera. They rejected some genera like Viscospora, Simiglomus, and Orbispora and accepted Dentiscutata, Sacculospora, and Corymbiglomus but required verification. Da silva *et al.* (2023) described two new genera, Dominikia and Kamienskia. Based on the phylogenetic analysis

performed by Sieverding *et al.* (2015), Rhizoglomus genus forms a separate clade within the family Glomeraceae.

Taxonomy and classification of this fungal group have been rapidly updated over the last years, and several reports have been made. Walker *et al.* (2018) classified the phylum Glomeromycota into a single class (Glomeromycetes) which includes four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales), 12 families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Geosiphonaecae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae, Pervetustaceae, and Sacculosporaceae), 34 generaand approximately 316 species. In the same year, the phylum Glomeromycota was divided by Tedersoo *et al.*(2018) into classes Glomeromycetes (Glomerale, Gigasporales, and Diversisporales), Paraglomeromycetes (Paraglomeromerales) and Archaeosporomycetes (Archaeosporales). Then, Wijayawardeneet *et al.* (2018) proposed another classification for Glomeromycota, which included two classes (Glomeromycetes, Archaeosporomycetes, and Paraglomeromycetes), four orders (Archaeosporales, Diversisporales, Glomerales, and Paraglomerales), 12 families and 38 genera and Gigasporales was not accepted. According to the latest unofficial classification presented on the website amf-phylogeny.com, AMF include four orders Diversisporales, Glomerales, Archaeosporales, and Paraglomerales, 12 families, 41 genera, and 342 species. Considering that new genera and species of this fungal group are being identified, such as Microkamienskia and Microkamienskia peruviana (Corazon-Guivin *et al.*, 2019). the classification of Glomeromycota is debatable. Nevertheless, the classification performed by Wijayawardene *et al.,* (2018) is used as a reference.

2.6. Morphological identification of AMF

Traditionally, AMF species have been identified by the morphological and anatomical characteristics of their spores (Traditionally, identification of AMF species has been made by spore extraction from soil and investigation of the morphological and anatomical characteristics of their spores) (Blaszkowski *et al.,* 2021). The morphological method is commonly used because it is fast, easy, cost effective, and economical and can identify at the genus level. In the absence of spores, the intraradical and extra-radical structures of AMF (arbuscules, vesicles, hyphae,) allow taxon identification at the family level (Corona *et al.*, 2023).

2.7. Roles of AM in ecosystems

The study by Brundrett (2002), on the co-occurrence of AM and ECM fungi in rainforest in Cameroon, provides a good example of a field study exploring possible functional roles of mycorrhizal fungi

2.7.1. Carbon transport

The fungal/plant interface provides a conduit for the movement of carbon from the plant to the fungus, and for movement between plants linked by mycelia (Hawkins *et al.,* 2023). The nature of the interface and its mode of regulation are still being elucidated (Hall and Williams, 2000). It is generally believed that mycorrhizal plants direct more of their photosynthates into the soil than nonmycorrhizal plants. This extra carbon accumulates in patches and at the edge of hyphal mats (Finlay and Read, 2005), and boosts the energy supply to the detrital food web, benefiting saprohytic microbes and other soil organisms (Barea, 2000). Because the chemical (Shahrokh *et al.,* 2020) and physical environment around mycorrhizas (the mycorrhizosphere) differs from nonmycorrhizas, presumably it provides microhabitats for soil biota that are not present in the rhizospere of nonmycorrhizal roots.Mycorrhizal fungi are estimated to consume from 15 to 50% of net primary production (Elliott *et al.,* 2022; Dell, 2002).

2.7.2. Nutrient cycling and nutrient conservation

Fungi are crucial components of ecosystems as they transport, store, release and cycle nutrients. A good example of the potential of mycorrhizal fungi to capture and deliver nutrients to their host comes from studies using inoculated eucalypts in field trials in sub-tropical China. Generally, trees in this region grow well below their potential. The main constraint to productivity appears to be low soil fertility (Dell *et al.,* 2001). Most of the land available for plantation forestry have been degraded over recent centuries with extensive loss of the A horizon caused by population pressure, inadequate management and over-harvesting (Xu *et al.,* 2004). Top soil crusting is common, contributing to enhanced erosion, reduced soil water storage, compaction and poor root development (Xu *et al.,* 2003). Low soil organic matter (SOM) content (<2%) also restrains productivity. As most soils for plantation eucalypts in southern China have lost their A_o layer, we need urgently to consider how to recover microbial biodiversity as there is no doubt that this will be important for improving long-term soil fertility. The capacity of some

ECM fungi to promote both early growth and survival of eucalypts is very important for commercial plantations on these disturbed and difficult sites. Significant effects of ECM fungal inoculation on growth of plantation eucalypts were obtained at two sites in southern China (Xu *et al.,* 2001). Effects were isolate dependent with some isolates stimulating tree growth and some isolates depressing tree growth. Similar results were obtained in a trial in the Philippines where two isolates increased survival while one isolate decreased survival of Eucalyptus urophylla (Corbeels *et al.,* 2003). The improvement in growth could be attributed to the acquisition of P as other essential mineral nutrients were supplied at establishment. Generally, inoculation only increased stand volume under P-limiting soil conditions (Hodge, 2014).

In forests, litter is an important nutrient reservoir. ECM fungi can mobilise P, N and other nutrients from litter to tree roots (Carballo *et al.,* 2022). Godbold *et al.*(2006), estimated that ECMs account for 43% of the annual turnover of N in a Pseudotsuga menziesii forest in Oregon. Litter type can affect the diversity and function of ECMs (Conn and Dighton, 2000). Bofante and Genre (2023), propose that the high diversity of fungal partners that a tree may have allows optimal foraging and mobilisation of various N and P forms from organic soil layers.

2.7.3. Soil structure

Mycorrhizal fungi, particularly ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi (AMF), play a crucial role in enhancing soil structure, which is vital for various ecological functions. Soil structure refers to the arrangement of soil particles and the spaces between them, influencing water infiltration, aeration, root penetration, and nutrient availability. Mycorrhizal fungi contribute significantly to soil aggregation through the production of extracellular polysaccharides and glomalin, which bind soil particles together, thereby improving soil stability and reducing erosion (Bedini *et al.,* 2009). Additionally, the extensive mycelial networks formed by these fungi connect individual soil particles and aggregates, facilitating nutrient exchange and enhancing water movement within the soil profile. Furthermore, mycorrhizal fungi influence the composition and activity of soil microbial communities, which are essential for organic matter decomposition and nutrient cycling, further enhancing soil structure and fertility. In agricultural soils, the presence of AM fungi has been shown to increase the formation of soil aggregates, leading to improved crop yields and resilience to drought (Bedini *et al.,* 2009). However, there is a notable scarcity of research focused on mycorrhizal fungi in tropical ecosystems, which may

limit our understanding of their ecological roles and contributions to soil structure. Given the high biodiversity of tropical ecosystems, understanding these interactions is crucial for conservation and sustainable land management.

2.8. Factors influencing mycorrhizal diversity

Mycorrhizal diversity is affected by many factors, including biotic and abiotic factors, and these factors can affect the interaction between the plant and mycorrhizal. In short, these factors are as follows:

2.8.1. Soil physical and chemical properites

Soil nutrients, especially phosphorous, are one of the most important abiotic factors that affect the mycorrhizal fungi. Both nitrogen and phosphorus significantly affect root colonies when they are present at high levels. Therefore, the state of balance for these two elements is a required condition, that is, the nutritional needs of the family. The plant and the nutritional needs of the living organism should not be within the critical sufficiency limit because this simply means entering into a kind of competition for the source of food, and this explains that some plants vaccinated with VAM may decrease their dry weight or growth rate because the VAM and the plant are included in this type from the competition so that the rate of net photosynthesis is insufficient for both the VAM and the plant, and thus the interference changes from Mutualism (+, +) to (−, +) Parasitism (Alkobaisy, 2020).

There is some information about the negative effects of nitrogen fertilizers on the formation of mycorrhizae, and it was found that nitrogen (ammonia nitrate) clearly reduces both the infection of mycorrhizae and the number of spores in wheat fields. As plants fertilized with high ratios of ammonium to nitrate have a higher phosphorous content in their tissues than plants fertilized with low ratios of (NH_4^+) to (NO_3^-), and these high concentrations of phosphorous in plant tissues inhibited infection. The reason for the inhibitory effect of ammonium may be attributed to the low pH in the area rhizosphere and to see the effect of fertilizers on mycorrhizae. The initial fertility of the soil must be known, because in poor soils the production of spores was limited to the total quantity and not the percentage of infected roots derived from plant growth(Alkobaisy, 2020).

As for phosphate fertilizers, some studies have shown that these fertilizers have negative effects on the internal mycorrhizal fungi. Increasing the processed phosphorous may reduce the infection of mycorrhizae to levels that are insufficient to encourage the absorption of other elements. Phosphorus and zinc of the shoots of pollinated plants grown in low fertility soils were more than that of unpollinated plants. Increasing the level of ready phosphorus is an inhibitor of the growth of mycorrhizal, unlike insoluble forms such as rock phosphate, which is not considered an inhibitor. The results on wheat plants confirmed this, as it was found that the addition of phosphorous levels of 60, 120, and 240 kg phosphorus/ha led to a reduction in the percentage of infected roots in the fertilized treatments compared to the non-fertilized treatments, where the level led to 240 kg phosphorus/ha to the absence of infection significantly and the removal of the beneficial effect of the mycorrhizal infection (DOI: http://dx.doi.org/10.5772/intechopen.108099).

The reason for the decrease in mycorrhizal infection as a result of the increase in phosphorous levels was shown by Cooper *et al.* (1984), that under conditions of phosphorus deficiency, the amount of phospholipids in the membranes of root cells decreases, leading to an increase in the permeability of these membranes, and this leads to an increase in the root secretion of reducing sugars. And amino acids lead to the formation of mycorrhizae, thus increasing the percentage of infected roots, but under conditions of availability of phosphorus, and the permeability of the membranes of the roots cells decreases due to the increase in phospholipids in them, and as a result, the secretions of the roots decrease from reducing sugars and amino acids, and this leads to a decrease in the percentage of infected roots. The decrease in the rate of infection may also be due to the increase in the concentration of phosphorus in the tissues of the plant, and the reason can be attributed to the fact that high levels of phosphorus may reduce the concentration of carbohydrates in the roots of plants, and as a result, the rate of infection is reduced. In general, high soil fertility leads to less mycorrhizal infection, so it is unlikely that we will find many mycorrhizae in densely cultivated soils. However, some crops are highly infested with fungi even in very fertile soils, as mycorrhizae are found in all poor and rich soils. Therefore, a low level of fertility is not always a condition for a significant development for mycorrhizal (De Sousa, 2023).

2.8.2. Temperature

Studies, such as those by Matsubara *et al.* (2000), have shown that temperature affects the formation of spores and colonies in greenhouse conditions, where the ideal temperature for mycelium on root surfaces is 20–30°C, while spore formation and the strength of spore cyst-forming species peak at 35°C, and the succession and decrease of temperatures enhance colony and spore formation.

2.8.3. Light

Light can indirectly affect soil microorganisms through its effects on plants, whose photosynthetic products are released from the roots (Dehlin *et al.,* 2008). The penetration of light through soil is important because of its effects on factors of ecological significance, such as spore germination, root growth, fungal growth, and formation of mycorrhizal and leguminous nodules. Light penetration can be affected by soil moisture content, soil type, cover material, and particle size. Phytochromes that are biliprotein photoreceptors enable some microorganisms to adapt to the light regime in the soil (Rottwinkel *et al.,* 2010). Fungi are unable to use light for photosynthesis; however, radiation plays a role in the biochemical and morphological responses of some fungi such as Phycomyces blakeslleanus, including their growth and differentiation. Physiologically and ecologically, a significant amount of light penetrates the soil approximately 4–5 mm from the surface, eliciting some phototrophic responses in plant roots. This information has led some VAM experts to hypothesize the function of LED on VAM formation. The induction of hyphal growth by light and chemicals, for example, the effect of blue light on hyphal branching, has been reported (Yaryura *et al.,* 2013).

2.8.4. The pH

The p^H level of the soil significantly influences the behavior and interaction of mycorrhizal fungi, with variations in the type of VAM fungi playing a critical role in determining the suitability of interactions. Studies have demonstrated that *Glomus mosseae* thrives in alkaline soils, a characteristic common to most soils in Iraq, where it germinates spores within a broad p^H range of 6 to 9. Conversely, *Gigaspora coralloida*, typically isolated from acidic fluoride soils, exhibits optimal spore germination within a p^H range of 4 to 6. This highlights *G. mosseae*as more compatible with alkaline environments compared to *G. mossea* .Additionally, *Glomus*

epigaeum has shown the ability to germinate spores across a wider p^H range, from 6 to above 8, surpassing the adaptability of the previously mentioned species. Research also indicates that *Glomus intraradices* and *G. mosseae* can colonize diverse soil types, with enhanced growth in neutral to alkaline soils, whereas *Gigaspora margarita* exhibits a preference for acidic conditions.These findings underscore the role of p^H in determining VAM spore germination and suggest a correlation between the pH suitable for host plant growth and that optimal for VAM development.Furthermore, soil pH not only influence AM root coloniization but also affects the growth and phosphatase activities of extraradical mycelium (Van Aarle *et al.,* 2002).

2.9. Application of AMF in agriculture and restoration

2.9.1. Improvement of Soil Quality and Health

Soil quality depends on the physical and chemical properties, and the diversity, distribution, and activity of the soil biota (Raut *et al.*,2020). Soil health refers to the ability of the soil to function as a living ecosystem. Soil health is governed by soil quality and thus influences human health via enhancing crop quality. As a beneficial biological tool, AMF maintains soil quality and health by influencing three main factors: soil structure, plant physiology, and ecological interactions (Figure 4) (Fall*et al.,* 2022).

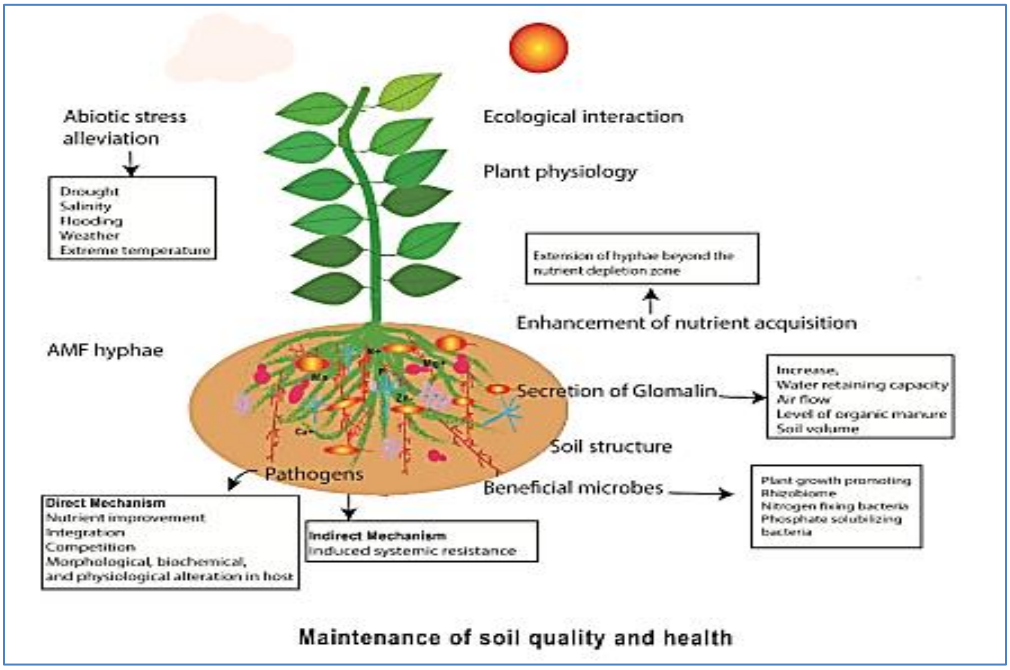

Figure 4. Mechanisms of maintaining soil quality and health by AMF to enhance plant productivity (Fall *et al.*, 2022)

Maintaining the soil structure is one of the important processes in agriculture. AMF form water-stable soil micro-aggregates by secreting hydrophobic glycoprotein, glomalin, which acts as along term binding agent (Leitheit *et al.,* 2014). AMF deposit glomalin between the outer hyphal walls and adjacent soil particles to form micro-aggregates and further macro-aggregates, thus forming the backbone for soil aggregation (Li *et al.,* 2022). This facilitates an increase in water retaining capacity, airflow, soil volume, and level of organic matter. In agricultural lands, to take more advantage of the maintenance these aggregates provide, lower disturbance and no-till fields are required (Bone *et al.,* 2008). AMF maintain both positive and negative ecological interactions in the rhizosphere. *AMF contact positively with beneficial microorganisms, including phosphate-solubilizing bacteria(PSB), nitrogen-fixing bacteria, and plant-growth-promoting rhizobacteria* (PGPR). Negatively, mycorrhizae interact to suppress both disease-causing soil-borne pathogens and pests (Nanjundappa *et al.,* 2019).

2.9.2. Drought Resistance

Drought stress in plants triggers a cascade of morphological and molecular changes, including increased ethylene production, which accelerates leaf senescence and reduces chlorophyll content, thereby impairing photosynthesis. Additionally, drought induces the accumulation of reactive oxygen species (ROS), leading to lipid peroxidation, protein misfolding, and compromised cell membrane integrity, ultimately causing cell death and diminished plant productivity. To counter these effects, plants activate adaptive mechanisms involving transcriptomic and proteomic modifications to regulate gene expression (Goicoechea *et al.,* 2020).

Supporting these natural defenses, arbuscular mycorrhizal fungi (AMF) enhance plant resilience by preserving chlorophyll levels and photosynthetic capacity under drought conditions. For instance, AMF-inoculated *Zea mays L.* plants under severe drought showed significant increases of 38.05% in total chlorophyll and 89.80% in carotenoids compared to non-inoculated controls (Folli-Pereira *et al.,* 2020).

AMF also modulate stress-related hormones, including abscisic acid, strigolactones, and jasmonic acid (JA), with JA elevation enhancing shoot carbohydrate levels and root osmotic potential. Furthermore, AMF improve plant water status by increasing stomatal conductance,

membrane conductivity (via aquaporin gene upregulation), and hydraulic conductivity, aided by their hyphal penetration into fine soil pores and the production of soil-aggregating glomalin. They also promote the synthesis of protective molecules, such as metallothioneins and polyamines, and osmolytes like proline, sugars, and glycine betaine, to mitigate osmotic stress. AMF further reduce oxidative damage by limiting ROS generation through enhanced water uptake or by increasing antioxidant enzyme activities, such as superoxide dismutase, peroxidase, and catalase. For example, inoculating the C_3 plant *Leymus chinensis* with AMF reduced malondialdehyde levels by up to 66% under mild to moderate drought, although this effect diminished under extreme drought (Li *et al.,* 2019). Collectively, AMF play a critical role in alleviating drought-induced damage, preserving plant health, and maintaining productivity.

2.9.3 Land Restoration

In general, mycorrhizae have positive effects on plants by maintaining all factors that are related to plant growth (Begum *et al.,* 2019) and influencing the community structure through community assembly and succession. Consequently, AMF have been applied to the restoration of ecosystems (De moura, 2022).

In central Asia, ephemeral plants supply higher fodder for livestock and play a role in reducing the occurrence of sandstorms. Over utilization of ephemeral plants causes a disturbance to desert vegetation (De Moura, 2022). As a solution to the slowing rate of natural restoration, the inoculation with AMF has been found to speed up the total cover, community productivity, and biodiversity of ephemeral plants over a three-year study period (Asmelash *et al.,* 2016). Long-term mining activities cause an adverse effect on soil and vegetation biodiversity. Observations have shown that the introduction of AMF leads to mine restoration and plant re-establishment, mainly by improving mineral acquisition and maintaining ecosystem stability and functioning (Wang, 2017).

2.9.4. Pathogen Biocontrol

A large number of pathogens, including above-ground and soil-borne organisms, cause adverse biotic stress on plant viability and functionality, and this results in a substantial yield loss in the cultivated fields. Like other beneficial microorganisms, mycorrhizae have the ability to

biocontrol pathogens by acting as a priming system in pathogen resistance. AMF identify the pathogens via chitin-related compounds, i.e., chito-oligosaccharides (COs) and lipo-chito-oligosaccharides (LCOs). Plants also produce microbe-specific molecular patterns on their receptor complexes. In the beginning, all chitin-related molecules are identified by the LysM–RLK complex. By combining this complex with other proteins, microbe identification is achieved. Along with that, mycorrhizae confer plant pathogen resistance via direct and indirect mechanisms. Indirectly, the AMF can trigger the induced systemic resistance (ISR) of plants. With that, the plants change their gene expression level, lignification incensement, and hormone levels (Schouteden *et al.*,2015), thus increasing pathogen resistance.

As direct mechanisms, AMF are involved in plant nutrient improvement, integration with other beneficial organisms, direct competition, morphological, biochemical, and physiological alteration of plants for pathogen biocontrol (Figure 4). By creating a competition for space, infection sites, and nutrients for the pathogen, and photosynthesis, the AMF control plant invasion by pathogens (Gupta *et al.,* 2020). For example, AMF competed with the major plant pathogenic nematode, Meloidogyne incognita, for the colonization in root nodules of the plant Prunus perscisa and inhibited gall formation (Chen *et al.,* 2018). Additionally, the formation of the underground common mycorrhizal network (CMN) that connects individual plants influences biocontrol by enhancing the available bioactive zone for plants (Young *et al.,* 2015). Interestingly, AMF can act as an early warning system for herbivore attacks via exchanging signals among plants. When a plant is attacked by the aphids, they cause changes in plant volatiles, such as methyl salicylate, and share the message of aphid attack with the neighboring plants via a common mycorrhizal network (Chen *et al.,* 2018).

2.10. The status of arbuscular mycorrhiza research in Ethiopia

Research on mycorrhiza in Ethiopia dates back to the mid-1980s, with significant contributions from Tekalegn Mamo and Killham (1987). However, it was not until the early 1990s that Anders Michelsen from Copenhagen University conducted pioneering research relevant to forestry. In 1993, Michelsen carried out nursery AM inoculation trials to assess the effects on the growth and field survival of Acacia abyssinica and Acacia sieberiana seedlings (Michelsen, 1993a). The following year, he, along with Sprent, examined the impact of AM fungi inoculation on the

nitrogen fixation of legume trees in Ethiopia (Michelsen & Sprent, 1994). Despite these early studies, there has been limited research on arbuscular mycorrhiza inoculation in tree species in Ethiopia. The latest significant contributions came from Emiru Birhane and colleagues, who conducted several studies between 2012 and 2015. In 2012, they investigated the effects of native AM consortia inoculation on the photosynthesis rate, water use efficiency, and growth of Boswellia papyrifera seedlings under simulated rainfall shortages, finding significant positive effects on all measured variables. In 2014, they explored the competitive ability of tree seedlings using Acacia etbaica and Boswellia papyrifera, concluding that AM inoculation did not enhance competitive ability. In 2015, they examined the effects of native AM consortia inoculation and moisture availability on nutrient uptake, biomass, and root mycorrhizal colonization in Acacia senegal, A. etbaica, and Boswellia papyrifera. Their findings indicated that at high moisture levels, fast-growing Acacia species benefited more from AM inoculation, while at lower moisture levels, the slower growing Boswellia papyrifera showed more benefits.

Michelsen (1993b) also made significant contributions by conducting ecosystem-level research on AM fungi associations in Ethiopian forests. He investigated the mycorrhizal status of 28 epiphytic plants in the Bale Mountains National Park, discovering that only 7 of these species were colonized by arbuscular mycorrhizal fungi, with the three facultative epiphytic species showing the highest colonization rates. Later, Tesfaye Wubet *et al.* (2003a) conducted research in three dry Afromontane forests in central and eastern Ethiopia, focusing on 11 ecologically and economically important native tree species. They reported that all these species were arbuscular mycorrhizal, generating data on the mycorrhizal status of seven species for the first time.

After a gap of about seven years, another important ecosystem-level AM research was conducted by Emiru Birhane *et al.* (2010) in three woodlands of northern Ethiopia, specifically in the Combretum-Terminalia and Acacia-Comiphora ecosystems. Their research aimed to determine whether the woody species in these ecosystems were arbuscular mycorrhizal and to morphologically identify the associated AM fungi genera. They found that all 43 woody plants studied were mycorrhizal, with Glomus being the predominant genus. Notably, 17 of the species were reported as arbuscular mycorrhizal for the first time.

Morphological studies on AM associations across different land uses and species were also conducted. Michelsen (1992) investigated the mycorrhizal status of tree nursery seedlings in

Ethiopia, while Zebene Asfaw (2003) examined AM associations within traditional agroforestry systems in southern Ethiopia. Diriba Muleta *et al.* (2008) studied factors affecting AMF spore abundance in Coffea arabica farming systems in southwestern Ethiopia, finding that various factors, such as sampling points, sites, depths, and shade tree species, significantly influenced AMF spore abundance. They noted that coffee agroforestry systems maintained higher AMF spore abundance compared to coffee monoculture systems, particularly at lower soil depths. Similarly, Tadesse Chanie and Fassil Assefa (2013) investigated AM fungi abundance and diversity in the rhizosphere of Coffea arabica shade trees in southwestern Ethiopia. In 2013, Zerihun Belay *et al.* conducted a morphological investigation of AM fungi associations with Acacias in the Rift Valley.

The following year, Mengsteab Hailemariam *et al.* (2013) studied the role of agroforestry trees in transferring infective AMF to associated annual crops. In 2014, they used maize as a trap plant to investigate the AMF infectivity of Faidherbia albida rhizospheric soil from various land-use types, including area exclosure, grazing, and cultivated lands.

Zerihun Belay *et al.* (2015) further explored the diversity and abundance of AM fungi across different land use types, comparing AMF abundance and diversity in soils from these land uses to trap culture, and found that land uses with greater plant diversity had better AMF abundance and diversity.In recent years, several additional AMF research activities have been conducted in Ethiopia, including studies by Beyene Dobo *et al.* (2016) and Emiru Birhane *et al.* (2017, 2018),as well as work by Yoseph Tewodros *et al.* (2017) and Fisseha Asmelash *et al.* (2019).

3. MATERIALS AND METHODS

3.1. Description of study area

This study was conducted in Lokka Abaya at randomly selected agroforest, permanent crop, forest and open grass land allotted for livestock grazing, western border of Sidama regional state of Ethiopia during dry season in January and February of 2016 E.C. Lokka Abaya is located at $6^0$17'25''N latitude and $37^0$49'44''E longitude (Figure 5). It is situated at 325 km southwest of the capital, Addis Ababa and 50 km southwest of regional city, Hawassa. The study area is characterised by bi-modal type of rainfall in which the short rainy season occurs from March to May, whilst the main rainy season occurs from June to September.

Mean annual rainfall varies from 700mm to 1877mm and mean annual temperature ranges from 26^0C to 35^0c. The area is also characterised by erratic rainfall, moisture stress and high temperature during the dry season (Central Statistical Agency (CSA, 2007). The altitude ranges from 1500 m to 1768 m above sea level. Agriculture is the principal source of livelihood for the most of the population in the district. The soil type is mainly grey sandy loam soil and it is susceptible to erosion (United States Agency for International Development (USAID), 2008).

The sedimentary rocks deposited one on top of another is found in some parts of the study area, sedimentary rocks are formed by layer and layers of material (sand or mud sediments). The black soil is known to retain huge amount of water during wet season while it cracks and loses its water contents at dry season. This type of soil is suitable for cultivation of Teff in the district. The clay soil type is used to cultivate Maize, Pepper and other crops.

The district covers about a total area of 119,000ha. Of which 20.2% is covered by farm land, 6.19% grazing land, 0.854% uncultivated land, 70% forest and bushes, 2.63% other vegetation and 0.126% of the land is not suitable for agriculture.

Loka Abaya national park and many other natural and man cultivated forests are found in the district. One among these forest is Segeno gale natural forest that contains many varieties of plant species. Plant species commonly distributed in the forest include Acacia *spp*, *Rasa abyssinica*, Euphorobia *spp*, *Prunus africana* and many others. The presence of these types of species in Segeno gale forest indicates that it is coverd by dense forest both broad leaved and

narrow leaved plants before they were cleared around forty years ago (personal communication) and Sebesebe Demissew and Friss, (2009).

The mixed crop cultivation is the main agricultural activities of the population of the district. The district farmer grow different types both food crops and cash crops. The major food crops that are cultivated in the district are *Zea mays* (Maize), *Eragrosis tef* (Teff), *Ensete vertricosum* (Enset) and others. Other field cash crops include *Catha edulis* (Khat), *Coffee arebica* and *Ipomoea batatas* (Sweet potato).

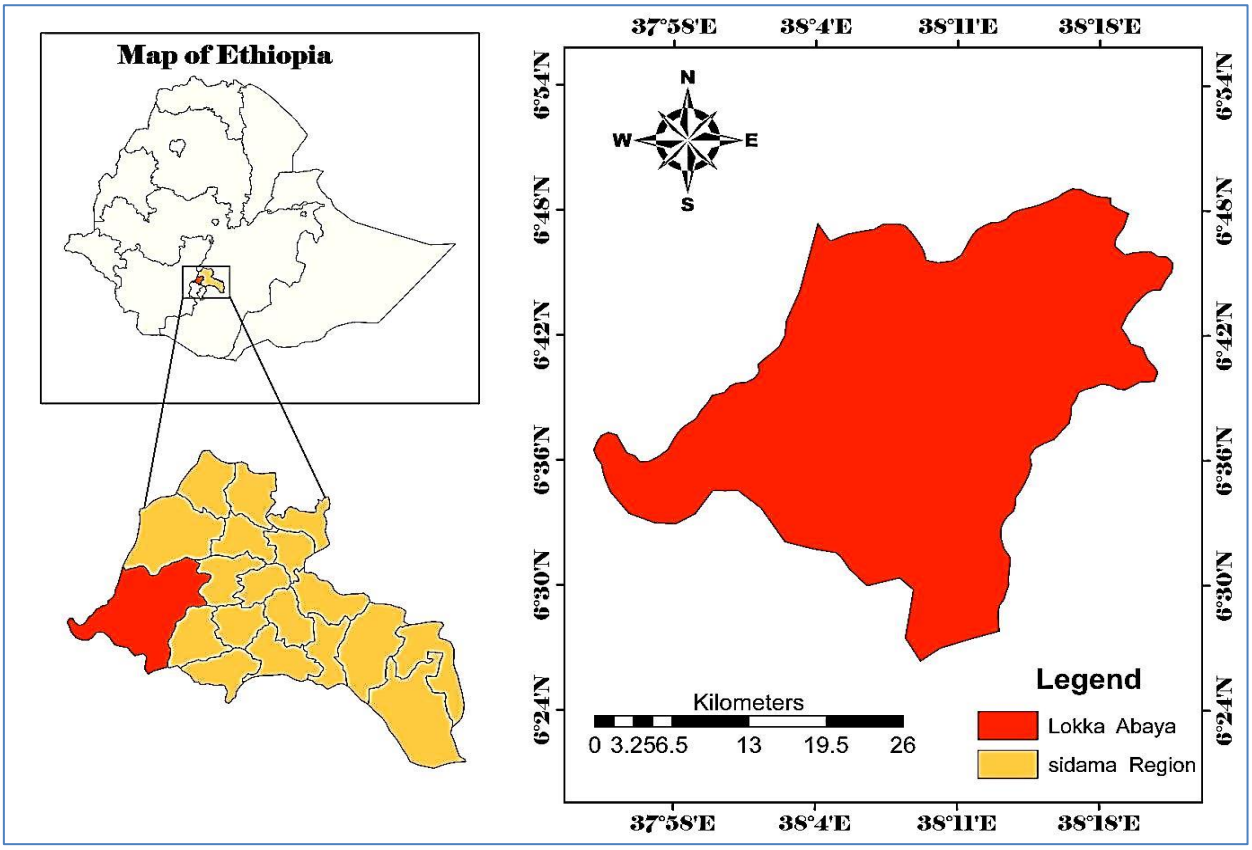

Figure 5. Map of the study area. (The map was created using shape file sourced from (https://amu-geo.maps.arcgis.com) and processed with ArcMap software, version 10.4.).

3.2. Study design

Descriptive cross-sectional field survey and laboratory analysis were employed to understand the rate of root colonization and diversity of AMF under the rhizosphere soils of different cropping system; such as agroforest, perennial crops, forest lands and open grazing fields.

3.3. Soil and root sample collection and analysis

Soil and root samples were collected to determine the AMF association and to evaluate root colonization respectively. Sampling was carried out in randomly selected annual crops, natural forest, the agroforestry and open grass land.

The main agricultural system in Loka Abaya is the home garden agroforestry system, commonly practiced in Sidama region. Some of the trees in agroforestry system, crop land, and in the natural forests, are given in table 2. In addition to agroforestry tree rhizosphere soil sampling, soil samples from grasslands, annual and perennial crop lands were also sampled.

All soil samples were collected during dry season (January to February) in year 2016 E.C from each sampling site. Soil sample collection was carried out at each plant rhizosphere close to the tree (0-1m distance), and underneath grass or the crop from each PAs in triplicate. The soil was sampled to a depth of 0-15cm using a soil auger. In each case a composite soil sample of 1kg was collected from each plant, grass land, and cropland species.

In total (23×6×3) or 414 soil samples were collected (from the plants' rhizosphere) for analysis from all the 6 PAs (sampling sites) from agroforestry, natural forest, the grasslands and the cropland uses to compare AMF distribution, abundance and diversity with the type of the land use.

Figure 6. Image of Soil and rhizosphere root sample collection in Loka Abaya District , Sidama Regional State

Immediately after collection, half of the sample was trap cultured using soghum bicolour as a host plant. The purpose of trap culturing techniques is to get abundant spore density and to initiate germination of dormant spores in a inherent soil. It may also help us to isolate a different species from the field soil samples.

The second half of the soil samples were air-dried at room temperature for two weeks and preserved at 4^0C for the analysis of soil physicochemical properties, AMF spore abundance and AMF diversity. About 0.5mg of fine root samples from each plant and the bulk soil was also collected, washed with tap water, preserve in 50% ethanol, and stored at 4^0C for the analysis of percentage root colonization.

Table 2. Plant species present in Loka Abaya Woreda open grass land, Permanet crop,forest and agroforestry systems the time of sampling according to geographical location and altitudinal ranges of the study site

Kebele	Land use type	Latin name	Family	Amharic	Sidamgna	Northing	Easting	Altitude
Danshe gambeltu	Agroforest	*Phaseolus vulgaris37*	*Fabaceae*	Boloke	Wahe	06^{0}38'644"	038^{0}15'657"	1687
		Zea mays37	*Poaceae*	Bokolo	Badalla			
		Citrus sinensis	*Rutaceae*	Burtukan	Burtukane	06^{0}38'645"	038^{0}15'627"	1678
		Coffea Arabica L.16	*Rubiaceae*	Buna	Buna	06^{0}38'645"	038^{0}15'627"	1678
		Carica papaya L.	*Caricaceae*	Papaya	Papaye	06^{0}38'638"	038^{0}15'650"	1679
		Gardenia volkensii k.schum	*Rubiaceae*	-	Gambella	06^{0}38'631"	038^{0}15'644"	1680
		Eragrosis tef15	*Eragrostis tef*	Teff	Gashe	06^{0}38'660"	038^{0}15'843"	1677
Segeno gale	Forest	*Ruclea schimperi (A.D.C.) Dandy*	*Ebenaceae*	-	Meessa	06^{0}44'833"	038^{0}15'583"	1783
		Calpurnia aurea (Alito)Benth	*Fabaceae*	Dingeta	Chekata	06^{0}44'51.94"	038^{0}15'35.79"	1770
		Lannea schimperi(Hochst .ex.A.R.C)	*Anacardiaceae*	-	Galicha	06^{0}44'51.20"	038^{0}15'36.03"	1765
		Acacia seyal Delile	*Fabaceae*	Girar	Wacho	06^{0}44'51.20"	038^{0}15'36.03"	1765
		Dodonaea angustifolia L.f.	*Sapindaceae*	-	Ittancha	06^{0}44'51.20"	038^{0}15'36.03"	1765
Aleta sodo	Agroforest	*Euphorbia abyssinica J.F. Gmal*	*Euphorbiaceae*	Qulqual	Caricho	06^{0}40'277"	038^{0}17'835"	1686
		Catha edulis (Vahl.) 28	*Celastraceae*	Chat	Catte	06^{0}40'277"	038^{0}17'835"	1686
		Cofea Arabica L.36	*Rabiaceae*	Buna	Buna	06^{0}40'277"	038^{0}17'835"	1686
		Albizia schimperiana oliv	*Fabaceae*	Sensel	Maticho	06^{0}40'277"	038^{0}17'835"	1686
		Balanites aegyptiaca	*Balanitaceae*	-	Godicho	06^{0}40'107"	038^{0}17'811"	1691
		Sacchanum officinarum L.18	*Poaceae*	Shenkor ageda	shonkora	06^{0}40'29.06"	038^{0}17'42.84"	1673
Kura	Grass land	*Pennisetum*	*Poaceae*	Sar	Buyyo	06^{0}45'40.65"	038^{0}20'29.17"	1749

Kebele	Land use type	Latin name	Family	Amharic	Sidamgna	Northing	Easting	Altitude
		sphacelatum1						
		Pennisetum sphacelatum3	*Poaceae*	Sar	Buyyo	$06^0$45'40.00"	$038^0$20'29.90"	1753
		Pennisetum sphacelatum2	*Poaceae*	Sar	Buyyo	$06^0$20'32.78"	$038^0$20'32.78"	1755
	Forest	*Balanites aegyptiaca*	*Balanitaceae*	-	Badana	$06^0$45'40.17"	$038^0$20'29.37	1752
		Carissa spinarum L.	*Apocynaceae*	-	Otlicho	$06^0$45'41.11"	$038^0$20'30.45"	1755
		Olea europaea L.	*Oleaceae*	Woiera	Ejerssa	$06^0$45'41.21"	$038^0$20'30.45"	1755
Cancho	**Agroforest**	*Ehretia cymosa Thonn*	*Boraginaceae*	Game	Gidincho	$06^0$44'28.15"	$038^0$19'20.81"	1676
		Cordia Africana Lam	*Boraginaceae*	Wanza	Wadicho	$06^0$44'30.20"	$038^0$19'22.49"	1681
		Ensete Ventricosum	*Musaceae*	Ensete	Wesse	$06^0$44'30.20"	$038^0$19'23.69"	1684
		Zea mays	*Poaceae*	Bokolo	Badalla	$06^0$44'24.28"	$038^0$19'14.15"	1667
		Phaseolus vulgaris64	*Fabaceae*	Boloke	Wahe			
		Psidium guajava L.	*Myrtaceae*	Zeytuna	Zayitone	$06^0$44'24.07"	$038^0$19'12.65"	1664
Desse	**Agroforest**	*Catha edulis (Vahl.)11*	*Celastraceae*	Chat	Catte	$06^0$43'37.54"	$038^0$18'48.32"	1639
		Capsicum frutescens	*Capsicum frutescens*	Berbere	Qare	$06^0$43'38.37"	$038^0$18'48.28"	1651
		Coffea Arabica L.30	*Rubiaceae*	Buna	Buna	$06^0$43'7.28"	$038^0$18'43.22"	1654
		Zea mays L.21	*Poaceae*	Boqolo	Badalla	$06^0$43'37.07"	$038^0$18'43.22"	1660
		Phaseolus Vulgaris21	*Fabaceae*	Boloqe	Wahe			

3.4 Soil physichochemical properties

Soil physicho-chemical analysis was undertaken at Hawassa University College of agriculture soil laboratory following standard procedures and methods: Soil textural fraction was analyzed following the hydrometric methods after removal of organic matter using H_2O_2 and dispersing the soils with sodium hexametaphosphate (Black *et al*., 1965). Soil p^H was determined by potentiometric methods using 1:2.5 soil : water ratio and Electrical conductivity was determined by EC meter . Soil organic carbon (SOC) was determined by the Walkey-Black oxidation method (Schnitzer, 1982). Total nitrogen (TN) was determined using the Kjeldahl distillation method (Bremner, 1982), and available phosphorus (A_P) was determined using Olsen's extraction method (uv/visible Spectrometer, Lambda EZ201) (Olsen *et al.*, 1965).

3.5. AMFspore density

From each soil sample 100g dry soil was mixed in a 2 L capacity beaker containing 1.5 L of water. The soil in water was agitated by stirring vigorously by hand and left to settle down for about five minutes. Each soil sample was mixed in a substantial volume of water and decanted through a series of sieves of 500, 106 and 50 μm. The contents from 106 and 50 μm sieves were mixed with water and centrifuged for 5 minutes at 2000 RPM. After having discarded the supernatant, the pellets were re-suspended in 60% sucrose solution and centrifuged for 1 minute as before. The supernatant was carefully poured through a 50μm sieve and carefully washed with water to remove the sucrose and transferred into plastic petri-dishes.

The AMF spore and sporocarps of each sample was counted under 4× stereomicroscope. The spore densities were expressed as the number of spores and sporocarps per 100 g^{-1} of dry soil. Representative Morphospecies were mounted on slides with polyvinyl-lactic acid- glycerol (PVLG) or PVLG mixed with Melzer's reagent (1:1v/v).

Quantitative study or spore enumeration work was done according to INVAM, http://*invam.caf.wvu.edu.*as follow. First using a fine ruler, the diameter of the ocular field of the stereomicroscope at a magnification of 40X was determined (where spores can be easily distinguished from mineral particles and organic debris). The area of the spherical field was calculated at that magnification (40X). It was having a diameter of 5 mm and the area of the field was 19.6 mm^2 (radius 2.5mm). Plastic Petri dishes were used to count spores because the base of the plate is flat. Because the dish also is hydrophobic, enough water is added to have complete

coverage of the base. Those dishes which were 85 mm across were used. That is the area of the base that was calculated to be 5672 mm^2. From this datum and the area of the ocular field (19.6 mm^2), and the total number of fields in the dish was: 5672/19.6. After that, the extracted spore suspension was added to a petri dish and then the dish was rotated randomly to spread out spores as evenly as possible. Finally, spores were counted in 40 fields randomly chosen over the area of the dish. An average number of spores per field was calculated and multiplied by 289 (#fields/dish).

3.6. Pearson's correlation coefficient between spore density, root colonization and soil parameters

Pearson's correlation analysis was used to study the relationship between AMF and soil parameters. This is to study whether the spore density and diversity and plant root colonization are dependent on soil physicochemical properties or not**.**

3.7. Spore identification and characterization

Isolated spores are grouped into morphotypes on the basis of their morphological characteristics. Semi-permanent slides are prepared for each different spore morphotype with a polyvinyl-alcohol Lacto Glycerol (PVLG) and polyvinyl-alcohol (IVAM, 2004). Spore identification was based mainly on spore size, shape, colour, wall structure, hyphal attachment (simple, swollen and bulbous) (INVAM, 2004). Taxonomic differentiation was made based on the description of the International Culture Collection of Vesicular/Arbuscular Mycorrhizal Fungi (http://invam.cafwvu.edu;2005), and following descriptions by Schenk and Perez (1990).

3.8. Isolation Frequency (IF%), Relative Abundance (RD%) and Importance Value (IV%) of AMF

The isolation frequency (IF) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all samples. Relative spore density (RD) was defined as the ratio between the spores' densities of a particular genus or species to the total AMF in a given soil (Li *et al*. (2007). The importance value (IV) was used to evaluate the dominance of AMF

species based on IF and RD and was calculated as IV= (IF+RD)/2. An IV≥50% indicated that a genus or species was dominant; whereas 10% <IV<50% and IV≤10% indicated common genera or species; and rare genera or species, respectively (Chen *et al*., 2012).

3.9. AMF Root colonization

The root pieces collected and preserved were stained according to Phillip and Hyman (1970) with some modifications. The root pieces were cleared in 10% KOH solution for 15 to 20 minutes at 90 ^{0}C in a boiling water bath. Pigmented roots were bleached in H2O2 for about 20 min and again washed with water Then they were rinsed in tap water several times and acidified with 1% HCL solution. Finally, the root segments were stained for 10 min in 0.05% trypan blue at 90 ^{0}C for about 10 min and subsequently distained at room temperature in acidic glycerol

The stained root segments were mounted in an acidic glycerol on slides. The roots are then observed under the compound microscope (200× objective magnification). The presence of colonization in a root segment is recorded if hyphae (only), vesicle or arbuscule are found. Total about 100 to 150 intersection were examined for each sample. Total root colonization is calculated using the following formula:

$$\% \textbf{ of colonization } = \frac{\textbf{Total number of positive segments}}{\textbf{Total number of segments}} \times \mathbf{100}$$

3.10. Data Analysis

Data analysis was carried out with SPSS software (Version 24). Data on percentage of AM root colonization was transformed by arcsine$_{\times}^{1/2}$ and spore densities were transformed by log (×+1) to fulfil the assumption of normality and homogeneity of variances before analysis of variance (Li *et al.,* 2007).

Significance of difference in AM fungal spore abundance and percentage of root colonization between the samples were tested using Duncan's Multiple Range Test at $p < 0.05$ after one-way analysis of variance (ANOVA) with the SPSS software package (Version 24) (Li *et al*., 2007). Pearson's correlation analysis was used to study the relationship between AMF and soil parameters.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Soil physicochemical properties under different plant species

Some of the soil characteristics including organic Carbon, N, P availability, p^H, Ec, & texture were measured using the method established by the Soil Research Institute. Soil samples were composite soil samples taken by purposive random sampling at Loka Abaya District from different points, at a depth of 0-15 cm. Soil characteristics of various types of land cover are shown in table 3. The soil in the study area exhibited a slightly acidic nature, with mean pH ranging from 5.73 to 7.56 indicating a transition towards neutral conditions. The total nitrogen content varied significantly, with mean concentration between 0.01 and 0.29%. Notably, p^H results were generally consistent across samples, with the exception of those associated with *Olea europaea L.* In terms of available phosphorus (Ap (Olson mg/g)), the highest concentration was recorded in the agroforest species *Cordia africana* (0.3120mg/g) while the lowest levels were observed in the natural forest species *Acacia seyal deli* (0.0100mg/g) and the cash crop *Sacchanum officinarum L*. (0.1240 mg/g).Electrical conductivity measurements indicated that the highest values were associated with the agroforestry species *Balanites aegyptiaca*, recording an EC values of 4.9200mS/cm. Conversely, the lowest conductivity was noted in the local variant of the same species found in the natural forest, which measured 1.2500 mS/cm. The maximum organic carbon content was observed in cash crop plants located in Dese kebele, specifically in *Catha edulis,* followed by *Balanites aegyptiaca* and *Acacia seyal deli* found in Kura kebele's natural forest. These findings highlight siginificant variation in soil chemical properties across different land uses and plant species within the study area.The analysis of soil texture classes across various land uses in the study area revealed distinct classifications associated with specific plant species. In Aleta sodo Kebele, the soil samples from *Albizia schimper* and *Euphorbia abyssinica* agroforestry systems were classified as clay loam. Similarly, *Gradenia volkensii* in Cancho kebele and *Zea may* and *Phaseolus vulgaris* cash crops in Danshe gambeltu kebele also exhibited clay loam charactersitics. In cancho, *Psidium guaja* agroforestry, along with *Zea mays* and *Phaseolus vulgaris* cash crops, displayed similar textural properties, as did *Capsicum frutescens* and *Zeamays* cash crops in Dese kebele. In the natural forest of segeno gale, *Lannea schimper* was identified within a clay texture class. Other species such as *Calpurnia aurea,*

Dodonaea angustifolia, Acacia seyal deli and *Olea europaea* were also classified as clay in Segene gale and Kura Kebele's natural forest. Additionaly, *Carissa spinarum* and *Balanites aegyptiaca* were noted in the same classification within these kebeles, while *Saccharus officinarum* cash crops in Aleta sodo exhibited similar clay textural properties. In terms of agroforestry systems, *Cordia Africana* and *Balanites aegptiaca* in Cancho and Aleta sodo were characterized as clay, alongside *Pennisetum* species grass land in Kura kebele. The sand loam texture class was represented by *Catha edulis* and *Enset ventricosum* cash crops in Dese and Cancho Kebeles, as well as *Ruclea schimper* in the natural forest at segeno gale and *Ehretia cymonas Thonn* in Cancho Kebele's agroforestry. Finally, loam texture was observed in *Coffee arabica* cash crops across Dese, Aleta Sodo, and Danshe gambeltu Kebeles, while *Citrus sinesis* agroforestry species were specifically identified in Danshe gambeltu Kebele.

Table 3. soil Physico chemical properties (mean±SE) under the six different kebeles agroforest, natural forest, cash crop and grass land in loka Abaya woreda Sidama regional states, Ethiopia

Plant species	pH(H_2O)	TN%	AP(Olson) (mg/g)	Ec	OC	Texture class
Albizia schimperiana	6.88 ±0.57^{a}	0.04±0.57^{b}	0.13±5.77^{c}	1.87±0.05^{e}	13.3±0.05^{e}	Clay loam
Gardenia volkensii	6.94 ±0.57^{a}	0.20±0.57^{b}	0.20±0.05^{bc}	3.91±0.57^{b}	14±0.57^{de}	Clay loam
Citrus sinensis	6.89 ± 0.05^{a}	0.01±0.05^{b}	0.10±5.77^{c}	2.41±0.05^{de}	15±0.57^{cd}	Loam
Psidium guajava L.	7.07±0.57^{a}	0.03±0.57^{b}	0.13±5.77^{bc}	3.7±0.05^{bc}	6.2±0.05^{bc}	Clay loam
Balanites aegyptiaca	7.04 ±0.57^{a}	0.23±0.57^{a}	0.24±0.05^{ab}	4.92±0.57a	17±0.57^{ab}	Clay
Ehretia cymosa Thonn	7.14± 5.77^{a}	0.20±0.05^{b}	0.16±5.77^{bc}	3.89±0.05^{b}	17±0.57^{ab}	Sand clay loam
Euphorbia Abyssinica	6.15± 0.57^{a}	0.09±0.57^{b}	0.18±5.77^{bc}	1.67±0.05^{e}	18.5±0.57^{a}	Clay loam
Cordia Africana Lam	7.56±0.57^{a}	0.06±0.05^{b}	0.31±0.05^{a}	2.98±5.77^{cd}	14.3±0.57^{de}	Clay
Balanites aegyptiaca	6.74±0.05^{ab}	0.25±0.57^{cd}	0.15±5.77^{b}	1.25±0.57^{d}	19.2±0.05^{a}	Clay
Olea europaea L.	5.0 ± 0.57c	0.12±0.57^{cd}	0.07±5.77^{d}	1.57±0.05^{d}	16.2±0.57^{b}	Clay
Acacia seyal Deli	7.22± 0.05^{a}	0.05±0.57^{a}	0.01±0.00^{e}	3.8±0.05^{ab}	19.2±0.05^{a}	Clay
Carissa spinarum L.	6.5±0.05^{abc}	0.16±0.05^{c}d	0.12±0.00^{c}	2.5±5.77^{c}	19.0±0.57^{a}	Clay
Ruclea schimper	6.11±5.77^{bc}	0.29±0.05^{cd}	0.15±5.77^{b}	4.30±5.77^{a}	10.2±0.05^{d}	Sand clay loam

Plant species	pH(H_2O)	TN%	AP(Olson) (mg/g)	Ec	OC	Texture class
Lannea schimper	6.77±0.05^{ab}	0.11±0.05^{b}	0.22±5.77^{a}	3.40±5.77^{b}	14.0±0.57^{c}	Clay loam
Calpurnia aurea	7.14±5.77^{a}	0.22±0.05^{bc}	0.08±0.00^{d}	3.4±0.05^{b}	18.0±0.57^{a}	Clay
Dodonae angusti folia L.f.	6.73±0.57^{ab}	0.16±0.05^{d}	0.12±0.00^{c}	2.7±5.77^{c}	16.0±0.57^{b}	Clay
Ensete ventricum	6.67±0.57^{a}	0.07±0.05^{a}	0.44±5.77^{a}	3.7±0.57^{a}	17.0±0.57^{bc}	Sand clay loam
Zea mays & Phaseolus vulgaris 21	6.5±0.57^{a}	0.28±0.57^{a}	0.15±5.77^{c}	3.7±0.05^{a}	18.5±0.57^{ab}	Clay loam
Chat edulis 11	6.84 ± 0.57^{a}	0.10±0.05^{a}	0.17±5.77^{bc}	3.7±0.57^{a}	19.3±0.05^{a}	Sand clay loam
Zea mays & Phaseolus vulgaris 31	6.50±0.57^{a}	0.18±0.57a	0.15±0.05^{c}	3.7±0.57^{a}	18.5±0.57^{ab}	Clay loam
Coffe arabica 30	6.77±0.57^{a}	0.08±0.57^{a}	0.15±0.05^{c}	3.86±0.57^{a}	17.6±0.57^{bc}	Loam
Capsicum frutescens	6.73±0.57^{a}	0.14±0.57^{a}	0.17±5.77^{bc}	1.75±0.57^{b}	18.5±0.57^{ab}	Clay loam
Zea mays & Phaseolus vulgaris 64	6.50±0.05^{a}	0.17±0.57^{a}	0.15±5.77^{c}	3.77±5.77^{a}	18.5±0.57^{ab}	Clay loam
Sacchanum officinarum L.	7.33± 0.05^{a}	0.26±0.05^{a}	0.12±5.77^{c}	3.47±0.57^{a}	16.5±0.57^{c}	Clay
Carica papaya L.	7.27±0.05^{a}	0.24±0.05^{a}	0.22±5.77^{b}	2.07±5.77^{b}	17.5±0.57^{bc}	Clay loam
Coffe arabica36	6.77±0.05^{a}	0.15±0.05^{a}	0.15±5.77^{c}	3.86±0.05^{a}	17.6±0.57^{bc}	Loam
Eragrosis teff15	6.52 ± 0.05^{a}	0.12±0.57^{a}	0.14±5.7^{c}	1.36±0.05^{b}	17.4±0.57^{bc}	Clay loam
Coffee arabica 16	6.85± 0.05^{a}	0.25±0.57^{a}	0.16±5.7^{bc}	1.89±0.57^{b}	18.0±0.57^{abc}	Loam
Pennisetum sphacelatum s3	6.25±0.577^{b}	0.06±0.57^{a}	0.10±0.57^{a}	1.20±0.00^{c}	18.2±0.57^{a}	Clay
Pennisetum sphacelatum s2	6±0.057^{c}	0.22±0.57^{ab}	0.20±0.57$_{a}$	1.3±0.005^{b}	17.0±0.57^{a}	Clay
Pennisetum sphacelatum s1	6.72±0.057^{a}	0.26±0.57^{b}	0.10±0.00^{a}	1.98±0.00^{a}	18.5±0.57^{a}	Clay

Key:OC, Organic carbon; TN, Total nitrogen; Ap, available phosphorus; Ec, Electrical conductivity. Similar letters in column show no significant difference between plant species at $p<0.05$

4.1.2. AMF root colonization and Spore density

In a study assessing arbuscular mycorrhizal fungi (AMF) colonization in various agroforestry plants, the highest percentage of root length colonization in field soil was observed in *Citrus sinensis*, with a notable 94.5%. Conversely, the lowest colonization rates were recorded in *Ehretia cymosa Thon* at 83.87% and *Euphorbia abyssinica* at 82.55%. All agroforestry species examined exhibited AMF infection, characterized by the presence of arbuscules and vesicles; however, *Euphorbia abyssinica* and *Ehretia cymosa Thon* demonstrated lower levels of colonization.

In a trap culture setting, the highest percentage of root length colonization was found in *Psidium guajava*, reaching 85.91%, while *Ehretia cymosa Thon* again exhibited the lowest colonization rates. Notably, the formation of arbuscules and vesicles was most pronounced in *Cordia africana*, with a colonization rate of 28%, and *Psidium guajava*, which reached 40%. The AMF spore densities associated with different agroforestry land use types were quantified from both field soil and trap cultures, as summarized in table 4. Notably, spore densities in trap cultures were observed to be up to twice as high as those recovered from field soil. Significant differences in spore counts between field soil and trap cultures were also noted.

In field soil, *Psidium guajava* exhibited the highest spore count at 2251 spores per 100 g of soil, followed by *Gardenia volkensii* with 780 spores per 100 g. Conversely, the highest spore counts in trap cultures were recorded for *Balanites aegyptiaca* (1228 spores), *Citrus sinensis* (1222 spores), and *Albizia schimperiana* (1060 spores) per 100 g of dry soil. In contrast, *Psidium guajava* (664 spores) and *Ehretia cymosa* (713 spores) exhibited the lowest spore counts per 100 g of soil in trap culture conditions.

Table 4. AMF spore density in agroforestry in the field and trap cultures soil samples

Plant species	Agroforestry							
	F				Tc			
	SD	Ac	Vc	RLC	SD	Ac	Vc	RLC
Psidum guajava L.	2251.7±11^{a}	26.8±0.5^{ab}	37.3±0.5^{c}	86.5±0.5^{c}	664±5.7^{e}	25.3±0.5^{b}	40.8±0.5^{a}	85.9± 0.5^{a}
Ehretia cymosa Thon	512±1.1^{e}	22.9±0.3^{c}	35.5±0.4^{d}	83.8±0.5^{d}	713±0.5^{e}	22±0.5^{c}	28.1±0.5^{g}	60.5± 0.5^{f}
Cordia africana	346.5±16.6^{f}	27.02±0.5^{a}	36.4±0.5^{cd}	87.8±0.5^{c}	919±5.7^{d}	28±0.5^{a}	30.1±0.5^{f}	70.08± 0.5^{e}
Balanite aepyptice a	550.03± 0.9^{d}	26.9±0.5^{a}	37±0.5^{cd}	84.2±0.5^{d}	1228± 5.7^{b}	21.9±0.5^{c}	36.5±0.5^{b}	81.7 ±0.7^{b}
Albizia schimperi ana oliv	173.00 ±5.1^{g}	25.8±0.5^{ab}	36.4±0.5^{cd}	88.2±0.5^{bc}	1060±5.7^{c}	25±0.5^{b}	33.3±0.5^{de}	80.9±0.5^{b}
Europhor bia abyssinini ca	525.00±2.7^{e}	22.09±0.5^{c}	39.5±0.5^{b}	82.5±0.5^{d}	3693±57.7^{a}	22.5±0.5^{c}	36.2±0.5^{bc}	75 ±0.5^{c}
Citrus sinensis	647.00± 0.5^{c}	25.2±0.5^{b}	42.8±0.5^{a}	94.5±0.5^{a}	1222±5.7^{b}	27.7±0.5^{a}	34.7±0.5^{cd}	80.5±0.5^{b}
Gardenia volkensiik .schum	780 ±5.7^{b}	26.4±0.5^{ab}	37.7±0.5^{c}	89.6±0.5^{b}	881±5.7^{d}	23±0.5^{c}	32.05±0.5^{e}	73.04± 0.5^{d}

Key: F- field sample; T- trap culture sample Ac, Arbuscular colonization; Vc, vesicular colonization; RLC, Total root colonization; SD,Spore density. Similar letters in columns show not significant difference between groups at $p<0.05$

4.1.3. AMF spore density in natural forest in the field and the trap culture soil samples

The root colonization by arbuscular mycorrhizal fungi (AMF) in various natural forest plant species is presented in table 5. The highest percentage of root length colonization in field soil was recorded in *Lennea schimper* (100%), while the lowest was observed in *Dodonaea angustifolia L.f.* (84%). All natural forest plants examined were found to be infected with AMF, characterized by both arbuscule and vesicle colonization.

In trap culture conditions, the highest percentage of root length colonization was recorded in *Calpurnia aurea* (85.90%), whereas the lowest was noted in *Balanites aegyptiaca* (68.7%). Regarding vesicle formation in trap culture, the highest colonization was found in *Carissa spinarum L.* (40%), followed by *Dodonaea angustifolia L.f.* (38.63%) and *Acacia seyal Delile*

(37.50%). The lowest vesicle colonization was observed in *Balanites aegyptiaca* (30.05%) and *Calpurnia aurea* (32.39%).

When considering arbuscule formation, the highest percentage was recorded in *Ruclea schimper* (27%) and *Lennea schimper* (25.80%), followed by *Carissa spinarum L*. (24%) and *Acacia seyal Delile* (23.43%). The lowest arbuscular colonization was noted for *Olea europaea L.* (20.25%). In most cases, arbuscular and vesicular colonization rates did not show significant differences at the $p < 0.05$ level.The spore abundance of AMF varied significantly among the different natural forest plant species.

The mean number of AMF spores in field soil ranged from 196 spores per 100 g dry soil in *Olea europaea L.* to 1172 spores per 100 g dry soil in *Ruclea schimper*. Conversely, the data indicated that spore populations in trap cultures were lower than those in field soil for certain species, such as *Ruclea schimper* (609 spores) and *Dodonaea angustifolia* (632 spores).

However, spore density in *Calpurnia aurea* (1734 spores), *Balanites aegyptiaca* (1445 spores), *Carissa spinarum* (1040 spores), and *Olea europaea L.* (1184 spores) was observed to be three times greater than that found in field soil samples. It seems that the mycorrhization intensities are based on phosphorus content in the soil (Table 3). The high mycorrhizal intensities was observed with low concentration of phosphorus (Table 3). The contents of vesicule and arbuscule also vary from 23.80 to 41.66 and 20.25 to 36.63 in field soil and trap cultures respectively.

Table 5**. AMF spore density in natural forest in the field and trap cultures soil samples**

Plant species	Natural forest							
	Field				Trap culture			
	AC	VC	RLC	SD	AC	VC	RLC	SD
Ruclea schimper (A.D.C) Dandy	21.3±0.6^{f}	42.7±0.1^{b}	86.5±0.6^{b}	1172.3±0.3^{a}	27.0±0.5^{a}	36.5±0.6^{c}	83.8±0.6^{b}	609±0.6^{e}
Calpurnia aurea (Alito) Benth	29.3±0.6^{a}	33.3±0.5^{d}	84±0.6^{e}	344.0±0.^{d}	25.4±0.5^{ab}	32.4±0.5^{d}	85.9±0.6^{a}	1734±5.8^{a}
Lennea schimper (Hochst.ex .A.R	28.4±0.6^{ab}	46.9±0.6^{a}	100±0.5^{a}	1157±0.6^{b}	25.9±0.5^{a}	34.1±0.5^{d}	77.6±0.6^{d}	1006±0.5^{d}
Acacia Seal Delile	29.3±0.6^{a}	41.3±0.5^{b}	88±0.6^{d}	210.3±4.2^{e}	23.4±0.5^{c}	37.5±0.5^{bc}	76.6±0.6^{de}	572±05.8^{e}
Dodonaea angustifolia L.F	27.1±0.6^{bc}	31.3±0.6^{e}	79±0.6^{f}	775±0.6^{e}	20.4±0.5^{d}	38.6±0.6^{ab}	75±0.6^{e}	632±0.6^{e}
Balanites aegyptiaca	25.2±0.6^{de}	39.6±0.6^{c}	94.5±0.5^{b}	197±1.2^{f}	21.1±0.5^{d}	30±0.6^{e}	68.7±0.6^{ef}	1445±0.6^{b}
Carissa spinarum L.	25.8±0.57^{cd}	38.8±0.57^{c}	90.58±0.57^{c}	196±0^{f}	24±0.5^{bc}	40±0.5^{a}	81.3±0.5^{c}	1040±05.7^{d}
Olea europacea L.	23.8±0.5^{e}	41.6±0.5^{b}	94±0.5^{b}	196±0^{f}	20.2±0.5^{d}	36.6±0.5^{c}	81±0.5^{c}	1184±5.7^{c}

Key:F- field sample; T- trap culture sample Ac, Arbuscular colonization; Vc, vesicular colonization; RLC, Total root colonization; SD,Spore densitySimilar letters in columns show not significant difference between groups at $p<0.05$

4.1.4. AMF spore density in Cash crop in the field and trap cultures soil samples

The root colonization by arbuscular mycorrhizal fungi (AMF) and spore density in both field soil and trap culture for various cash crop plant species are summarized in table 6. In field soil, the highest percentage of root length colonization was observed in *Coffee arabica*$_{36}$ (100%) and *Carica papaya* (92.75%), while the lowest colonization was recorded in *Zea mays*$_{37}$ (55.87%)

and *Phaseolus vulgaris*$_{37}$(55.87%), as well as *Saccharum officinarum* (56.17%). All examined cash crops exhibited AMF infection characterized by both arbuscules and vesicles.

In terms of arbuscule formation, the highest colonization was found in *Catha edulis*$_{28}$(30.01%) and *Coffee arabica*$_{30}$(29.35%), while the lowest was noted in *Saccharum officinarum* (22.72%) and both *Zea mays* and *Phaseolus vulgaris* (19%). Regarding vesicle formation, the highest percentages were recorded in *Coffee arabica*$_{36}$(48.54%), *Coffee arabica*$_{30}$(43.76%), and *Catha edulis*$_{28}$(43%). Conversely, the lowest percentages were observed in *Zea mays* and *Phaseolus vulgaris*$_{64}$ (22.03%).

In trap culture conditions, the maximum root length colonization was again recorded in *Coffea arabica*$_{16}$(100%), followed by *Saccharum officinarum* (90.36%) and *Catha edulis*$_{28}$ (87.5%), with the minimum observed in *Eragrostis teff*$_{15}$ (53.2%). For arbuscule formation in trap culture, the highest colonization was discovered in *Ensete ventricosum* (32.11%), while both *Zea mays* and *Phaseolus vulgaris*$_{37}$ showed a colonization rate of 29.3%. The lowest arbuscule formation was noted in *Eragrostis teff*$_{15}$ (21.1%).

The spore population per 100 g of dry field soil varied among species, with the highest density recorded in the rhizosphere of *Coffee arabica* (838 spores), followed by *Catha edulis* (734 spores). The lowest spore density was found in *Saccharum officinarum*$_{18}$ (284 spores/100 g soil). In trap culture, the highest spore density was registered in *Saccharum officinarum*$_{18}$(2921 spores), followed by *Zea mays* and *Phaseolus vulgaris* (1137 spores), *Capsicum frutescens* (1106 spores), and *Coffee arabica* (1054 spores). The lowest spore population in trap culture was reported for *Ensete ventricosum* (303 spores).

Table 6. AMF spore density in Cash crop in the field and trap cultures soil samples

Plant species	Cash crop							
	Field				Trap culture			
	Ac	Vc	RLC	SD	Ac	Vc	RLC	SD
Enset ventricusum	23.4±0.57^{hi}	37.6±0.57^{ef}	74.02±0.57^{g}	394.3 ±5.48^{i}	32.1±0.57^{a}	38.05±0.57^{cd}	75.6± 0.57^{f}	303 ±57.73^{h}
Coffee arabica 36	27.1±0.57^{cd}	48.5±0.57^{a}	100 ±0.57^{a}	399 ±1.73^{i}	25.6±0.57^{de}	33.7± 0.57^{f}	79.7± 0.57^{e}	1103 ±0.57^{c}
Catha edulis 11	27.8±0.57^{bc}	38.8±0.57^{de}	87.5±0.57^{d}	683.5±5.66^{c}	25.2±0.57^{e}	39.08± 0.57^{cd}	81.6±0.57^{d}	832 ±5.77^{f}
Coffee arabica 16	28.1±0.57^{bc}	32.4±0.57^{gh}	83.2±0.57^{e}	457.3 ±2.60^{h}	27.1±0.57^{cd}	48.5±0.57^{a}	100 ± 0.57^{a}	1054 ±5.77^{cd}
Sacchanum officinarum L.	22.7±0.57^{i}	31±0.57^{h}	56.1±0.57^{j}	475.6 ±1.33^{g}	25.3±0.57^{e}	36.1± 0.57^{e}	90.3±0.57^{b}	884 ±5.77^{ef}
Sacchanum officinarum L. 18-	25.3±0.57^{efg}	36.1±0.57^{f}	90.3±0.57^{c}	284.06±2.8^{k}	22.6±0.57^{fg}	39.2± 0.57^{cd}	76.1±0.57^{f}	2921 ±5.77^{a}
Catha edulis 28-	30.0±0.57^{a}	43 ±0.57^{bc}	87.7±0.57^{d}	734 ±0.00^{b}	27.7±0.57^{bc}	38.8±0.57^{cd}	87.5± 0.57^{c}	867 ±5.77^{ef}
Capsium frutescens	24.3±0.57^{ghi}	36.58±0.57^{f}	79.2±0.57^{f}	640.6± 1.20^{d}	25.6±0.57^{de}	37.8±0.57^{d}	86.4± 0.57^{c}	1106 ±0.57^{c}
Carica papay L.	26.08±0.57^{def}	42.02±0.57^{c}	92.7±0.57^{b}	548.7± 5.48^{e}	27 ±0.57^{cd}	31.7± 0.57^{g}	80 ±0.57^{de}	875 ±5.77^{ef}
Zea mays & Phaseolus vulgaris	19 ±0.57^{j}	29.02±0.57^{i}	63 ±0.57^{i}	343.3±0.33^{j}	23.5±0.57^{f}	39.7± 0.57^{bc}	80.8±0.57^{de}	896 ±57.73^{ef}
Eragrosis teff 15	24.7±0.57^{fgh}	39.5±0.57^{d}	79.01±0.57^{f}	402 ±0.57^{i}	21.1±0.57^{g}	39 ±0.57^{cd}	53.2± 0.57^{j}	924 ±5.77^{e}
Zea mays & Phaseolus vulgaris64	26.50±0.57^{cde}	22.03±0.57^{j}	68.5±0.57^{h}	535.6±0.88^{f}	26.5±0.7^{cde}	22.03±0.57^{h}	68.5±0.57^{h}	997 ±0.57^{d}
Zea mays & Phaseolus vulgaris 37	24 ±0.57^{ghi}	33.5±0.57^{g}	55.8±0.66^{j}	838.6±0.66^{a}	29.3±0.49^{b}	49.1± 0.57^{a}	71.5± 0.57^{g}	1173 ±5.77^{b}
Coffe arabica 30	29.35±0.57^{ab}	43.76±0.57^{b}	81.7±0.57^{e}	838.6±0.66^{a}	28.7±0.57^{bc}	41.1±0.57^{b}	66.700±0.57^{i}	760.00±5.77^{g}

Key: F- field sample; T- trap culture sample Ac, Arbuscular colonization; Vc, vesicular colonization; RLC, Total root colonization; SD,Spore density Similar letters in columns show not significant difference between groups at $p<0.05$

4.1.5. AMF spore density in grass land in the field and trap cultures soil samples

Table 7 presents the findings on AMF root colonization and spore populations in both field soil and trap culture grassland. The highest percentage of root length colonization observed in field soil was associated with *Pennisetum sphacelatum*$_1$, at 65.04%, while the lowest was recorded for *Pennisetum sphacelatum*$_3$, at 52.76%. All three grassland species exhibited infection by AMF, characterized by arbuscular and vesicular colonization. Notably, the trap culture demonstrated a higher rate of colonization compared to the field soil.

In terms of spore population density, the highest number of spores per 100 grams of dry field soil was found in the rhizosphere of *Pennisetumsphacelatum*$_1$ (1096 spores) and *Pennisetum sphacelatum*$_2$ (1006 spores), while the lowest density was noted in *Pennisetum sphacelatum*$_3$(944 spores). Conversely, in the trap cultures, the spore populations per 100 grams were generally lower, with the exception of *Pennisetum sphacelatum*$_3$, which recorded a spore population of 956.

Table 7.AMF spore density in grass land in the field and trap cultures soil samples

Plant species	Grass land							
	Field				Trap culture			
	Ac	Vc	RLC	SD	Ac	Vc	RLC	SD
Pennisetum sphacelatum1	21 ±0.6^b	28 ±0.6^a	65 ±0.6^a	1962 ±0.0^a	24 ±0.6^a	39.2±0.6^a	76 ±0.6^b	947 ±5.8^a
Pennisetum sphacelatum2	20 ±0.6^b	22 ±0.6^c	58.4±0.8^b	1006.2±3^b	22.5±0.6^a	32.5±0.6^b	78.8±0.6^a	832 ±5.8^b
Pennisetum sphacelatum3	27 ±0.6^a	25 ±0.6^b	52.8±0.6^c	944.3±12^c	24.3±0.6^a	30 ±0.6^c	75.7±0.6^b	956 ±25.2^a

Key:F- field sample; T- trap culture sample Ac, Arbuscular colonization; Vc, vesicular colonization; RLC, Total root colonization; SD,Spore density Similar letters in columns show not significant difference between groups at $p<0.05$

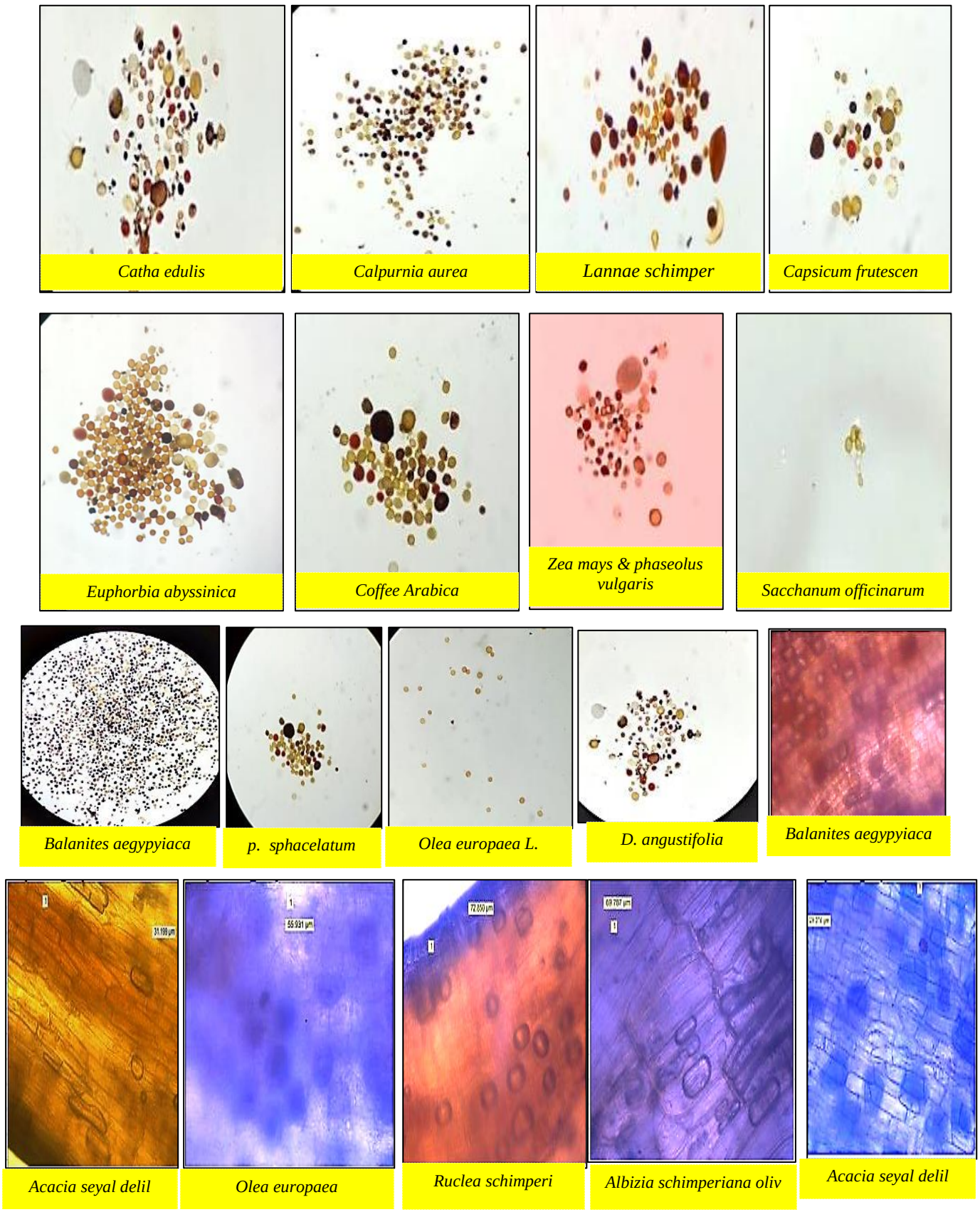

Figure 6. Image of spore density and root colonization observed on Loka Abaya woreda Sidama National Regional states Sampling sites included six Kebele's (i.e Kura, Cancho, Dese, Danshe gambeltu, Segeno gale and Aleta sodo respectively).

4.1.6. AMF Spore Community Composition

The study assessed the diversity of Arbuscular Mycorrhizal Fungal (AMF) genera and morphospecies associated with various agroforestry trees, perennial, and annual crops in Loka Abaya districts (Table 8). A total of 30 AMF morphospecies, belonging to eight genera (*Acaulospora, Glomus, Claroideoglomus, Rhizophagus intraradices, Rhizophagus clarus, Scutellospora sp$_1$, Scutellospora sp$_2$, Enterophospora, and Funneliformis*), were identified in the rhizosphere of plants across six kebele land-use types.

The findings revealed a varying distribution of AMF genera among plant species, indicating a diverse symbiotic relationship. Among the identified AMF genera, *Glomus* exhibited the highest colonization rates across most plant species, dominating *Saccharum officinarum* (60.87%), *Ensete ventricosum* (68.75%), and *Carica papaya* (63.63%). Similarly, *Acaulospora* was frequently detected in species such as *Pennisetum sphacelatum* (66.67%) and *Carissa spinarum* (43.86%).

In terms of spore production, Glomus accounted for the highest number of spores (43.50%), followed by Acaulospora (32.33%), Claroideoglomus (8.46%), and Enterophospora (4.23%). The genus Glomus also contained the highest number of morphospecies (12), followed by Acaulospora (10), while Claroideoglomus and Scutellospora each had two species. Conversely, Scutellospora sp1, Scutellospora sp2, Funneliformis sp., and Rhizophagus clarus contributed less than 5% to the total spore count.

Notably, the study revealed that land-use type played a significant role in AMF diversity. The agroforestry system exhibited the highest number of AMF morphospecies (25), while cash cropping systems harbored the least (11). In forest systems, 15 morphospecies were detected, whereas common agroforests, cash crops, and grasslands displayed morphospecies diversity ranging from 21 to 25.Individual plant species also exhibited variations in AMF diversity. *Coffea arabica* harbored the highest number of morphospecies (>25), followed by *Catha edulis* (23 morphospecies). *Psidium guajava* and *Capsicum frutescens* each supported 16 morphospecies, while *Eragrostis teff, Carica papaya, Gardenia volkensii, and Euphorbia* were associated with 11 morphospecies each. The lowest number of morphospecies was recorded for *Balanites aegyptica* 9 morphospecies.

Table 8. Percentage AMF diversity genera/ morphospecies in different agroforestrytrees, perennial andannual crops of Loka Abaya

S. N	Plant species	AMF Genera/Morphospecies (%)									
		Ac.	*Glo*	*Claro.*	*R.intra*	*R.Clarus*	*Scut1*	*Scut2.*	*Entero*	*Funnel*	*Spp.No*
1	*Catha edulis*	17.39	47.83	13.04	8.70	-	-	-	35	8.70	23
2	*Carissa spinarum.*	43.86	42.86	7.14	-	-	7.14	-	-	-	14
3	*Eragrostis teff.*	27.27	42.86	-	-	-	-	-	-	18.18	11
4	*Saccharum officinarum*	17.39	60.87	35	8.70	35	35	-	-	-	23
5	*Psidium guajava*	31.25	56.25	6.25	-	-	-	6.25	-	-	16
6	*Albizia schimperi*	30.0	30.0	20	-	10	-	-	-	10	10
7	*Phaseolus vulgaris*	30.0	60.0	-	10	-	-	-	-	-	10
8	*Olea europacea*	35.71	35.71	7.14	7.14	7.14	-	-	7.14	-	14
9	*Carica papaya L.*	27.27	63.63	9.09	-	-	-	-	-	-	11
10	*Dodonae angustifolia L.F.*	35.71	42.86	-	-	-	7.14	-	-	14.29	14
11	*Gardenia Volkensii*	36.36	36.36	18.18	-	-	-	-	9.09	-	11
12	*Ensete Vertricosum*	31.25	68.75	-	-	-	-	-	-	-	16
13	*Ehretia cymosa thonn*	30.77	46.15	-	7.69	-	-	-	15.38	-	13
14	*Acacia seyal Delil*	40	26.67	13.33	6.66	6.66	6.66	-	-	-	15
15	*Zea mays*	36.84	31.58	10.53	5.26	-	-	-	15.79	-	19
16	*Balanites aegyptia*	33.33	55.56	-	11.11	-	-	-	-	-	9
17	*Euclea schimperi*	36.36	36.36	9.09	-	-	-	18.18	-	-	11
18	*Pennisatum sphacelatum*	66.67	14.29	9.52	-	9.52	-	-	-	-	21
19	*Capsicum frutescens*	18.75	31.25	25	-	-	-	-	25	-	16
20	*Coffea Arabica*	40	44	4	-	8	4	-	-	-	25
21	*Citrus sinesis*	12.5	43.75	12.5	12.5	-	-	6.25	6.25	6.25	16
22	*Cordia africania*	30.77	38.46	15.38	-	7.69	-	-	7.69	-	13

Key: Ac., *Acaulospora*; Glo., *Glomus* species; Claro., *Claroidioglomus*; R.in;*Rhizophagus intraradices*; R. Clar., *Rhizophagus Clarus*; Scu.Sp_1., *Scutelospora* species$_1$; Scu. Sp_2; *Scutelospora* species$_2$; Enter.,*Enterophora* species; Fulle; *Funneloformis* species.

4.1.7. Pearson's correlation coefficient natural forest

Pearson's correlation coefficient (Table 9) showed that p^H and Nitrogen have a positive correlation (r= 0.5074), indicating that as pH increases, Nitrogen content tends to increase.p^H shows a moderate negative correlation with Phosphorus (P) (r= -0.1051), suggesting that higher p^H values are associated with lower phosphorus levels.

A positive relationship exists between p^H and EC (r = 0.2646), indicating a slight increase in conductivity with higher p^H. p^H has a weak positive correlation with organic carbon (OC) (r = 0.4454) and negative correlations with RLCT (r = -0.2223) and RLCF (r = -0.1826), indicating that higher p^H is associated with a reduction in root length colonization both in the trap and field.

The correlation between p^H and SDT is weakly positive (r = 0.0968), whereas it is weakly negative with SDF (r = -0.0977).

Nitrogen shows a negative correlation with Phosphorus (r = -0.3030), suggesting that an increase in nitrogen content is associated with a decrease in phosphorus. Nitrogen has a moderate positive correlation with EC (r = 0.4988) but a weak correlation with other variables. Organic carbon has a strong negative correlation with Phosphorus (r= -0.4907) and EC (r = -0.4512), indicating that higher organic carbon content is associated with lower phosphorus and EC levels.

RLCT has a moderate positive correlation with spore density in trap (SDT) (r= 0.4043) and weak correlation with spore density in the field (SDF).

RLCF shows a positive correlation with SDT (r = 0.3423), suggesting that spore density in the trap increases with field colonization. There is a strong negative correlation between SDT and SDF (r = -0.4336), indicating that spore density in the trap and field are inversely related.

Table 9. Pearson's correlation coefficients in between AMF parameters and soil chemical properties in Natural forest

Parameter	p^H	N	P	EC	OC	RLCT	RLCF	SDT	SDF
p^H	1								
N	0.5074	1							
P	-0.1051	-0.3030	1						
EC	0.2646	0.4988	-0.0119	1					
OC	0.4454	0.1771	-0.4907	-0.4512	1				
RLCT	-0.2223	-0.1102	-0.1819	0.4733	-0.3655	1			
RLCF	-0.1826	0.1809	0.4100	-0.3070	0.0231	-0.1602	1		
SDT	0.0968	-0.2095	0.0707	-0.4305	0.4043	0.0898	0.3423	1	
SDF	-0.0977	-0.0424	0.6779	0.5574	-0.8636	0.1611	-0.0202	-0.4336	1

Key:pH, power of hydrogen ; N, Nitrogen; P,phosphorus; EC, Elecrical conductivity; OC, organic carbon; RLCT, Root length colonization trap; RLCF, Root length colonization field ; SDT, Spore density trap; SDF, Spore density field

4.1.8. Pearson's correlation coefficient Agroforestry

Pearson's correlation coefficient (Table 10) the p^H shows a moderate positive correlation with nitrogen content (r = 0.3854) and phosphorus (r = 0.3108), indicating that in agroforests, higher p^H is linked to increased levels of nitrogen and phosphorus. p^H has a weak positive correlation with electron conductivity (r = 0.2242) but shows a weak negative correlation with spore density in traps (SDT) (r = -0.3791), suggesting that p^H affects nutrient availability more than spore density in traps.

Nitrogen content demonstrates a moderate positive correlation with electron conductivity (r = 0.5167) and a weak positive correlation with phosphorus (r = 0.4484), reflecting the interconnectedness of these nutrients in agroforests. However, nitrogen shows weak negative correlations with root length colonization in both traps (RLCT) (r = -0.1254) and the field (RLCF) (r = -0.2467), indicating that higher nitrogen content may slightly inhibit root colonization

Phosphorus also shows weak negative correlations with root colonization in traps (r = -0.3067) and in the field (r = -0.3297), though it maintains a weak positive correlation with electron conductivity (r = 0.2592). These correlations suggest a limited role for phosphorus in promoting root colonization in agroforests.

Electron conductivity (EC) has a moderate positive correlation with nitrogen (r = 0.5167) and a weak positive correlation with phosphorus (r = 0.2592). However, EC shows a weak negative correlation with root colonization in the field (r = -0.1892) and a moderate negative correlation

with spore density in traps (r = -0.5144), indicating that higher conductivity is associated with reduced root colonization and spore density in traps.

Organic carbon exhibits weak correlations with most parameters, though it has a moderate positive correlation with spore density in traps (r = 0.5090), suggesting that organic carbon supports spore density in agroforest environments. OC shows a moderate negative correlation with root length colonization in field (r = -0.6820), indicating that higher organic carbon may hinder root colonization in these conditions.

Root length colonization in traps is negatively correlated with organic carbon (r = -0.2033) and electron conductivity (r = -0.1892), suggesting that higher carbon and conductivity may reduce root colonization. However, RLCT shows weak positive correlations with spore density in the field (r = 0.4510) and pH (r = 0.0510), implying that root colonization in traps benefits from increased pH and spore density under field conditions.

Root length colonization in the field exhibits weak negative correlations with phosphorus (r = -0.3297) and nitrogen (r = -0.2467), and a moderate negative correlation with spore density in traps (r = -0.3942), reflecting that nutrient content and spore density in traps may hinder field colonization.

Finally, spore density in the field (SDF) is moderately positively correlated with RLCT (r = 0.4510) and weakly with electron conductivity (r = 0.2823), suggesting that field spore density benefits from root colonization in traps and increased conductivity. However, SDF shows a weak negative correlation with spore density in traps (r = -0.2355), indicating an inverse relationship between spore densities in traps and field conditions

Table 10. Pearson's correlation coefficients in between AMF parameters and soil chemical properties in Agroforestry

Parameter	p^H	N	P	EC	OC	RLCT	RLCF	SDT	SDF
p^H	1								
N	0.3854	1							
P	0.3108	0.4484	1						
EC	0.2242	0.5167	0.2592	1					
OC	-0.1959	0.1871	0.0387	0.2267	1				
RLCT	-0.1241	0.1254	-0.3067	-0.0806	-0.2033	1			
RLCF	0.0510	-0.2467	-0.3297	-0.1892	-0.6820	0.2940	1		
SDT	-0.3791	-0.0997	-0.0183	-0.5144	0.5090	0.0324	-0.3942	1	
SDF	0.0162	0.0123	-0.2344	0.2823	0.1550	0.4510	-0.0043	-0.2355	1

Key:p^H, power of hydrogen ; N, Nitrogen; P,phosphorus; EC, Elecrical conductivity; OC, organic carbon; RLCT, Root length colonization trap; RLCF, Root length colonization field ; SDT, Spore density trap; SDF, Spore density field

4.1.9. Pearson's correlation coefficients Cash crop

Pearson's correlation coefficient (Table 11) p^H and N show a moderate positive correlation (r=0.4065), which may indicate that soils with higher p^H levels also tend to have higher nitrogen content. pHand EC are moderately correlated (r =0.2536), suggesting a relationship between soil acidity and conductivity, which could be influenced by soil mineral composition.

N and OC have a positive correlation (r =0.3058), potentially reflecting a link between nitrogen availability and organic matter levels in soil. OC and SDF are positively correlated (r=0.4139), implying that higher organic carbon levels could be associated with greater spore density in field settings.

Many of the other parameters exhibit weak correlations, often less than 0.2 in absolute value, indicating weak or no association. For instance, p^H and RLCT (r=0.0694) and P and RLCF (r=0.0263) suggest little relationship between p^H or phosphorus with root length colonization in either trap or field conditions.

Negative correlations, such as between SDT and P (r=−0.4347), could indicate an inverse relationship in specific soil conditions.

Table 11. Pearson's correlation coefficients in between AMF parameters and soil chemical properties in Cash crop

Parameter	p^H	N	P	EC	OC	RLCT	RLCF	SDT	SDF
p^H	1								
N	0.4065*	1							
P	0.1745	0.0270	1						
EC	0.2536*	0.1703	0.0623	1					
OC	0.1848	0.3058*	0.0288	0.2775	1				
RLCT	0.0694^{ns}	-0.1203	0.0398	-0.0720	0.1833	1			
RLCF	0.0946	0.0482	0.0263^{ns}	-0.1752	-0.0249	0.2966	1		
SDT	0.2219	-0.0245	-0.4347^{ns}	0.0128	-0.2125	0.0238	-0.4479	1	
SDF	-0.0901	0.0601	-0.1208	0.1424	0.4139*	0.0231	-0.1217	-0.1096	1

Key: p^H, power of hydrogen ; N, Nitrogen; P,phosphorus; EC, Elecrical conductivity; OC, organic carbon; RLCT, Root length colonization trap; RLCF, Root length colonization field ; SDT, Spore density trap; SDF, Spore density field.

*- Correlation is significant at the 0.05 level (2-tailed); ns- Not significantly different.

4.1.10. Pearson's correlation coefficients Grass land

Pearson's correlation coefficient (Table 12) pH has a strong negative correlation with Phosphorus (P) (r= -0.5391) and a moderate negative correlation with Nitrogen (r= -0.3912), suggesting that as pH increases, both phosphorus and nitrogen decrease.

p^H shows a very strong positive correlation with EC (r= 0.6899), meaning that as pH increases, electroconductivity also increases. pH has a positive relationship with organic carbon (r= 0.5370), indicating a moderate increase in OC with higher pH values.

There is a strong negative correlation between pH and root length colonization in trap (r= -0.6257), while it shows a positive correlation with root length colonization in the field (r= 0.6474), implying opposite effects on root colonization under trap and field conditions. p^H has a strong positive relationship with spore density in field (r= 0.8991) and a moderate positive correlation with spore density in trap (r= 0.6279).

Nitrogen has a strong negative correlation with Phosphorus (r= -0.3096) and a strong negative correlation with EC (r= -0.7317), suggesting that increases in nitrogen are associated with decreases in both phosphorus and electroconductivity. Nitrogen is strongly negatively correlated with spore density in field (r= -0.6997), indicating that higher nitrogen content tends to lower spore density in the field.

Phosphorus has strong negative correlations with RLCT (r= -0.6842) and SDT (r= -0.6581), indicating that higher phosphorus levels are linked to lower root length colonization and spore density in the trap. Phosphorus is weakly negatively correlated with most other variables.

EC shows a strong positive correlation with organic carbon (r= 0.3484) and pH, but has negative correlations with P (r= -0.2946), RLCT (r= -0.2749), and RLCF (r= -0.0255). It has very strong positive correlations with SDF (r= 0.9968), suggesting that higher EC values correspond with greater spore density in the field.

OC has a moderate positive correlation with EC (r= 0.3484) and SDT (r= 0.3855), indicating that organic carbon tends to increase with electroconductivity and spore density in the trap. It has weak negative correlations with RLCT (r= -0.2519) and is weakly correlated with other variables. RLCT has a strong negative correlation with SDT (r= -0.9415), suggesting that increased root colonization in the trap reduces spore density in the trap.

RLCF has a very strong positive correlation with SDF (r= 0.9012), indicating that higher root colonization in the field is associated with higher spore density in the field. Spore density in trap and spore density in field have a moderate positive correlation (r= 0.3603), suggesting a somewhat positive relationship between spore densities in both environments.

SDF shows a very strong positive correlation with pH, EC, and RLCF, suggesting that higher spore density in the field is closely tied to these variables.

Table 12. Pearson's correlation coefficients in between AMF parameters and soil chemical properties in Grass land

Parameter	**pH**	**N**	**P**	**EC**	**OC**	**RLCT**	**RLCF**	**SDT**	**SDF**
pH	1								
N	-0.3912	1							
P	-0.5391	-0.3096	1						
EC	0.8699	-0.7317	-0.2946	1					
OC	0.5370	-0.3696	-0.1201	0.3484	1				
RLCT	-0.6257	-0.2951	0.6884	-0.2748	-0.2519	1			
RLCF	0.6474	-0.8757	-0.0255	0.9269	0.2415	0.1046	1		
SDT	0.6279	0.1747	-0.6581	0.2974	0.3855	-0.9415	-0.0592	1	
SDF	0.8991	-0.6997	-0.3495	0.9968	0.3869	-0.3287	0.9012	0.3603	1

Key: p^H, power of hydrogen ; N, Nitrogen; P,phosphorus; EC, Elecrical conductivity; OC, organic carbon; RLCT, Root length colonization trap; RLCF, Root length colonization field ; SDT, Spore density trap; SDF, Spore density field

4.1.11. IsolationFrequency (IF %), Relative Abundance (RD%) and Importance Value (IV%) of AMF in rhizosphere soils of culturally protected forest, agroforestry and crop land

The dataset presents the occurrence and distribution of various arbuscular mycorrhizal fungi (AMF) species and genera based on three key parameters: Isolation Frequency (IF), Relative Abundance (RA), and Importance Value (IV) in (Table 13). These parameters collectively

determine the ecological significance and status of each species, categorized as either Common or Rare.

A total of 30 AMF species across different genera were recorded, with a majority classified as Common and a few as Rare. Among them, the genus *Glomus* exhibited the highest species richness, followed by *Acaulospora, Claroideoglomus, Rhizophagus, Scutellospora, Funelliformis, and Entrophospora*.

Glomus sp. $_2$ demonstrated the highest IF (59.1%), RA (7.33%), and IV (33.22%), indicating its dominance in the ecosystem. Other highly abundant species included *Glomus* sp.$_3$ (IF: 54.55%) and *Glomus* sp.$_1$(IF: 50%), reinforcing the significance of *Glomus* in the AMF community.Several *Acaulospora* species also exhibited considerable prevalence, with *Acaulospora* sp.$_4$ and *Acaulospora* sp.$_2$ showing high IF values (40.91%), highlighting their substantial contribution to soil mycorrhizal networks.

Claroideoglomus sp.$_1$(IF: 45.45%, IV: 25.3%) and *Rhizophagus intraradices* (IF: 36.36%, IV: 19.84%) were also noteworthy contributors to the AMF community.Several species exhibited low frequency and abundance, classifying them as Rare. Notable among these were *Glomus pustulatum* (IF: 9.09%), *Scutellospora* sp.$_2$(IF: 9.09%), and *Glomus* sp.$_8$ (IF: 18.18%). These species, despite their low representation, play critical ecological roles in specific soil conditions and plant associations

The AMF community in this dataset is dominated by *Glomus* and *Acaulospora* species, with *Glomus* sp. 2 emerging as the most influential taxon. While common species maintain a significant presence, the occurrence of rare species underscores the biodiversity and potential niche specialization within the ecosystem. Understanding these species' distribution patterns can aid in optimizing soil health and plant-microbial interactions for ecological restoration and agricultural productivity.

Table 13. Isolation Frequency (IF%), Relative Abundance (RD%) and Importance Value (IV%) of AMF in rhizosphere soils of culturally protected forest, agroforestry and cropland.

AMFspcies/Genera	IF	RA	IV	Status
Acaulospora scorbiculata	27.27	2.72	15	Common
Acaulospora myriocarpa	11.11	1.21	6.16	Rare
*Acaulospora sp.*1	31.82	4.53	18.18	Common
*Acaulospora sp.*2	40.91	4.23	22.57	Common
*Acaulospora sp*3	36.36	3.63	20	Common
*Acaulospora sp.*4	40.91	5.14	23	Common
*Acaulospora sp.*5	27.27	3.02	15.15	Common
*Acaulospora sp.*6	27.27	3.93	15.6	Common
Acaulospora sp.7	13.63	0.91	7.27	Rare
*Acaulospora sp.*8	31.92	4.23	18.1	Common
Glomus mosseae	40.91	4.23	22.57	Common
Glomus etuncatum	31.82	3.93	17.88	Common
Glomus luteum	13.64	1.51	7.58	Common
Glomus Pustulatum	9.09	0.91	5	Rare
Glomus sp. 1	50	4.83	27.42	Common
Glomus sp. 2	59.1	7.33	33.22	Common
Glomus sp. 3	54.55	8.16	31.36	Common
Glomus sp. 4	36.36	3.93	20.15	Common
Glomus sp. 5	31.82	4.23	18.03	Common
10 Glomus sp. 6	31.82	3.02	17.42	Common
Glomus sp. 7	13.64	1.81	7.73	Rare
*Glomus sp.*8	18.18	1.51	9.85	Rare
*Scutelospora sp.*1	13.64	0.91	7.28	Rare
*Scutelospora sp.*2	9.09	0.6	3.23	Rare
Funelliformis	27.27	3.02	15.15	Common
Entherophospora	40.91	4.53	22.72	Common
Rhizophugus clarus	27.27	2.42	14.85	Common
Rhizophugus intraradices	36.36	3.32	19.84	Common
*Claroideoglomus sp.*1	45.45	5.14	25.3	Common
Claroideoglomus sp .2	31.82	3.32	15.79	Common

4.2 Discussion

4.2.1. AMF Spore density in the rhizosphere soil

The species diversity and spore density of arbuscular mycorrhizal fungi (AMF) were studied in soils from various land use types, including agroforestry, natural forest, cash crop systems, and grassland, in Loka Abaya District, Sidama National Regional State, Ethiopia.

The spore densities recovered through direct counts from field soil across various land use types ranged from 173 to 2,251 spores per 100g of soil (Table 4), 196 to 1,172 spores (Table 5), 284.06 to 838.67 spores (Table 6), and 944 to 1,962 spores (Table 7), respectively. Trap cultures established from the same land use types showed higher spore numbers, ranging from 664 to 3,693 (Table 4), 609 to 1,734 (Table 5), 303 to 2,921 (Table 6), and 832 to 956 spores per 100g of dry soil (Table 7).

For the current experimental kebeles, the spore density of arbuscular mycorrhizal fungi (AMF) species in the rhizosphere soil across different land use types was also determined by measuring spore density per 100g of sampled soil. The AMF in the rhizosphere soil of *Euphorbia abyssinica* and *Balanites aegyptiaca* trees exhibited the highest spore density compared to all other land uses, with populations ranging from 1,228 to 3,693 spores per 100g in trap cultures (Table 4). Conversely, the lowest AMF spore density was recorded in the rhizosphere soil of the cash crops *Zea mays* and *Phaseolus vulgaris*, with a spore density of 535.67 in the field and 997 in trap cultures per 100g of dry soil.

Similarly, a study by Cesra Patuicio *et al.*, (2009) and Bordoloi *et al.*,(2015) described significant variation in spore density across different cropping systems. The mixed cropping system (cabbage + sunflower + maize) had a spore density of 929 spores per 100g of soil. Likewise, cash crops like *Catha edulis* had the highest spore abundance, ranging from 867 to 734 spores in trap culture and field soil, respectively. In the agroforestry systems of Aleta Sodo kebeles, high spore density in both field soil and trap cultures was observed, second only to the Dese kebeles for *Catha edulis.*

A study in South Ethiopia's Sidama area and Senegal by Beyene Dobo *et al.* (2016), indicated that the highest AMF spore population was recorded in the rhizosphere soils of *Croton macrostachyus* (1,066 spores per 100g of soil) and *Catha edulis* (1,054 spores), while the lowest spore density was recorded for *Dioscorea alata* (100 spores per 100g of dry soil). When comparing similar land covers between the current study and that of Beyene Dobo *et al.* (2016),

notable variations emerge. For example, the current study found spore densities ranging from 394 to 1,103 for *Enset ventricum*, 457 to 1,054 for *Coffea arabica*, and 343 to 896 for *Zea mays* in both field soil and trap culture.

In general, spore recovery from agroforestry and cash crop plant species was 6 to 9 times higher in trap cultures than in direct soil counts. Trap culturing enhanced spore density significantly, especially for agroforestry, natural forest, and cash crop land use types. This finding aligns with Zerihun Belay *et al.* (2015), who reported that spore densities recovered through direct soil counts varied between 2.8 and 6.1 spores per gram of soil, while trap cultures from the same land use types showed higher numbers, ranging from 2.5 to 11.4 spores per gram of trap culture soil.

An inverse relationship between soil phosphorus (P) content and spore density was observed across different land use types. The highest spore densities in both field and trap cultures were found in agroforestry, natural forests, and cash crops associated with low P content, while the lowest densities were recorded in a few cash crops characterized by high soil phosphorus content. This negative relationship between spore density and phosphorus content has been observed in other studies, such as those in Ethiopian, Finnish, and Swedish soils. These studies suggest that certain AMF species tend to sporulate abundantly under conditions of low phosphorus availability (Zerihun Belay *et al.*, 2015; Xu *et al.*, 2018).

Regarding *Olea europaea* and similar crops, the present study found that mycorrhization depended on spore density and root length colonization, ranging from an average of 196 to 11,840 spores per 100g of soil and root colonization rates of 23.80% to 41.66%, with corresponding root length colonization from 20.25% to 36.63%. Warda Kachkouch *et al.* (2012) similarly described infection percentages increasing from 30% (Tamzazit) to 96% (Knichale) in cultivated olive trees, indicating a good potential for mycorrhization in those soils. In Morocco, Warda Kachkouch *et al.* (2012) recorded spore densities in the rhizosphere of *Olea europaea* ranging from 364 (Brifougass) to 168 spores per 100g of soil (Ouezzane).

4.2.2.Root colonization by AMF

The root colonization of different arbuscular mycorrhizal (AM) structures varied among plant species, both within and across land use types. This result aligns with previous findings (Li *et al.*, 2007). Vesicular colonization was notably high, ranging from 31.25% to 46.91% in field soil, and 32.39% to 40% in trap culture within natural forests. The high rate of vesicular colonization may be due to vesicles' role in accumulating storage products in many AMF associations, where

they can persist in roots for months or even years (Xu*et al.*,2018). Arbuscules, on the other hand, are short-lived.

The average AMF colonization levels were higher in agroforestry and natural forests (89.91%) compared to cash crops (53.20%). This finding is consistent with previous studies conducted in various parts of Ethiopia (Zenebe Asfaw, 2003; Zerihun Belay *et al.*, 2014). Total root length colonization (RLC) varied depending on the land cover, with ranges of 82.55% to 94.50% and 60.50% to 85.91% (Table 4), 79.00% to 100% and 68.70% to 85.90% (Table 5), 55.87% to 100% and 53.20% to 100% (Table 6), and 52.76% to 65.04% and 75.71% to 78.75% (Table 7) in both field soil and trap culture, respectively. This variability Supports findings from earlier studies highlighting that land cover and soil conditions significantly affect arbuscular mycorrhizal fungi (AMF) colonization (Smith and Read, 2008; Brundrett, 2009).

Among the specific plant species, the highest AMF colonization rate was found in *Acacia Seal Delile*, with a colonization rate of 88% in field soil, followed by *Pennisetum sphacelatum3* (52.8%). These results differe from the findings of Zerihun Belay *et al.* (2013), who reported the lowest AM fungal colonization in *Acacia seyal* (67.3%) in open grazing fields at Zeway. The high AMF colonization in *C. sinensis* may be attributed to its perennial nature and extensive root system, which provides a favorable environment for AMF symbiosis. Correspondingly, the lowest AMF colonization in the present study was recorded in inorganically cultivated *Zea mays* and *Phaseolus vulgaris* (55.87%), *Saccharum officinarum* (56.17%), and *Pennisetum sphacelatum*$_3$ (52.8%). These findings suggest that AMF colonization is influenced not only by land cover type but also by plant species and agricultural practices.

Similarly, Beyene Dobo *et al.* (2016b) reported RLC ranging from 55.69% in Ensete ventricum (monocropping) to 90.52% in *Coffea arabica* (agroforestry). Mycorrhization was highest in natural forests (100%), followed by agroforestry (94.5%), while cash crops (55.86%) and grasslands (52.76%) exhibited significantly lower colonization. Despite variations between studies, the general trend of higher AMF colonization in perennials and agroforestry systems is consistent, reinforcing the influence of land use on mycorrhizal associations.

Differences in RLC can be attributed to edaphic factors, crop type, and management practices. Soil p^H, nutrient availability, and organic matter content significantly shape plant-AMF interactions (Conti *et al.*, 2025). Plant host identity plays a crucial role, with Vályi *et al.*(2021) demonstrating that AMF colonization is primarily driven by plant species rather than management practices. Additionally, Kabir and Koide (2020) highlighted the impact of tillage,

crop rotation, and cover cropping on AMF abundance, root colonization, and crop performance. These findings underscore the importance of sustainable land management in maintaining AMF diversity and function.

In terms of specific crops, the current study found the following mycorrhization rates: *Coffea arabica* (100%, 83.25%, 81.7%),*Citrus sinensis* (94.5, 80.6),*Cordia africana* (87.83%), *Zea mays* and *Phaseolus vulgaris*(63%, 68.5%, 55.87%), *Saccharum officinarum* (90%), *Catha edulis* (87.5%, 87.71%), and *Ensete ventricum* (74.02%). In contrast, Beyene Dobo *et al.* (2016b) reported slightly different values: 80.14% in *Coffea arabica*, 72.33% in *Cordia africana*, 80% in *Zea mays*, 73.27% in *Phaseolus vulgaris*, 68.87% in *Saccharum officinarum*, 84.74% in *Catha edulis*, and 85.73% in *Ensete ventricum*. This variation in AM fungal colonization patterns across crop types may reflect the inherent heterogeneity in root systems and environmental factors such as soil physicochemical properties.

For *Citrus sinensis*, trees without AMF inoculation exhibited 24.88% root colonization and 0.28 cm hyphal length per gram of soil, while inoculated trees showed higher colonization, ranging from 42.57% to 55.70%, and hyphal lengths between 3.66 cm and 5.57 cm per gram of soil in China. The present study indicates that both field and trap cultures significantly increased root vitality, with an observed improvement of 94.50% and 80.55%, respectively. These findings are consistent with those of Qiang-sheng Wu *et al.* (2020), who reported that AMF-inoculated *Citrus sinensis* trees exhibited superior growth, soil quality, nutrient acquisition, and stress tolerance (Wu *et al.*, 2017b). Additionally, Songa Chan *et al.* (2015) noted AMF colonization in about 39.11% of fine root segments in *Citrus sinensis.* These results further reinforce the conclusions drawn by Wu *et al.* (2017), who highlighted that citrus species, due to their limited and shorter root hairs, are highly dependent on AMF. Mycorrhizal associations not only enhance plant nutrition and soil fertility but also influence root system architecture, improve soil structure, and strengthen plant resilience against environmental stresses. The significant increase in root vitality, colonization, and hyphal length observed in *Citrus sinensis* following AMF inoculation exemplifies the critical role of mycorrhizas in optimizing *citrus* growth. These interactions demonstrate how AMF facilitate nutrient uptake and root development, supporting Wu *et al.* (2017)’s assertion that mycorrhizas contribute to a broader range of physiological benefits beyond mere nutrient acquisition.

The extent of mycorrhizal infection in root systems is influenced by environmental and physiological factors, including the age of the plants, soil phosphate levels, and the population of mycorrhizal propagules in the soil (Yago *et al.*, 2009)

According to Girma Zeleke (2023), the highest hyphal colonization was recorded in *Miscanthus* species (85.41%), followed by *Capsicum annuum* monocrop (65.9%). Similarly,Booniue *et al.* (2011) reported the highest and lowest AMF colonization in *Capsicum frutescens* as 59.2% and 45.7%, respectively. The present study found 79.26% colonization in *Capsicum frutescens*. Moges Shenkutie (2014) recorded the highest colonization in *Capsicum frutescens* (60.3%) and the lowest in *Teff* (*Eragrostis tef*) (21.71%), whereas in the current study, *Zea mays* and *Phaseolus vulgaris* exhibited colonization rates of 55.87% and *Saccharum officinarum* 56.17%. Interestingly, *Teff* showed 79.01% colonization in this study, contrasting with other studies where *Teff* had lower rates: 58% (Cesra *et al.*, 2009) and 31% (Tekalign Mamo & Killhalm, 1987).

Notably, low AMF colonization (10–20%) in *Capsicum frutescens* was previously reported by Castilo *et al.* (2013). In contrast, in this study, *Zea mays* recorded 63% colonization in intercropping with *Phaseolus vulgaris* compared to other reports of 73.4% (Moges Shenkutie, 2014) and 40–44% (Sasvari & Posta, 2010).All investigated cash crops in this study displayed the three main AMF structures arbuscules, vesicles, and hyphae.

In conclusion, plants in natural protected areas showed higher colonization rates by AMF structures (arbuscules, vesicles, hyphae) and total root colonization (RLC) compared to inorganically grown cash crops and grasslands. This is consistent with previous studies, which suggest that modern agricultural practices, such as fertilization, biocide application, and monoculture cultivation, have been shown to negatively impact the composition and diversity of arbuscular mycorrhizal fungi (AMF) communities. A study by Manoharan *et al.* (2017) demonstrated that conventional farming practices reduce AMF diversity, whereas organic farming sustains greater AMF diversity, which is beneficial for sustainable cereal production. Similarly, research by Vályi *et al*. (2020) found that diverse plant mixtures maintain greater AMF spore viability compared to monocultures, even after 12 years.

Similary,the overuse of biocides can have detrimental effects on arbuscular mycorrhizal fungi (AMF). They highlight that biocides, designed to target harmful organisms, can also harm beneficial soil microorganisms like AMF, which are essential for plant nutrient acquisition. The application of these chemicals can reduce AMF colonization in plant roots and lower spore

populations in the soil, ultimately disrupting the symbiotic relationship that supports plant health and soil biodiversity. This disruption can lead to decreased plant growth and soil fertility (Smith and Read, 2019).In this study, arbuscular and vesicular colonization of AMF showed a statistically significant positive correlation ($p < 0.001$). Correlation analysis further revealed a strong positive relationship between arbuscular colonization and both hyphal and vesicular colonization, as noted by Lingfei *et al.* (2005).

The increase in spore density, along with an increase in soil available phosphorus observed in agroforestry and natural forest systems, may be due to the phosphorus concentration not being high enough to inhibit mycorrhizal development (Muleta *et al.*, 2007). Regarding soil texture, the soil sample from the study area was primarily sandy clay loam. In Cancho Kebele, agroforestry plant species included *Ensete ventricosum* and *Ehretia cymosa*. In Segeno Gale Kebele, natural forest species such as *Ruclea schimperi* were observed, while in Dese Kebele, *Catha edulis* was prominent.

In Aleta Sodo Kebele, agroforestry and cash crop systems included plants like *Saccharum officinarum* and *Balanites aegyptiaca*, both found in clay-rich soils. Additionally, in Segeno Gale Kebele, natural forest species such as *Calpurnia aurea*, *Dodonaea angustifolia*, and *Acacia seyal* were identified. In Kura Kebele, natural forest species like *Olea europaea*, *Carissa spinarum*, and *Balanites aegyptiaca* were present, whereas agroforestry species like *Cordia africana* and grassland species such as *Pennisetum sphacelatum* were observed in Cancho and Kura Kebeles, respectively.

Clay loam soils were found in Aleta Sodo Kebele, where agroforestry species like *Albizia schimperiana* and *Euphorbia abyssinica* were prevalent. In Danshe Gambeltu, *Gardenia volkensii* was present in agroforestry systems, while *Zea mays* and *Phaseolus vulgaris* were grown as cash crops. Similarly, in Dese Kebele, cash crops such as *Zea mays*, *Phaseolus vulgaris*, and *Capsicum frutescens* were common. In Cancho Kebele, *Psidium guajava* was observed in agroforestry systems, alongside *Zea mays* and *Phaseolus vulgaris* as cash crops.

Similar to our findings, Carrenho *et al.* (2007) reported that certain soil types, particularly sandy clay loam and clay loam, are favorable for mycorrhizal development. Additionally, the soil pH in the study area ranged from 5.7 to 6.9, which is conducive to AMF development. Berruti *et al.* (2016) also highlighted the crucial role of soil texture in the establishment and functionality of arbuscular mycorrhizal fungi (AMF). They emphasized that balanced soil structures, such as

sandy clay loam and clay loam, create optimal conditions for AMF by ensuring adequate aeration, moisture retention, and nutrient availability. These soil types promote AMF colonization and spore proliferation, ultimately enhancing plant nutrient uptake and overall soil health. This study underscores the importance of AMF in sustainable agriculture, advocating for soil management practices that preserve and promote their activity.

4.2.3. AMF Spore Community Composition

In this study different plants species from different land use types in reference to the Agroforest, Forest, Cash crop and Grass lands were sampled and studied for mycorrhizal diversity, root colonization and spore density.Based on spore morphology a total of 30 AMF species belonging to eight genera;*Acaulospora* species, *Glomus* species,*Claroi* species,*Rhizophagus intraradices*, *Scutolospora* sp_1 &$_2$, *Enterophophora* and *Funneloformis* species were detected.

The thirty species isolated from different land uses in Loka Abaya District is higher compared to 23 AMF morphospecies conducted in Hawassa area districts of Sidama Regional state (Girma Zeleke *et al.,* 2023), 15-18 AM species recorded in the acid soils of Western Kenya and agroforestry systems in the Miombo ecozone of Malawi (Mathimaran *et al.,* 2007), 17 AMF species from soil fertility met systems in Nigeria (Emmanuel *et al.,* 2010)and 29 morphospecies in South Ethiopia, Sidama area at agroforestry practing lands of Shebedino and Wensho districts (Beyene Dobo *et al.,*2016b) and Lower compared to the similar studies; 42 species recorded from the different land use tpes from a dry and humid agroecosystem of Bishoftu and Shoa Robit, Ethiopia (Zerihun *et al.,* 2013; Zerihun *et al.,* 2015), 43 species were identified from the different cropping systems from Jabi Tehnan Woreda, West Gojam, Ethiopia (Moges Shenkutie ,2014), 64 virtual taxa recorded in Cameroon (Mount Cameroon and Mount Manengouba) and Kenya (Chuka and Malava) (Yves *et al.,*2023*),* 66 AM fungal species from Southeast, China (Wang*et al.,*2015), 44 from semi-arid grass lands of Nambia (Uhlmann *et al.,* 2004) and 60 from sub-saharan Savannas of Benin, West Africa (Tchabi *et al.,* 2008). Like wise, 43 AM species were isolated from Wesrtn Brazilian Amazon (Sturmer and Siqueira,2011).

Spores of eight genera; *Glomus* species *Acaulospora* species, *Claroi* species , *Enterphophora*, *Radiphagus intraradices* , *Radiphagus clarius, Funnelform Scutelospora* $sp._1$&$_2$had higher spore production, account for 43.5%, 32.33%, 8.46%, 4.23%, 3.62%, 2.72%, 2.42%,1.51% and 1.21% of the total number of spores respectively. These results were comparable to the diversity of AMF described in Ethiopia (Beyene Dobo *et al.,*2016; Zerihun *et al.,* 2012) and Senegal

(Catford *et al.*,2003; Chen *et al.;* 2012) andother countries. The AMF species diversity observed in this study was much higher than the 17 speies identified in *Acaulosporaceae* (5), *Glomeraceae* (4), *Gigasporaceae* (5) and others (3) from different land use types in Kenya (Jewfa *et al.;* 2009).Common genera *Acaulospora, Glomus, Claroideoglomus, Funneliformis*have been discovered in study out put of Abdelhalim *et al.,* (2013) and that of the present one.In general, AMF-crop associations may not always be specific, as other influencing factors, such as the physicochemical properties of the soil, must also be considered.

Overall, the results highlight the ecological significance of AMF in different land-use types and plant species. The dominance of *Glomus* and *Acaulospora* suggests their crucial role in plant-soil interactions, influencing soil fertility, plant health, and agroforestry sustainability. These findings provide essential insights for optimizing land-use strategies, enhancing crop productivity, and maintaining ecosystem stability in Loka Abaya.

4.2.4. Isolation Frequency (IF%), Relative Abundance (RD%) and Importance Value (IV%) of AMF in rhizosphere soils

The relative abundance (RA) literally refers to the spore production potential of AMF. In this regard the current study, based upon the importance value (IV) , the differenet AMF genera were generally categorized into "commonly distributed" and" rarely distributed" species across the different land use types (Table 13). However, there were no species found as dominat (IV >50%) that was distributed amongst all plants land systems(Table 13) This is quite different from the report of Zerihun Belay *et al.,* (2015), where the genera *Claroideeoglomus* and *Funneliforms* was categorized into the dominant genera with 63 and 56, respectively.

Further more, most species from *Acaulospora* species and a species from *Glomus* , *Funneliforms* ,*Rhizophagus inntraradices* and *Claroideoglomus* Sp1 & 2 were categorized into the common group (with IV 15%<×<50%) together with many species of *Glomus* (33%), *Etherophospora* (22%) and *Acaulospora* (18%) and that were distributed in many of the land system. However, A few species from *Glomus pestulatum* were "Rare (IV<10%) and distributed in one or in the other land use types (Table-13). The output of the present study was in agreement with the findings by Wang*et al.,* (2015) and Oehl *et al.,* (2009) who reported *Glomus* species as a high spore producing genera in a shorter times than other genera such as *Funneloformis* species and *Scutelospora* species 2. These common genera could therefore be selected for future studies in

AMF inoculum preparation. After conducting further testing in their compatability with different crops and checking their persisitence in the field. These AMF genera would be an alternative microbiological technology inputs to highcost seeking and environmentally unfriendly inorganic input crop production, particularly of horticulture production in Ethiopia. The other species , *Acaulosporascorbiculata* , *Acaulospora* species, *Glomus mosseae*, *Glomus etuncatum*, *Glomus luteum*, *Glomus* species, *Funnelliforms*, *Etherophospora*, *Rhizophagus clarus* , *Rhizophagus intraradicesClaroideoglomus* sp1 &2 were detected from most of the sites , and the other group (5%) were found across atleast two or more of the land systems (data not shown).

4.2.5. Correlation of Soil Physico- Chnemical Properties with AMF Spore density and Root length colonization

The Pearson's correlation coefficients presented in Table 9 and Table 10 highlight key relationships between soil chemical properties and arbuscular mycorrhizal fungi (AMF) parameters in both natural forests and agroforestry systems. In natural forests, a positive correlation between soil p^H and nitrogen (r = 0.5074) suggests that increased p^H enhances nitrogen availability, aligning with previous findings from Ethiopian ecosystems (Tadesse *etal.*, 2019; Chanie & Assefa, 2021). However, the negative correlation between p^H and phosphorus (r = -0.1051) indicates that higher pH may reduce phosphorus availability, likely due to precipitation of phosphorus in alkaline soils. This inverse relationship has been noted in other studies, including work by Girma *et al.* (2020) and Tisdale *et al.* (1993). In agroforestry systems, a moderate positive correlation between p^H and both nitrogen (r = 0.3854) and phosphorus (r = 0.3108) suggests that higher p^H levels improve nutrient availability, enhancing AMF colonization, as seen in Ethiopian agroecosystems (Tadesse *et al.*, 2019; Chanie & Assefa, 2021). Additionally, both organic carbon and electrical conductivity (EC) exhibit correlations that influence AMF colonization, with organic carbon showing weak negative correlations with root length colonization in the field (r = -0.6820) and in traps (r = -0.2033), suggesting that excessive organic matter may hinder AMF colonization, possibly due to shifts in microbial competition (Mekonnen *et al.*, 2021). EC, on the other hand, is negatively correlated with root colonization and spore density in traps (r = -0.5144), reflecting the inhibitory effects of high salt concentrations on AMF activity (Abbott & Murphy, 2007). Interestingly, nitrogen and phosphorus exhibit weak negative correlations with AMF colonization in both the field and trap cultures, aligning with the findings of Birhane *et al.*(2017) and Wubet *et al.* (2003), who noted that nutrient enrichment can reduce AMF dependency. The strong negative correlation between

spore density in trap cultures and in the field (r = -0.4336) also highlights the environmental differences between controlled and natural settings, which can influence AMF proliferation and distribution. These findings underscore the complex interactions between soil properties and AMF dynamics, with significant implications for managing soil fertility and supporting healthy AMF communities in both natural forests and agroforestry systems in Ethiopia.

The analysis of Pearson's correlation coefficients between arbuscular mycorrhizal fungi (AMF) parameters and soil chemical properties in cash crop systems and grassland ecosystems in Table 11 and 12 reveals several noteworthy relationships. In cash crop systems, a moderate positive correlation between soil p^H and nitrogen content (r = 0.4065) indicates that higher p^H levels are associated with increased nitrogen availability, consistent with findings by Beyene Dobo *et al.* (2016). Similarly, a positive correlation between organic carbon (OC) and spore density in the field (SDF) (r = 0.4139) suggests that elevated organic carbon levels may enhance AMF spore density, aligning with research on the influence of organic matter on AMF proliferation (Yimer *et al.,* 2023). Weak correlations were found between other soil parameters and AMF colonization metrics, such as the minimal relationship between pH and root length colonization in traps (RLCT) (r = 0.0694), and phosphorus (P) and root length colonization in the field (RLCF) (r = 0.0263), indicating that soil properties may not have a straightforward effect on AMF colonization. The negative correlation between spore density in traps (SDT) and phosphorus (r = −0.4347) further suggests that higher phosphorus levels may suppress AMF spore production, as observed in previous studies (Beyene Dobo *et al.,* 2016). In grassland ecosystems, soil p^H exhibited significant negative correlations with phosphorus (P) (r = -0.5391) and nitrogen (N) (r = -0.3912), indicating that increasing soil p^H may reduce the bioavailability of these nutrients, as reported by Dobo and Belay (2016) and Zhao *et al.* (2021). A strong positive correlation between p^H and electrical conductivity (EC) (r = 0.8699) suggests that higher p^H levels may lead to an increase in soluble salts, impacting ion exchange in soils, which is consistent with findings from Yimer *etal.* (2023) and Singh *et al.* (2020). Additionally, p^H exhibited contrasting effects on AMF colonization, being negatively correlated with root length colonization in traps (RLCT) (r = -0.6257) but positively correlated with root length colonization in the field (RLCF) (r = 0.6474), suggesting field conditions may support AMF colonization better than controlled trap conditions, a trend also observed by Elhassan *et al*. (2019). Nitrogen showed strong negative correlations with phosphorus (r = -0.3096), EC (r = -0.7317), and spore density in the field (r = -0.6997), which suggests that excess nitrogen can suppress AMF spore production and colonization, a

phenomenon documented in Kenya (Mutai *et al.,* 2021) and other global studies. Phosphorus also negatively correlated with root colonization and spore density in both trap cultures and the field (r = -0.6842, r = -0.6581), supporting findings from Tadesse *et al*. (2022) and Chaudhary *et al.* (2021). The study also found a strong positive correlation between EC and spore density in the field (SDF) (r = 0.9968), indicating that mycorrhizal spore density increases with higher soil salinity or mineral content, a trend observed in studies from China and Sudan (Wei *et al.,* 2023; Ahmed *et al.,* 2017). Organic carbon had moderate positive correlations with EC (r = 0.3484) and SDT (r = 0.3855), suggesting that soil organic matter enhances AMF proliferation, as noted in Kenya (Koech *et al.,* 2020). Additionally, spore density in the trap (SDT) showed a strong negative correlation with root length colonization in traps (RLCT) (r = -0.9415), implying a trade-off between spore production and root colonization, a trend observed globally (Zerihun Belay *et al.,* 2015; Sharma *et al.,* 2019). Overall, these results demonstrate the complexity of interactions between soil chemical properties and AMF dynamics across different ecosystems.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study provides fundamental insights into the status of arbuscular mycorrhizal (AM) associations, AMF colonization, and spore density across different land use types, including agroforestry, natural forests, cash crop lands, and grasslands in the Loka Abaya district of the Sidama Region, Ethiopia. The findings highlight the significance of AMF associations in the six kebeles studied. The results indicate that AMF colonization and spore density decline due to continuous cropping and soil disturbances in cash crop lands. Agroforestry systems exhibited the highest spore density, whereas naturally protected forests had the greatest root colonization. Additionally, AMF diversity varied across different plant species, with agroforests showing the most extensive associations with diverse AMF species, followed by natural forests. In contrast, cash crop lands had the lowest AMF associations, likely due to the use of inorganic agricultural inputs, emphasizing the advantages of organic farming in preserving below-ground AMF diversity.

AMF crop associations are not always specific, as various factors such as soil physicochemical properties, land management practices, and site slope may influence them. Among the identified AMF genera, *Glomus* and *Acaulospora* were the most frequently recovered, appearing in 43.50% of the rhizosphere soil samples. In contrast, *Funneliformis* and *Scutellospora* were the least encountered genera, present in only 2.42% and 1.21% of the soil samples, respectively. Although not statistically significant, a positive correlation was observed between spore density, root colonization, and soil properties such as organic carbon content and optimal total nitrogen levels.

Overall, multipurpose agroforestry systems and naturally protected forests play a crucial role in maintaining environmental sustainability, improving soil structure, and restoring AMF associations compared to cash crop lands. Thus, promoting crop diversification can enhance soil biological and chemical properties, ultimately improving agricultural productivity.

5.2 Recommendations

This study was based on a two-month sampling period (January–February), which may not fully capture the seasonal variations in AMF dynamics. Therefore, the following recommendations are provided for relevant stakeholders, including the Loka Abaya District Agriculture Bureau, farmers, government bodies, and non-governmental organizations:

- The diversity and abundance of AMF communities were found to be negatively affected by the extensive use of agricultural inputs. To safeguard below-ground AMF associations and maintain their ecological benefits, proper management of inorganic inputs is essential. Additionally, further research is needed to assess the specific effects of various agricultural inputs on AMF associations in the Loka Abaya district.
- Glomus, Acaulospora, Claroideoglomus, Entrophospora, and Rhizophagus intraradices were identified as the five AMF genera with high spore production potential. Therefore, it is recommended to further investigate their inoculum potential across different land use systems and soil physicochemical conditions.
- This study relied on morphological characteristics for spore identification rather than molecular techniques. To enhance the accuracy of species identification and reveal greater diversity, future studies should incorporate advanced molecular techniques.
- Organic carbon (OC%), total nitrogen (TN%), and pH levels were found to influence AMF diversity. Low phosphorus (P%) and total nitrogen (TN%) levels favored AMF growth, whereas higher pH levels were associated with reduced AMF density. To strengthen these findings, further research is needed to explore the detailed relationships between AMF and soil physicochemical properties across a broader geographic area, incorporating different land use systems and plant species over an extended period.

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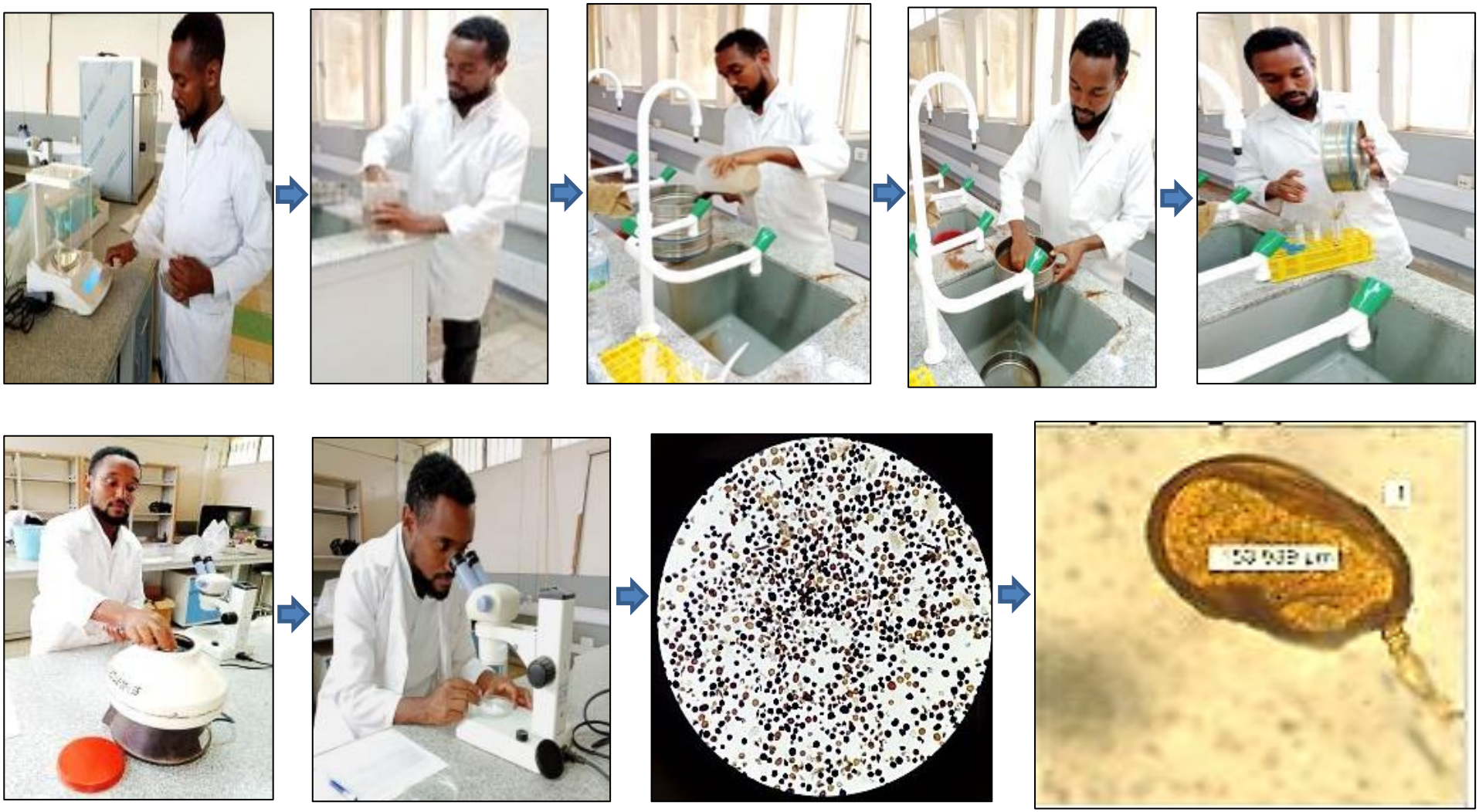
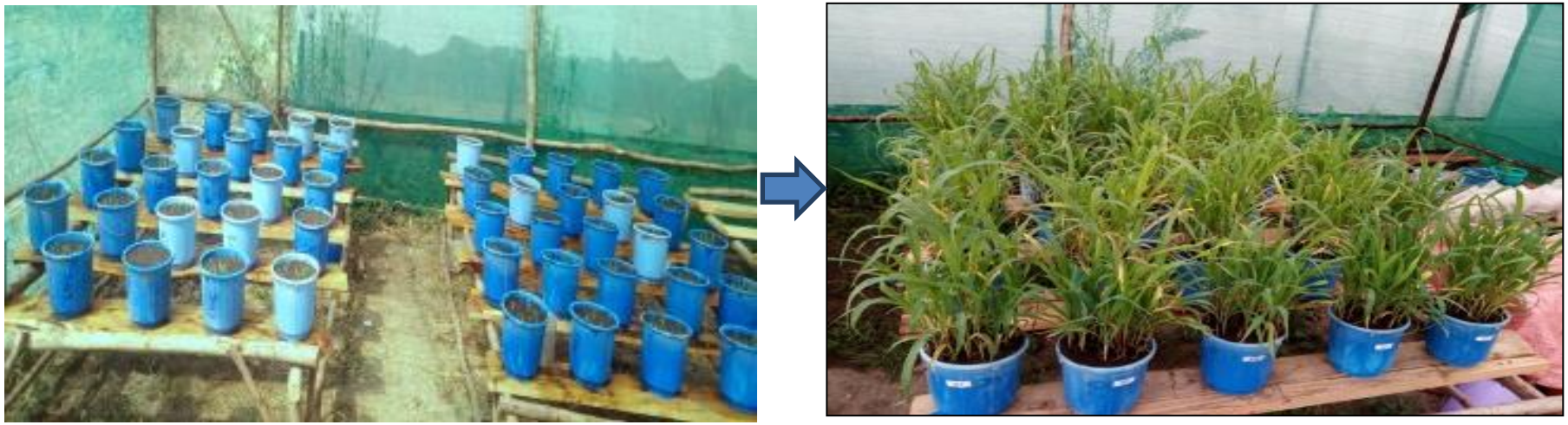
LIST OF APPENDICES

Appendix 1: Counting of spores of different species in field soil sample and trap cultures using Microscope from Hawassa University Molecular Biology Laboratory



Appendix 2: Soil sample preparation for physciochemical analysis

Appendix 3: Trap cultures (Sorghum bicolorCultures Grown in Greenhouse in three months)



Appendix 4: Mounting root on Glass Slides, Slides with spore samples and Long Term StorageEthanol-Glycerin mix

Appendix 5: Soil texture analysis

Appendix 6: Soil Physico chemical properties (mean±SE) under the six different kebeles agroforest, natural forest, cash crop and grass land in loka Abaya woreda Sidama regional states, Ethiopia

Plant species	pH(H_2O)	TN%	AP(Olson) (mg/g)	Ec	OC	%texture		
						Sand %	Clay%	Silt%
Albizia schimperiana	6.88 ±0.57^{a}	0.04±0.57^{b}	0.13±5.77^{c}	1.87±0.05^{e}	13.3±0.05^{e}	40.6	33.4	26
Gardenia volkensii	6.94 ±0.57^{a}	0.20±0.57^{b}	0.20±0.05^{bc}	3.91±0.57^{b}	14±0.57^{de}	34.6	37.4	28
Citrus sinensis	6.89 ± 0.05^{a}	0.01±0.05^{b}	0.10±5.77^{c}	2.41±0.05^{de}	15±0.57^{cd}	38.6	25.4	36
Psidium guajava L.	7.07±0.57^{a}	0.03±0.57^{b}	0.13±5.77^{bc}	3.7±0.05^{bc}	6.2±0.05^{bc}	44.6	33.4	22
Balanites aegyptiaca	7.04 ±0.57^{a}	0.23±0.57^{a}	0.24±0.05^{ab}	4.92±0.57a	17±0.57^{ab}	32.6	51.4	16
Ehretia cymosa Thonn	7.14± 5.77^{a}	0.20±0.05^{b}	0.16±5.77^{bc}	3.89±0.05^{b}	17±0.57^{ab}	46.6	31.4	22
Euphorbia Abyssinica	6.15± 0.57^{a}	0.09±0.57^{b}	0.18±5.77^{bc}	1.67±0.05^{e}	18.5±0.57^{a}	42.6	33.4	24
Cordia Africana Lam	7.56±0.57^{a}	0.06±0.05^{b}	0.31±0.05^{a}	2.98±5.77^{cd}	14.3±0.57^{de}	36.6	43.4	20
Balanites aegyptiaca	6.74±0.05^{ab}	0.25±0.57^{cd}	0.15±5.77^{b}	1.25±0.57^{d}	19.2±0.05^{a}	42.6	45.4	12
Olea europaea L.	5.0 ± 0.57c	0.12±0.57^{cd}	0.07±5.77^{d}	1.57±0.05^{d}	16.2±0.57^{b}	36.6	43.4	20
Acacia seyal Deli	7.22± 0.05^{a}	0.05±0.57^{a}	0.01±0.00^{e}	3.8±0.05^{ab}	19.2±0.05^{a}	36.6	45.4	18
Carissa spinarum L.	6.5±0.05^{abc}	0.16 ±0.05^{cd}	0.12±0.00^{c}	2.5±5.77^{c}	19.0±0.57^{a}	34.6	53.4	12
Ruclea schimper	6.11±5.77^{bc}	0.29±0.05^{cd}	0.15±5.77^{b}	4.30±5.77^{a}	10.2±0.05^{d}	54.6	31.4	14
Lannea schimper	6.77±0.05^{ab}	0.11±0.05^{b}	0.22±5.77^{a}	3.40±5.77^{b}	14.0±0.57^{c}	44.6	37.4	18
Calpurnia aurea	7.14±5.77^{a}	0.22±0.05^{bc}	0.08±0.00^{d}	3.4±0.05^{b}	18.0±0.57^{a}	32.6	45.4	22
Dodonae angusti folia L.f.	6.73±0.57^{ab}	0.16±0.05^{d}	0.12±0.00^{c}	2.7±5.77^{c}	16.0±0.57^{b}	34.6	51.4	14
Ensete ventricum	6.67±0.57^{a}	0.07±0.05^{a}	0.44±5.77^{a}	3.7±0.57^{a}	17.0±0.57^{bc}	50.6	23.4	26
Zea mays & Phaseolus vulgaris37	6.5±0.57^{a}	0.28±0.57^{a}	0.15±5.77^{c}	3.7±0.05^{a}	18.5±0.57^{ab}	38.6	35.4	26
Chat edulis 11	6.84 ± 0.57^{a}	0.10±0.05^{a}	0.17±5.77^{bc}	3.7±0.57^{a}	19.3±0.05^{a}	48.6	31.4	20
Zea mays & Phaseolus vulgaris 21	6.50±0.57^{a}	0.18±0.57^{a}	0.15±0.05^{c}	3.7±0.57^{a}	18.5±0.57^{ab}	38.6	35.4	26
Coffe arabica 30	6.77±0.57^{a}	0.08±0.57^{a}	0.15±0.05^{c}	3.86±0.57^{a}	17.6±0.57^{bc}	38.6	25.4	36
Capsicum frutescens	6.73±0.57^{a}	0.14±0.57^{a}	0.17±5.77^{bc}	1.75±0.57^{b}	18.5±0.57^{ab}	34.6	37.4	28
Zea mays & Phaseolus vulgaris 31	6.50±0.05^{a}	0.17±0.57^{a}	0.15±5.77^{c}	3.77±5.77^{a}	18.5±0.57^{ab}	38.6	35.4	26
Sacchanum officinarum L.	7.33± 0.05^{a}	0.26±0.05^{a}	0.12±5.77^{c}	3.47±0.57^{a}	16.5±0.57^{c}	26.6	51.4	22
Carica papaya L.	7.27±0.05^{a}	0.24±0.05^{a}	0.22±5.77^{b}	2.07±5.77^{b}	17.5±0.57^{bc}	38.6	29.4	32
Coffe arabica 36	6.77±0.05^{a}	0.15±0.05a	0.15±5.77^{c}	3.86±0.05^{a}	17.6±0.57^{bc}	38.6	25.4	36
Eragrosis teff15	6.52 ± 0.05^{a}	0.12±0.57^{a}	0.14±5.7^{c}	1.36±0.05^{b}	17.4±0.57^{bc}	28.6	33.4	38
Coffee arabica 16	6.85± 0.05^{a}	0.25±0.57^{a}	0.16±5.7^{bc}	1.89±0.57^{b}	18.0±0.57^{abc}	38.6	25.4	36
Pennisetum sphacelatum s1	6.25±0.577^{b}	0.06±0.577^{a}	0.10±0.57^{a}	1.20±0.00^{c}	18.2±0.577^{a}	34.6	53.4	12
Pennisetum sphacelatum s3	6±0.057^{c}	0.22±0.577^{ab}	0.20±0.57$_{a}$	1.3±0.005^{b}	17.0±0.577^{a}	34.6	53.4	12
Pennisetum sphacelatum s2	6.72±0.057^{a}	0.26±0.5773^{b}	0.10±0.0^{a}	1.98±0.00^{a}	18.5±0.577^{a}	34.6	53.4	12

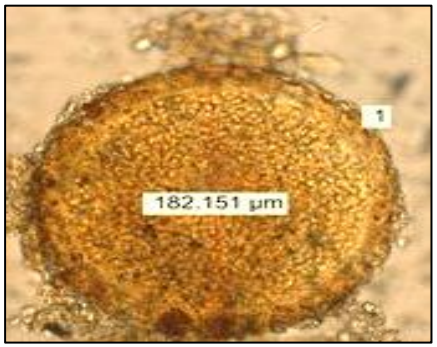
Appendix 7: Isolation Frequency (IF%), Relative Abundance (RD%) and Importance Value (IV%) of AMF in rhizosphere soils of culturally protected forest, agroforestry and cropland.

AMFspcies/Genera	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	IF	RA	IV
Acaulospora scorbiculata	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-	-	27.27	2.72	15
Acaulospora myriocarpa	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	11.11	1.21	6.16
Acaulospora sp.1	+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	+	31.82	4.53	18.18
Acaulospora sp.2	+	-	-	+	-	+	-	-	+	-	-	-	+	-	+	-	-	+	-	+	+	-	40.91	4.23	22.57
Acaulospora sp3	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	-	-	+	36.36	3.63	20
Acaulospora sp.4	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	+	-	+	+	-	+	40.91	5.14	23
Acaulospora sp.5	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	27.27	3.02	15.15
Acaulospora sp.6	-	+	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	27.27	3.93	15.6
Acaulospora sp.7	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	13.63	0.91	7.27
Acaulospora sp.8	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-	-	+	31.92	4.23	18.1
Glomus mosseae	+	-	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-	40.91	4.23	22.57
Glomus etuncatum	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	+	-	+	31.82	3.93	17.88
Glomus luteum	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	13.64	1.51	7.58
Glomus Pustulatum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	9.09	0.91	5
Glomus sp. 1	+	+	+	-	-	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	-	50	4.83	27.42
Glomus sp. 2	+	+	+	+	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-	+	+	+	59.1	7.33	33.22
Glomus sp. 3	+	-	+	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+	+	-	+	54.55	8.16	31.36
Glomus sp. 4	-	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	-	-	+	-	36.36	3.93	20.15

Glomus sp. 5	+	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	+	-	31.82	4.23	18.03
Glomus sp. 6	-	+	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	31.82	3.02	17.42
Glomus sp. 7	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	13.64	1.81	7.73
Glomus sp.8	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	18.18	1.51	9.85
Scutelospora sp.1	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	13.64	0.91	7.28
Scutelospora sp.2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	9.09	0.6	3.23
Funelliformis	+	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	27.27	3.02	15.15
Entherophospora	+	+	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	40.91	4.53	22.72
Rhizophugus clarus	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	+	-	+	27.27	2.42	14.85
Rhizophugus intraradices	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	36.36	3.32	19.84
Claroideoglomus sp.1	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	+	+	-	45.45	5.14	25.3
Claroideoglomus sp .2	+	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	31.82	3.32	15.79

Key:-1.*Catha edulis* 2. *Carissa spinarum* 3. *Eragrostis teff* 4. *Saccharum officinarum* 5. *Psidium guajava* 6. *Albizia schimperiana* 7. *Phaseolus vulgaris* 8. *Olea europaea L.* 9. *Carica papaya* 10. *Dodonae angustifolia* 11. *Gardenia volkensii* 12.*Ensete ventricotum 13. Ehretia cymosa Thonn* 14.*Acacia seyal Delil* 15. *Zea mays* 16.*Balanites aegyptiaca* 17.*Lannea schimperi* 18.*Pennisetum Sphacelatum* 19. *Capsicum fructscens* 20. *Coffea Arabica* 21.*Citrus sinensis* 22.*Cordia Africana :+ Present; - Absent*

Appendix 8: Leica DM4B microscopic view of some Glomeromycotan species identified from rhizosphere soil samples collected from agroforestry, natural forests, cash crops, and grasslands at Hawassa University College of Agriculture Laboratory. Each spore was mountedin PVLG.

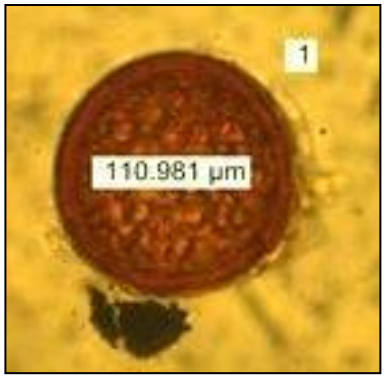


A.scorbiculat

A.sp1

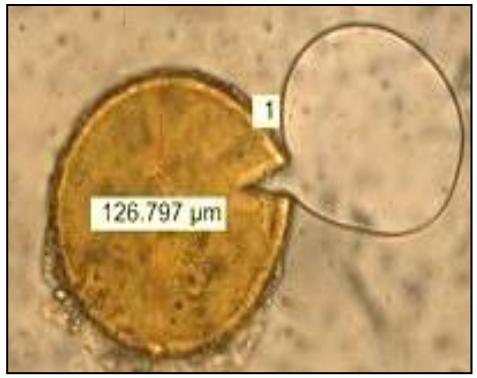
A.sp2

A.sp3



A.sp.4

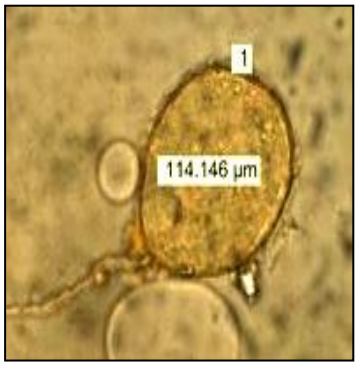
A.sp.5



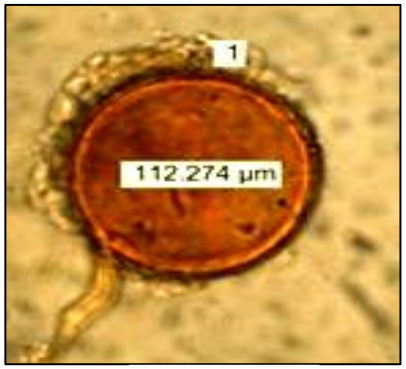
A. sp.6

A. myriocarp

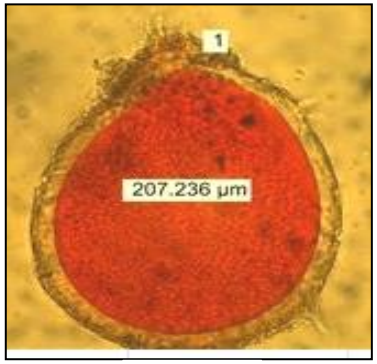
A.sp.7



G. Pustulatum



G.luteum

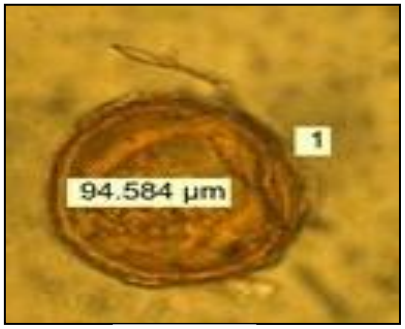


G. sp1

G.etuncatum

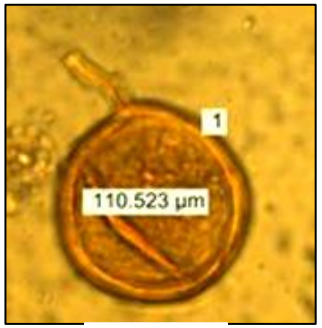
G.sp.2

G.sp.3



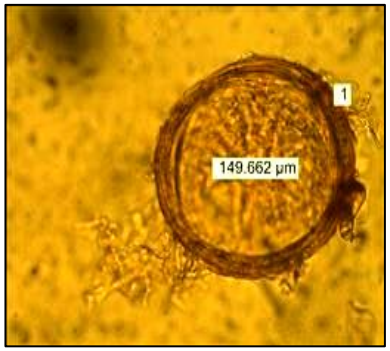
G.sp.4

G.sp.5

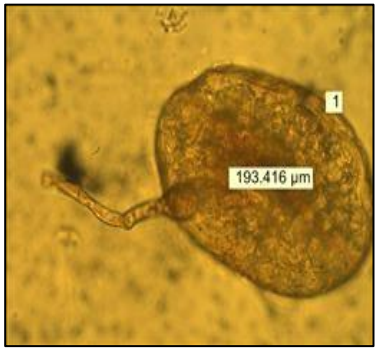


G.sp.6

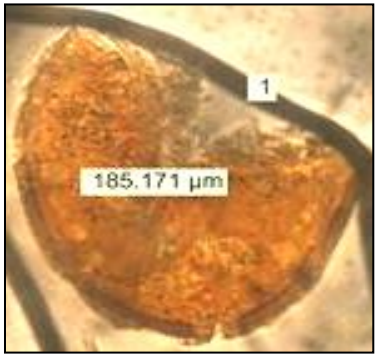
G.sp.7



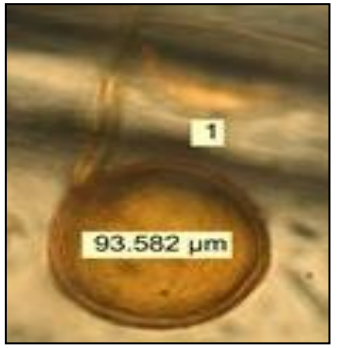
G.sp.



Scute.sp



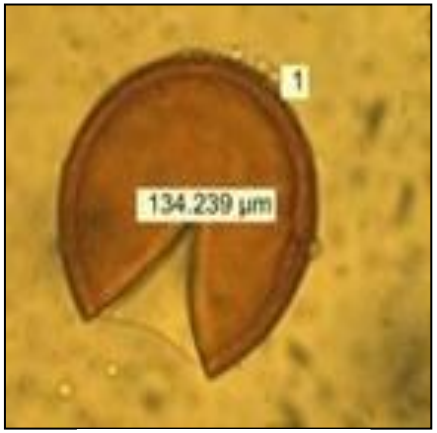
G.mosseae



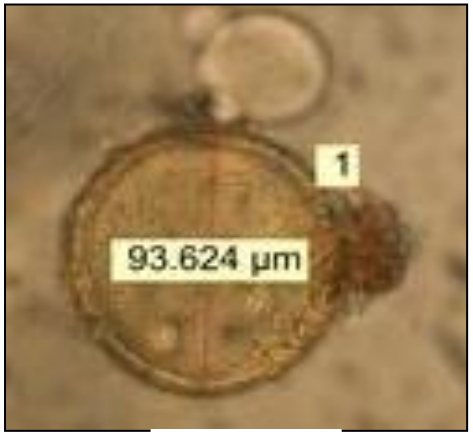
G.sp.8

A.sp8

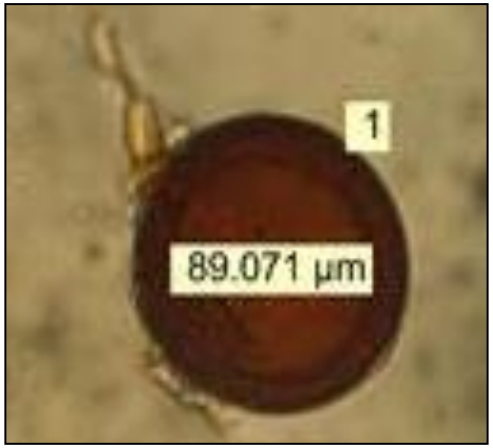
Funneloformis sp.1



Rh.interaradicies

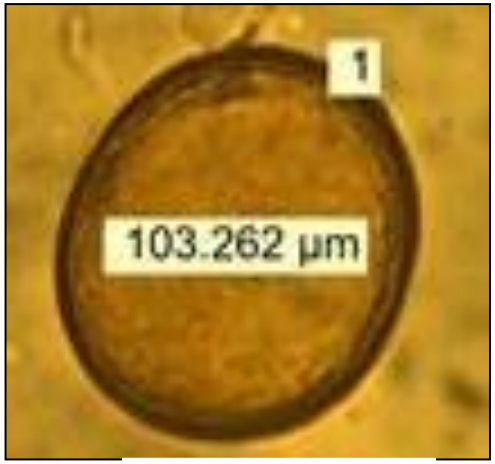


Rh. clarus



Scut.sp.1

Entrophospora sp 1



Claroideoglomus 2

Appendix 9: Images of soil Physico-chemical preparation undertaken at Hawassa University College of agriculture soil laboratory(A) soil texture fraction analysisby hydrometric method; (B)Available phosphorus extraction using UV/Visible spectrometer; (C)pH and EC by potentiometric method and EC meter; (D),(E)Total nitrogen determined by the Kjeldhal distillation method; (F), (G)and(H)Soil organic carbondetermined by the walkey black oxidation method