UNIVERSITY OF NAIROBI

ARBUSCULAR MYCORRHIZA FUNGI COMMUNITIES AND ASSOCIATED COMMON TREE SPECIES IN CHAWIA, FURURU AND NGANGAO FORESTFRAGMENTS OF THE TAITA HILLS FOREST, KENYA

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NOVEMBER 2023

DECLARATION

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged or referenced in accordance with the University of Nairobi's requirements

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DEDICATION

“This thesis is dedicated to my parents for their support throughout my studies”

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS AND ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
MIP	Mycorrhizal Infective Propagules
MPN	Most Probable Number
P	Phosphorous
K	Potassium
N	Nitrogen
ANOVA	Analysis of Variance

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are forms of symbionts that are ubiquitous in natural ecosystems. They are crucial in maintaining plant communities by enhancing uptake of nutrient and water as well as acting as major drivers of seedling recruitment and sustainability. This research study intended to (1) To evaluate arbuscular mycorrhiza fungi status of common tree species, (2) To establish diversity of arbuscular mycorrhiza species and, (3) To determine arbuscular mycorrhiza propagules infectivity potential of the forest soils in selected forest fragments in Taita Hills. In circular plots of 15m radius, data on tree species, altitudes, levels of disturbances and fine root samples of seedlings of common tree species were collected. In addition, 500g of soil samples were collected 10cm of each circular plot at 30cm depth. Two grams of fine root samples of the common tree species were stained in trypan blue for morphological assessment and two hundred milligrams of the fine roots were grounded and Arbuscular mycorrhizal DNA exacted using Zymo plant/seed DNA extraction kit. One hundred grams of soil samples were extracted using wet sieving and decantation methods and the most probable number method was used in the assessment of Mycorrhizal infective propagules. The Mycorrhizal colonization status varied across eight tree species with colonization intensity ranging between 43.33% and 90.50%. Genetic diversity of AMF colonizing common tree species revealed a predominance of *Glomus* spp. The spore density of AMF in soils differed across forest fragments. The calculated Shannon diversity index across forest fragments ranged between 1.376 and 1.504 with no variation recorded in AMF diversity across forest fragments. Mycorrizal infective propagules differed significantly across forest fragments. Positive correlation between AMF diversity and tree diversity was recorded while altitudes negatively correlated with AMF diversity. Disturbances positively associated with AMF diversity. In conclusion, the selected tree species were mycorrhizal and the diversity of AMF in the Taita hills forest was within the recommended range of AMF diversity in tropical forests.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Mycorrhizas are mutual beneficial associations where fungi are involved in nutrients and water uptake in exchange of photosynthetic carbon for growth and survival (Brundrett, 2004). The four main types of mycorrhizal associations recognized based on anatomy and partners' identity include arbuscular mycorrhiza fungi (AMF), ectomycorrhizal (EM), ericoid (Er) and orchid (Or) mycorrhizal fungi (Soudzilovsakaia *et al.,* 2020). According to Van der heijden *et al.,* 2015, eachtype of mycorrhiza associates with specific taxa of fungi. Arbuscular mycorrhizal associates with Glomeromycota; ectomycorrhizal and ericoid associate with taxa in Basidiomycota and Ascomycota respectively, whereas orchid mycorrhiza with some taxa in Basidiomycota collectively referred to as Rhizoctonia (Van der heijden *et al.,* 2015).

Arbuscular mycorrhizal fungi are the most ancient and are considered to have coevolved with terrestrial plants over 500 Million years ago (Redecker *et al.,* 2000). The AMF belong to the phylum Glomeromycota (Stürmer, 2012) categorized into three (3) classes, five (5) orders, 14 families, 29 genera and c230 species (Oehl *et al.,* 2011). Arbuscular mycorrhiza fungi form a symbiosis with approximately 90% of plant species (Soudzilovskaia *et al.,* 2020) and are crucial in ecosystem functioning (Lee *et al.,* 2013). The ecosystem services provided by AMF include conferring pest and pathogen resistance to plant communities, aiding the absorption of nutrients, (80% N, 90% P) (Marihno *et al.,* 2018), phytoremediation (Tuheteru *et al.,* 2020), improvement of soil aggregations (Soteras *et al.,* 2015) and increase in plant diversity (Zhang *et al.,* 2020). Arbuscular Mycorrhiza fungal communities are

dominant in tropical forests (Camenzind *et al.,* 2014). Tropical forests inhabit 50% of the total biodiversity globally including fungal species (Aerts *et al.,* 2011). 59% of tropical forest vegetation is dependent on arbuscular mycorrhiza fungal species (Averill *et al.,* 2014; Holste *et al.,* 2016). According to Teucher *et al.,* (2020), Kenya has diverse forest types including afromontane, rain forests, lowland and dry coastal forests. Afromontane forests are widely distributed across the African highland and are concentrated on elevation of between 1200m and 2500 meters (Abiem, 2020). The Taita Hills cloud forest fragment is the only remaining part of the original Afromontane forest in Kenya with vegetation confined to segregated mountain peaks (Teucher *et al.,* 2020). Taita Hills afromontane forest is biodiversity hotspot due to its high endemism in plant and animal species (Teucher *et al.,* 2020). Taita Hills Afromontane forest was once continuous; however, human settlement has fragmented the forests into small relics (patches) which are subjected to different anthropogenic pressure (Omoro *et al.,* 2010). Subsequently, there are distinct changes in the plant communities in Taita ecosystem. AMF communities are affected by disturbances (Soka and Ritchie, 2018) as depicted by changes in land uses and deforestation resulting to changes in forest structure (Jefwa *et al.,* 2009; Soteras *et al.,* 2015). The study aimed at investigating the level of AMF association of the selected tree species through determinationof the level of colonization, and by the use of molecular techniques to identify whether tree species have preference for AMF species. The outcome of this study has the potential applicationin restoration of degraded sites through mycorrhization of seedling prior to establishment. The mycorrhization of seedlings will guarantee subsequent survival and growth of seedlings particularly in highly degraded parts of the forest fragments for optimization of nutrient cycling ecosystem functioning.

1.2 Statement of the problem

Despite tropical forests harboring the most biodiversity globally (de Assis *et al.,* 2018), these ecosystems are subject to tremendous biodiversity loss and degradation (Hansen *et al.*, 2020). Tawaraya and Turjaman (2014) reported alarming disappearance of tropical forests at 13.5Million hectares per year, through deforestation and changes in land uses. Disturbances lead to biological loss caused by long-term effects on species diversity, community dynamics and ecosystem processes (Wekesa *et al.,* 2019). Belowground biodiversity decline with increase in anthropogenic activities (Okoth *et al.* 2009), Maina *et al. (*2016), Jefwa *et al.* (2009) and Jefwa *et al.* (2012). Anthropogenic activities have led to the fragmentation of the once intact Taita hills forest into nine smaller relics (Pellikka *et al.,* 2013).

However, the extent of anthropogenic activities on Arbuscular Mycorrhizal Fungi is not well documented (Rudolf *et al.,* 2013). Studies on AMF undertaken in tropical forests in Kenya predominantly relate to aspects of diversity and land use (Wandeto, 2014) and (Jefwa *et al.,* 2012). Whereas these studies represent AMF status, there are yet studies to be conducted on the occurrence of AMF in the different forest fragments, the AMF status of dominant tree species and the AMF species associated with the tree species. The small relics in the Taita hills are subjected to different anthropogenic pressure. In order to restore the small forest relics, additional AMF parameters need to be considered. The importance of belowground biodiversity, particularly AMF is often overlooked when planning for restoration, and yet there is clear relationship between the below ground diversity and above ground biodiversity (Birhane *et al.,* 2018).

1.3 Justification of the study

Sridhar, (2013) suggests fungal communities in biodiversity hotspot of the world as of intrinsic value requiring documentation in view of conservation strategies. Taita hills forest therefore, being a biodiversity hotspot (Teucher *et al.,* 2020) calls for attention. Additionally, fungal communities are sensitive to anthropogenic activities (Alem *et al.,* 2021). According to KNBS, (2019), the human population size in Taita hills increased from 111,000 to 340,671 between years 1969-2019. The increment in the population has resulted to reduction of the quality of forest habitat and in turn compromising the species inhabiting the forest (Teucher *et al.,* 2020). The anthropogenic pressure cause loss of fungal communities (Heilmann-Clausen*et al.,* 2015) therefore, affecting ecosystem functioning and maintenance of ecosystem resilience.

Approximately 2.2 to 3.8 million species of fungi have been described, out of which 120,000 species are currently accepted (Hawksworth and Lucking, 2017) with more species described from the temperate regions (Kinge *et al.,* 2017). In tropical forests, 228 AMF species have been documented (Marinho *et al.,* 2018). AMF provide all the four ecosystem services (Bakker *et al.,* 2019) but essentially in regulation and supporting services (Heilmann-Clausen *et al.,* 2015). Subsequently, AMF form major part of soil microbial community (Soka and Ritchie, 2018) hence important in maintaining ecosystem functioning (de Assis *et al.,* 2018). Outcome of this study will fill in the knowledge gaps and advise on restoration of degraded landscapes with reemphasis on tree-fungi interactions. This will provide paramount guidance for the development of national restoration policy in the Kenya that will guarantee subsequent survival and growth of seedlings particularly in highly

degraded parts of the forest fragments for optimization of nutrient cycling ecosystem functioning.

1.4 Objectives

1.4.1 General objective

To determine the status of Arbuscular mycorrhiza fungi in common tree species and soils of Taita Hills, Kenya.

1.4.2 Specific objectives

i. To evaluate arbuscular mycorrhiza fungi status of common tree species in the Chawia,Fururu and Ngangao forest fragments of Taita hills.

ii. To establish diversity of arbuscular mycorrhiza species in the Chawia, Fururu and Ngangao forest fragments of Taita hills.

iii. To determine arbuscular mycorrhiza propagules infectivity potential of the forest soils of Taita hills.

1.5 Study hypothesis

i. The arbuscular mycorrhiza fungi root colonization status differ across common tree species in Chawia, Fururu and Ngangao forest fragments.

ii. The diversity of Arbuscular mycorrhiza fungi communities differ in the soils of Chawia, Fururu and Ngangao forest fragments.

iii. The Mycorrhizal infective propagules are variable in Chawia, Fururu and Ngangao forest fragments.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of tropical montane forests

Montane forests are inimitable ecosystems with high diversity (Mestre *et al.,* 2017) comprising flowering plants, birds, amphibians, reptiles, mammals (Bruijnzeel *et al*., 2010) and vast AMF species (Duenas *et al.,* 2020). These forests play key roles in the provision of forest goods and services for instance timber, carbon sequestration (Martinez *et al.,* 2009) and sustaining water cycles (Bruijnzeel *et al.,* 2011). Montane tropical forests consist of non-cloudy and cloudy forests (Bruijnzeel *et al.,* 2010) occurring in altitudes of between 1500m to 2500m (Doumenge *etal.,* 1995). The montane forests account for 2.5% of the total tropical forest worldwide with the cloud forests estimated to cover 6.6% of the total montane forests (Bruijnzeel *et al.,* 2010). The increase in anthropogenic activities such as clearing for cultivation, human settlement and urbanization, deforestation, change in land uses and mining have been subjected to a decrease in forest cover (Teucher *et al.,* 2020) leading to a decline in biodiversity (Wekesa *et al.,* 2019). The remnants of cloud montane forests globally are restricted to Asia, America and Africa with 56%, 41%, and 16%, respectively (Bruijnzeel *et al.,* 2010).

2.2 Montane forests in Africa

Afromontane forests are widely distributed across African highlands (Abiem *et al*., 2020) characterized with vegetation restricted on mountain peaks (Teucher *et al.,* 2020). Afromontane forests include Ethiopian highlands, the Albertine Rift Mountains and the Eastern Arc highlands. The forests occur above 1500m in elevation. Afromontane forests

harbour distinct biodiversity with high levels of endemic species (Birhane *et al*., 2018). In Kenya, Taita Hills Afromontane Forest forms the Northern most part of the Eastern arc highlands.

2.3 Taita hills forest

Taita hills forest harbour high biological diversity (Teucher *et al.*, 2020). The unique plant diversity in the area includes 2% of plant taxa endemic to the region namely (*Psychotria crassipetala, Psychotria petitii, Meineckia ovata* and *Memecylon teitense*), 13% of plant species restricted to Kenya (*Coffea fadenii* and *Ocotea kenyensis)*. 22% of plant species are restricted to Kenya and Tanzania (Omoro *et al*., 2010; Thijs *et al*., 2014). Loss of forest cover has been highly pronounced in the region since the 1960's with Chawia and Ngangao estimated to have lost (85%) and (50%) of the forest cover (Beentje, 1988). Taita Hills forest consist of both exotic and indigenous tree species such as *Cupressus lusitanica, Pinus patula, Acacia mearnsii and Eucalyptus saligna, Macaranga conglomerata, Syzygium guineese, Tabernamontana stapfiana, Albizia gummifera, Pleiocarpa pycnantha, Phoenix reclinata, Maesa lanceo- lata, Oxyanthus speciosus, Xymalos monospora,* and *Cola greenwayi* (Omoro *et al*., 2010).

2.4 Mycorrhizal associations

Mycorrhizas are a type of interdependent association between plants and fungi (Brundrett, 2004).Four (4) main types of mycorrhizal associations are recognized based on anatomy and partners' identity (Soudzilovsakaia *et al*., 2020). They include arbuscular mycorrhiza fungi (AMF), ectomycorrhizal (EM), ericoid (Er) and orchid (Or) mycorrhizal fungi. According to Van der Heijden *et al*., (2015), each type of mycorrhiza is specific to the fungi they

associate with. For instance, Arbuscular mycorrhizal is associated with Glomeromycetes, ectomycorrhizal and Ericoid with basidiomycetes and ascomycetes while orchid mycorrhiza with basidiomycetes (Vander Heijden *et al*., 2015).

2.5 Role of Arbuscular mycorrhiza fungal association and life cycle

Arbuscular mycorrhiza fungi in the phylum Glomeromycota is the ubiquitous form of symbiosis (Schüßler, 2002; Fernandez and Kennedy, 2016; Chen *et al.*, 2018) found in natural terrestrial ecosystems, agro-ecosystems, grasslands (Shi *et al.*, 2019) and in tropical forests (Camenzind *et al.*, 2014). AMF form positive interactions (Keller *et al.*, 2011) with approximately over 80% of the plant species (Horn *et al.*, 2017) specifically the angiosperm group (Krüger *et al.*, 2017). Tropical soils are suggested to be low in phosphorus and nitrogen (Nottingham *et al.*, 2013;Belay *et al.*, 2020) and plants benefit from the nutrients (Hart *et al.*, 2003) mediated by the fungi when the roots are colonized by fungal propagules in form of hyphae, vesicles and arbuscules (Ramos-Zapata *et al.*, 2011). Subsequently, fungi gain 2-4% of the carbon from the plant. Similarly, AMF is crucial in ecosystem functions namely nutrient cycling (Bakker *et al.*, 2019), increasing plant resistance to diseases and pests (Fuchs and Haselwandter, 2008), enhancingplant nutrition and growth and, enabling forest succession through seedling regeneration and recruitment (Birhane *et al.*, 2018).

The life cycle of AMF is composed of three stages namely asymbiotic phase, presymbiotic phase and symbiotic phase (Piliarova *et al.*, 2019). In asymbiotic phase, AMF spores germinate in soils under suitable conditions spores in absence of host plant. The AMF hyphal branches extend in presence of a host plant and lastly, the hyphal interacs with the roots of plants and through the hyphae, AMF penetrates to the cortex to form AMF structures such as vesicles, arbuscules and coils (Giovannetti *et al.*, 1994).

2.6 Anthropogenic activities linked to AMF diversity loss

Diversity of Arbuscular Mycorrhizal fungal species shifts due to changes in plant diversity (Goldmann *et al.,* 2015). Deforestation cause direct loss of mycorrhizal communities and rhizosphere (Shi *et al.,* 2019). Other disturbances include changes in land uses (Jefwa *et al.,* 2012), mining and overharvesting of forest goods leading to soil erosion and infertility (Birhane *et al.,* 2018). The loss of biodiversity in tropical forests is majorly due to deforestation and change in land uses hence the decline in associated ecosystem services (Aerts *et al.,* 2011). Plant diversity are selective to AMF partners hence loss of AMF affects plants individually rather than the total diversity (Fuchs and Haselwandter, 2008) thus a shift in plant community structure (Birhane *et al.,* 2018). The diversity and distribution of AMF are highly influenced by the diversity of tree species as well as habitat conditions (Birhane *et al.,* 2020). Understanding the diversity and the level of mutualism in plant and AMF communities is paramount in the mitigation of further biodiversity loss.

2.7 Mycorrhizal infective propagules

AMF comprise different infective propagules including spores and mycelium (extraradical phase) which is highly infective and, the intraradical phase which is composed of arbuscules, vesicles and coils (Allen and Allen, 1991). The initiation of root colonization by AMF communities is dependent on the presence of propagules, environmental conditions and the competitive ability of AMF species (Solaima and Mackin, 2014). The mycorrhizal propagule includes spores, dead and live root fragments and extraradical mycelium (Solaiman and Mickan, 2014). These mycorrhizal structures are important in the establishment of a new colonization of the roots (Klironomos and Hart, 2002).

Soil infectivity is the ability of the soil fungi and bacteriato initiate infections on the roots of

host plants (Plenchette *et al.,* 1989). AMFs are critical indicators of soil health and sustainability (Klironomos and Hart, 2002). Disturbances on the ecosystem such as changes in land uses and clearing for settlement affect the fungal communities (Alem *et al.,* 2021), resulting in low mycorrhizal infectivity in forest soils, thus affecting the quality of soil (Jasper *et al.,* 1991; Gadzag *et al.,* 2019). Subsequently, the presence of the infective propagules is essential in forest recruitment of plant diversity for conservation and restoration purposes. Understanding the spatial variation in the ability of mycorrhizal propagules to form associations in natural habitats of Chawia, Ngangao and Fururu fragments was therefore crucial.

2.8 Molecular and morphological identification of AMF communities

AMF communities have been traditionally identified morphologically to the family level by the use of spore characters (size, type of hyphae and the number of germination walls) and intraradical structures (Redecker, 2003) such as vesicles and hyphae. However, this approach has several shortcomings that led to the adoption of molecular markers in AMF taxonomy (Wubet *et al.,* 2004). The emergence of several lineages of AMF that could not detect staining procedures coupled with parasitized spores from the field made morphological identification difficult (Redecker, 2003).

The small subunit ribosomal RNA gene is used for AMF-specific primers since it allows for adequate resolution in identification to the species level (Lee *et al*., 2008). Additionally, the development of AMF-specific primers and proper protocols has enabled the identification of AMF communities within the roots of plant species collected from the field (Young, 2012). Molecular markers have also facilitated the understanding of genetic diversity as well as ecosystem functioning (Young, 2012).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Site description

The study was conducted in three indigenous forest fragments (Ngangao, Chawia and Fururu) forming the Dabida massif (Thijs *et al.*, 2014) of the Taita Hills cloud forest. Ngangao relic is located at 030 21'S, 380 20'E, Chawia 030 28'S, 380 20'E and Fururu 030 25'S, 380 20'E (Figure 3.1). The sites lie at altitudes of 1750-1900, 1500-1600 and 1650-1750, respectively. The forest remnants receive an average rainfall of 2000mm which follow a bimodal pattern with long rains experienced in (March-May) and short rains (November-December). The forest patches differ in the level of disturbances ranging from low to high. A high level of disturbances is reported in the Chawia fragment (Omoro *et al*. (2010). Ngangao and Fururu patches are gazetted and managed by the Kenya Forest Service (Kenya Gazette supplement, 2016). However, Fururu have moderate disturbances (Teucher et al., 2020) and Ngangao has low levels of disturbances (Thijs *et al*., 2014). The three forest fragments cover 120 ha, 86 ha and 5 ha, respectively and are surrounded by high population pressure relying on subsistence agriculture threatening theforest's existence (Teucher *et al.,* 2020). The mean annual temperatures in Taita Hills range from 16 to 18 °C. The soils are Humic Nitisols (Jefwa *et al.,* 2012) with deep and well drainage. Among the dominant indigenous tree species are *Phoenix reclinata, Syzigium guinense, Macaranga conglomerata, Albizia gummifera, Strombosia schefffleri, Oxyanthus speciosus, Xymalos monospora, Pleiocarpapycnantha* and *Tabernaemontana stapfiana*.

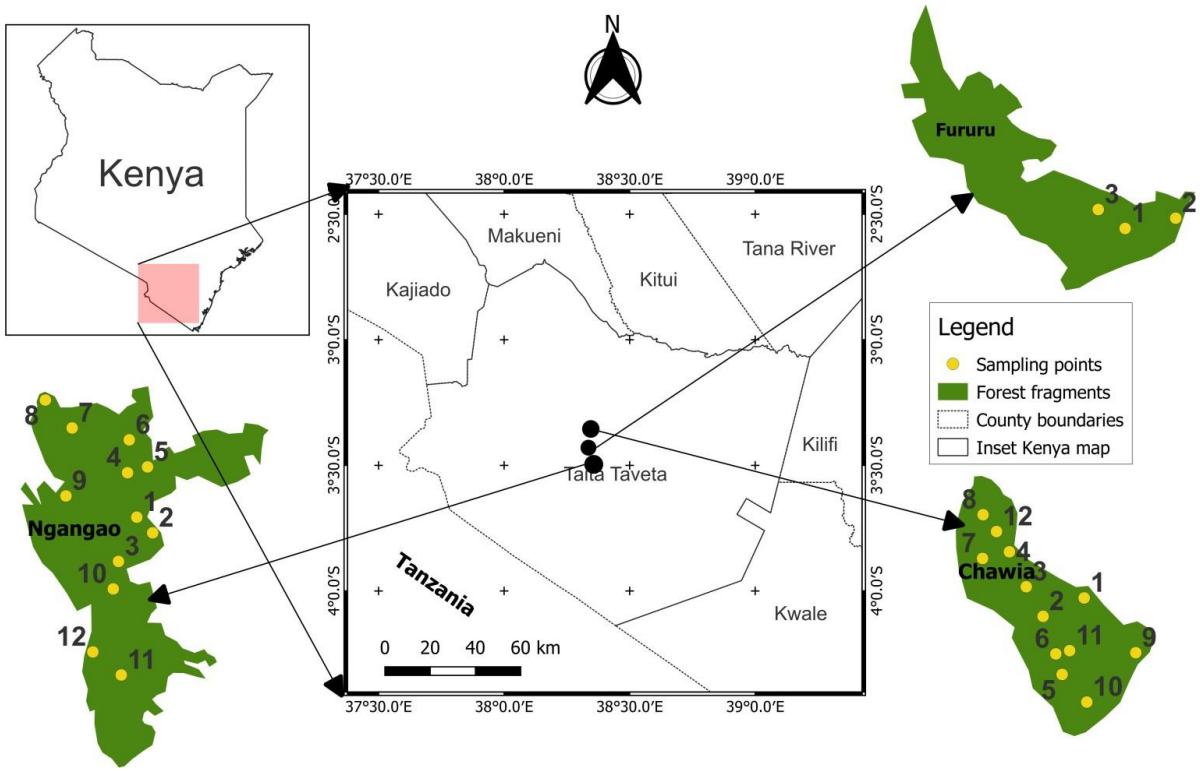

Figure 3.1 Map of selected forest fragments (Generated in QGIS software)

3.2 Study design

This study was conducted in September 2021, and the sites were selected based on altitudinal differences and level of disturbances. Circular plots of 15 meters radius were made with 300m intervals apart, and a 100m buffer was left at the forest edge to reduce edge effects. A 5m buffer was left within the 15m circular plots for the collection ofsoil samples. A total of 27 circular plots of 15m radius were randomly made across the three indigenous fragments. Three plots were made in Fururu and twelve plots each in chawia and Ngangao forest fragments. The sampling strategy considered the accessibility of the forest relics.

3.3 Data Collection

3.3.1 Records on tree diversity, altitudes and disturbances across selected forest fragments

All tree species were counted in the 27 circular plots of 15 meters across the forest fragments. Altitudes were recorded using Georeferencing Positioning System (GPS) and disturbances such as forest trails, tree stumps and exotic plant species were observed and countedper plot. The degree of disturbances was categorized into four groups. Where less than five (<5) features were recorded, the levels of disturbanes was assigned as low, observations ranging between five and ten (5-10) were classified as moderate and, features that were greater than ten (>10) were categorized as intense.

3.3.2 Status of arbuscular mycorrhiza fungi of eight common tree species across the forest fragments

With the help of a plant taxonomist, root samples from the seedlings of eight common and abundant tree species across forest fragments were predetermined on encounter either away or close to the mother tree species. Where seedlings were clustered, random sampling was done. The common tree species included *Pleiocarpa pycnantha, Xymalos monospora, Syzigium guineense, Oxyanthus speciosus, Tabernaemontana stapfiana, Macaranga conglomerata, Leptonychia usambarensis* and *Phoenix reclinata.* Upon encounter of targeted seeding, a block was created around the seedlings and he roots excavated using a clean hoe where the roots were deep while a shovel were for close to the rhizosphere.. Approximately two grams of less than 0.5mm fine roots were sampled from seedlings of each tree species. To avoid cross-contamination, the fine roots were cut using a clean scalpel blade and stored in separate bags. For morphological analysis, two grams of cut fine roots were preserved in 50ml glass bottles filled with 50% absolute ethanol for mycorrhizal characterization status at the National Museums of Kenya, Mycology laboratory. Subsequently, about one gram of the fine roots of seedlings for each tree species was stored in small envelopes and preserved in silica gel for molecular analysis at Nairobi University, Geneticslaboratory.

3.3.3 Morphological assessment; root staining and assessment

Roots were stained using a modified technique (Koske and Gemma, 1989). The fine roots from different tree species were washed thoroughly in tap water to remove the 50% ethanol used in preservation and cut into segments of 1cm. Clearing was done by placing fine segments in 10% aqueous Potassium hydroxide (KOH) and autoclaved at 1210 for 5 minutes. The cleared roots were then rinsed and bleached in alkaline Hydrogen peroxide (3ml, 30% Ammonia solution, in 10ml, 30%, Hydrogen peroxide) for 2 hours, a modification for roots of tree species. Thereafter, the roots were rinsed again and acidified in 1% Hydrochloric acid (HCl), stained using 0.25g trypan blue in acidic glycerol solution (250m Glycerol, 25ml 1% HCl and 527ml disilled water), and autoclaved for 3minutes. After the staining stage, the roots were de-stained in acidic glycerol and 30 pieces of 1cm fragments of each tree species were mounted on microscopic slides.

The presence of mycorrhizal features including extra-intraradical hyphae, vesicles, arbuscules and coils was assessed under x40 Olympus light microscope for qualitative and quantitative purposes. The percentage of root colonization was calculated as the number of root fragments colonized by AM fungi divided by the overall number of root fragments assessed multiplied by 100. The colonization features were estimated as the total percentage area occurrence of a feature, i.e. arbuscules, out of all the root fragments assessed on a slide.

3.3.4 Molecular characterization of AMF species colonizing roots of common tree species across fragments

3.3.4.1 DNA extraction

One gram of the fine roots of the selected common tree species was weighed and ground inliquid nitrogen using a mortar and pestle (Goswami *et al.,* 2018). The DNA was extracted using Quick- DNA plant/seed Miniprep extraction kit (ZYMO RESEARCH, USA) following the manufacturer's protocol for the isolation of DNA from plant tissues. However, two elution steps with 100 µl of elution buffer were used. The DNA was stored at –20° C for further analysis.

3.3.4.2 Nested PCR amplication of small subunit rDNA

Partial small subunit rDNA fragments were amplified using nested PCR with the universal eukaryotic primers NS1 (5'GTA GTC ATA TGC TTG TCT C-3') and NS4 (5'CTT CCG TCA ATT CCT TTA AG-3') (White *et al.,* 1990). DNA was amplified in 25 µl reaction volume composed of 8.5 µl of nuclease-free water, 12.5 µl of Go Taq G2 Hot Start Green Master Mix, 0.5 µl of 25mM Magnesium Chloride, 0.5 µl of forward and reverse primers and 2 µl of DNA template. Gene Amp PCR System 2400 (Perkin-Elmer Corporation, Norwark, CT, USA) was used for PCRs as described by Redecker (2000). Thermocycler conditions consisted of an initial denaturation of 3 minutes at 95 ºC, 20 seconds at 94 ºC, 35 seconds at 44 ºC, 1 minute at 72 ºC and 10 minutes at 72 ºC for 35 cycles. Thereafter, the first PCR products were used as a templatefor the second PCR amplification.

Second PCR step

The second step of PCR was to increase the specificity of the amplification reaction. This step was conducted using a combination of Glomales- specific primers (White *et al.,* 1990) with universal primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and ITS4R (CAG ACT T (G/A)TA(C/T)ATG GTC CAG). Specific primers were combined in three sets (Redecker, 2003). Set1 was composed of GLOM 1310F (AGC TAG GYC TAA CAT TGT TA) and GLOM 5.8R (TCC GTT GTT GAA AGT GAT C); the reverse specific primers (GLOM5.8R, GIGA5.8R (ACT GAC CCT CAA GCA KGTG) in combination with ITS1F and; the forward specific primers ACAU1660F (TGA GAC TCG GAT CGG), LETC1670F (GAT CGG CGA TCG GTG AGT) combined with ITS4R. The annealing temperature for (GLOM 1310F, GLOM 5.8R), (GIGA5.8R, ITS1F), (ACAU1660F, ITS4R) and (LETC1670F. ITS4R) was 44^0C, 49^0C, 43^0C and 45^0C, respectively. The PCR products were analyzed by electrophoresis on a 1% agarose gel in Tri-acetate EDTA (TAE) buffer pre-stained with Ethidium bromide solution, and viewed using 2UV Transilluminator (Model LM-20E, UVP Upland, CA 91786 USA). Thermo Scientific 1kb Gene ruler was used in the verification of the band sizes.

3.3.4.3 Sequencing arbuscular mycorrhizal fungi DNA

Integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye and PCR products purified using ExoSAP Protocol following the manufacturers' guidelines. The purified products were injected into an Applied Biosystems ABI 3500XL Genetic Analyser or Applied Biosystems ABI 3730XL Genetic Analyser with a 50cm array, using POP7 and fragments sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions.

3.4 Diversity of arbuscular mycorrhizal species in the forest fragments

3.4.1 Soil collection

Soils were collected towards the beginning of short rains from the three forest fragments. The soils were sampled from the rhizosphere of 27 circular plots at four different points of the 2/3m of the 15m radius circular plot using a soil auger at depths of 30 cm using a tranverse method (https://eoai-africa.org/wp-content/uploads/2020/03/BioVision-Soil-Sampling-Poster.pdf). A total of four soil samples were collected per plot and composited to form one sample which accounted for the 27 soil samples collected across forest fragments. The soil samples were packaged in sealable bags for further laboratory assessment of Arbuscular mycorrhizal spores.

3.4.2 Soil extraction

AMF spores were extracted using the wet sieving and decanting method followed by the sucrose centrifugation technique (Gerdemann and Nicolson, 1963). A total of one hundred grams of air- dried soil was mixed in 500ml of water in a 1000ml beaker and allowed to settle for 10 minutes. The soil mixture was passed through 710 and 45 micrometers mesh sieves and the liquid mixture was centrifuged for 5 minutes at 1750 rotations per minute (rpm). The supernatant was gently discarded. Subsequently, the spores were re-suspended in 50% sucrose solution and subjected to centrifugation for one minute at 1750 rpm. The supernatantwas quickly poured on 45micrometers sieves and carefully rinsed with tap water and the residue on the sieve was washed into a petri dish for observation under a dissecting microscope.

3.4.3 AMF species assessment

Arbuscular mycorrhizal spores were enumerated and categorized into morphotypes under a dissecting microscope. Diagnostic features such as spore colour, size, hyphal attachments and surface appearance (Brundrett *et al*., 1996) were recorded. The spore colour was determined by a colour chart (Edinburgh, 1969) and voucher specimen prepared by mounting each morphotype using Polyvinyl lactic acid glycerine (PVLG) and Melzer +PVLG reagents on microscopic slides. The AMF species were identified under the Olympus compound microscope using germination characteristics.

3.5 To determine the arbuscular mycorrhiza propagules infectivity potential of the forest soils

3.5.1 Most probable number experiment

The Most Probable Number (MPN) method proposed by Porter (1979) was used for the estimation of the number of infective propagules in the forest soils. The composite soil samples were passed through the 2mm sieve. To determine the infectivity of propagules, a fourfold dilution series with five replicates each and five dilutions were prepared in 50ml pots. The 100 was the original forest soil while 10^1 to 10^4 were diluted with sterile soil (sand). The diluent (sand) was autoclaved at 121^0C for 1 hour. A highly mycotrophic plant (*Sorghum bicolor*) was used as a host. Three seeds were planted on each pot to prevent cross-root infections. The plants in the greenhouse were watered three days a week and plants were harvested six (6) weeks after planting. The harvested roots were stained using the method proposed by Koske and Gemma (1989). 1cm root fragments were mounted on slides using polyvinyl alcohol-lactic acid-glycerol (PVLG), and the mycorrhizal colonizationwas observed under the Olympus light compound microscope.

3.6 Phylogenetic analyses

Low quality chromatograms from the submitted sequences were edited and trimmed using Chromas software. The incorrect base calls were edited after alignment and consensus sequences were created using the Bioedit software. A search for similar sequences using BLASTN (Altschul *et al.,* 1990) was performed, and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org) against the nearest neighbours. The evolutionary history was inferred using the Neighbor-Joining method

(Saitou and Nei, 1987) combined with a bootstrap test from 1000 replicates (Felsenstein, 1985). The evolutionarydistances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969) and sequences were treated with a similarity value of ≥ 95% of partial small subunit rDNA. The analyses included 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions for each sequence pair were removed by the pairwise deletion option. A total of 1889 positions were recovered in the final dataset. *Batrachochytrium dendrobatidis* was used as an out-group and a phylogenetic tree was generated using MEGA11 (Tamura *et al*., 2021).

3.7 Data analysis

Data were tested for normality using the Shapiro test. Data on spore abundance and AMF speciesabundance was log-transformed to assume normality using the log1p function in the R programme for statistical analysis (R Development Core Team, 2022). Comparison of dilution factor on infective propagules, spore density and colonization intensity of mycorrhizal features of sorghum from the MPN experiment and tree species across forest fragments and were analyzed using one-way Analysis of Variances in R software.

Two-way ANOVA was performed for analysis of the interaction of forest fragments and abundance of AMF species and, common tree species. Shannon diversity index was calculated using the below formula; H'= - P In Pi; where P i= is the relative abundance of each identified species per sampling site and calculated by the following formula, P= n i ; where n is the spore numbers of a species and N is the total number of identified species per sampling sites was used to calculate the diversity of both tree species and AMF communities. Additionally, correlation analysis was performed to assess the relationship

between AMF communities to tree species diversity, altitudes and disturbances. Correlation analysis was interpreted using methods by (Chan, 2003). All the tests of statistical significance were decided at $P < 0.05$ confidence level.

CHAPTER FOUR: RESULTS

4.1 Diversity of tree species across the selected three forest fragments in Taita hills forest

Ngangao fragment recorded the highest diversity of tree species, followed by Chawia and Fururu forest fragments with means of 1.926600, 1.692772 and 1.218358, respectively (Table 4.1). Significant difference in tree diversity was recorded across forest fragments ($p < 0.05$).

Table 4.1 Diversity of tree species in the selected forest fragments in Taita hills

Forest fragments	Shannon diversity index
Ngangao	1.926600[a]
Chawia	1.692772[ab]
Fururu	1.218358[b]
P-value	0.0264 *
±SE	0.431

Means with the same letter are not significantly different at alpha=0.05

Intense level of disturbances was only recorded in two plots in Chawia forest fragment. Moderate level of disturbance was recorded one plot each in Ngangao and Fururu fragments and; four plots in Chawia forest fragments. Low levels of disturbances were observed in five plots each in both Ngangao and Chawia fragments. Disturbances were not recorded in six and one plots in Chawia and fururu fores fragments (Table 4.2).

Table 4.2 Levels of disturbances recorded across the selected forest fragments in Taita hills

	Disturbance Intensity			
	None (0)	**Low (1)**	**Moderate (2)**	**Intense (3)**
Forest fragments				
Chawia	1	5	4	2
Ngangao	6	5	1	0
Fururu	0	2	1	0

4.2 Mycorrhizal colonization intensity in selected tree species across selected forest fragments in Taita hills forest

Mycorrhizal colonization intensity of the selected common tree species was significantly different ($P < 0.05$) (Table 4.3). *Macaranga conglomerata* recorded the highest percentage colonization intensity across forest fragments and least colonization intensity recorded in *Pleiocarpa pycnantha*. *Macaranga conglomerata, Leptonychia usambarensis* and *Oxyanthus speciosus* were significantly different from *Syzigium guineense*, *Xymalos monospora*, *Tabernaemontana stapfiana*, *Phoenix reclinata* and *Pleiocarpa pycnantha*.

Table 4.3 Mycorrhizal colonization intensity across selected common tree species

Tree species	Plant families	Mean Col/intensity (%)
Macaranga conglomerata	Euphorbiaceae	75.62[a]
Leptonychia usambarensis	Malvaceae	69.53[ab]
Oxyanthus speciosus	Rubiaceae	69.25[ab]
Syzigium guineense	Myrtaceae	56.75[bc]
Xymalos monospora	Monomiaceae	55.73[bc]
Tabernaemontana stapfiana	Apocynaceae	53.07[bc]
Phoenix reclinata	Arecaceae	52.22[bc]
Pleiocarpa pycnantha	Apocynaceae	48.33[c]
P-value		0.0445*

Means with the same letter are not significantly different at alpha=0.05

The colonization intensity of selected tree species in the Ngangao forest fragment was not significantly different (Table 4.4). *Leptonychia usambarensis* recorded the highest colonization intensity, followed by *Phoenix reclinata*. Mycorrhizal colonization intensity of selected common tree species in the Chawia forest fragment was not significantly different. However, *Macaranga conglomerata* differed significantly with *Xymalos monospora*, *Syzigium guineense* and *Pleiocarpa pycnantha*. *Macaranga conglomerata* had the highest colonization intensity. Significant difference was not recorded in the selected common trees species in Fururu forest fragment. *Oxyanthus speciosus* was highly colonized followed by *Macaranga conglomerata*. *Oxyanthus speciosus* was significantly different from *Phoenix reclinata* and *Pleiocarpa pycnantha*.

Table 4.4 Mycorrhizal colonization intensity of common tree species in Ngangao, Chawia and Fururu forest fragment in Taita hills

Tree species	Ngangao	Chawia	Fururu
Leptonychia usambarensis	68.62[a]	64.95[ab]	75[ab]
Phoenix reclinata	62.77[a]	46.23[b]	49.16[b]
Oxyanthus speciosus	61.23[a]	62.78[ab]	83.7[a]
Macaranga conglomerata	59.8[a]	90.50[a]	76.54[ab]
Syzigium guineense	56.94[a]	54.72[ab]	58.61[ab]
Tabernaemontana stapfiana	52.03[a]	55.24[ab]	51.94[ab]
Pleiocarpa pycnantha	48.88[a]	47.23[b]	48.88[b]
Xymalos monospora	43.33[a]	48.01[b]	75.84[ab]
P value	0.7958*	0.239*	0. 182*

Means with the same letter are not significantly different at alpha=0.05

All eight tree species had mycorrhizal features of AMF colonization. All AMF features occurred in *Leptonychia usambarensis, Xymalos monospora and Oxyanthus speciosus* (Table 4.5). Hyphae and appressoria were present in all tree species. The occurrence of arbuscules, vesicles and coils varied across tree species and forest fragments. The hyphal coils were recorded least among all the features, with occurence noted in *Oxyanthus speciosus, Xymalos monospora* and *Syzigium guineense* in Fururu and, *Leptonychia usambarensis* in Ngangao forest fragments. Arbuscules were recorded in *Leptonychia usambarensis, Xymalos monospora, Pleiocarpa pycnantha* and *Tabernaemontana stapfiana*. *Phoenix reclinata* and *Pleiocarpa pycnantha* were the only tree species with no vesicles. *Tabernaemontana stapfiana*, *Leptonychia usambarensis* and *Syzigium guineense* expressed vesicles irrespective of forest fragments. Arbuscules and vesicles were common in

Macaranga conglomerata and *Leptonychia usambarensis* in Ngangao, *Tabernaemontana stapfiana* in Chawia and, *Xymalos monospora* and *Leptonychia usambarensis* in Fururu forest fragments.

Table 4.5 Mycorrhizal features of common tree species across selected Taita forest fragments

Tree species	Forest fragment	Arbuscules	Vesicles	Hyphae	Appresoria	Coils
Macaranga conglomerata	Chawia	-	-	+	+	-
Macaranga conglomerata	Ngangao	+	+	+	+	-
Macaranga conglomerata	Fururu	-	+	+	+	-
Xymalos monospora	Ngangao	+	-	+	+	-
Xymalos monospora	Chawia	+	-	+	+	-
Xymalos monospora	Fururu	+	+	+	+	+
Oxyanthus speciosus	Ngangao	-	+	+	+	-
Oxyanthus speciosus	Chawia	-	-	+	+	-
Oxyanthus speciosus	Fururu	-	-	+	+	+
Syzigium guineense	Chawia	-	+	+	+	-
Syzigium guineense	Fururu	-	+	+	+	+
Syzigium guineense	Ngangao	-	+	+	+	-
Leptonychia usambarensis	Chawia	-	+	+	+	-
Leptonychia usambarensis	Ngangao	-	+	+	+	+
Leptonychia usambarensis	Fururu	+	+	+	+	-
Phoenix reclinata	Chawia	-	-	+	+	-
Phoenix reclinata	Fururu	-	-	+	+	-
Phoenix reclinata	Ngangao	-	-	+	+	-
Tabernaemontana stapfiana	Chawia	+	+	+	+	-
Tabernaemontana stapfiana	Fururu	-	+	+	+	-
Tabernaemontana stapfiana	Ngangao	-	+	+	+	-
Pleiocarpa pycnantha	Chawia	+		+	+	-
Pleiocarpa pycnantha	Ngangao	-	-	+	+	-
Pleiocarpa pycnantha	Fururu	-	-	+	+	-

There was no significant difference across single selected common tree species across the selected forest fragments in Taita hills. *Macaranga conglomerata* recorded the highest colonization intensity in the Chawia followed by Fururu and Ngangao forest fragments (Table 4.6). *Leptonychia usambarensis* was highly colonized in Fururu followed by Ngangao and Chawia forest fragments. *Oxyanthus speciosus* recorded the highest in Fururu followed by Chawia and Ngangao forest fragments.

Table 4.6 Comparison of mycorrhizal colonization intensity of selected tree species across forest fragments in Taita hills

Forest fragments	Tree species	Col/ Intensity %
Chawia	*Macaranga conglomerata*	90.50[a]
Fururu	*Macaranga conglomerata*	76.54[a]
Ngangao	*Macaranga conglomerata*	59.81[a]
P-value		0.238
SE		7.19
Fururu	*Oxyanthus speciosus*	83.75[a]
Chawia	*Oxyanthus speciosus*	62.77[a]
Ngangao	*Oxyanthus speciosus*	61.23[a]
P-value		0.276
SE		5.54
Fururu	*Xymalos monospora*	75.84[a]
Chawia	*Xymalos monospora*	48.01[a]
Ngangao	*Xymalos monospora*	43.33[a]
P-value		0.208
SE		7.95
Fururu	*Leptonychia usambarensis*	75.00[a]
Ngangao	*Leptonychia usambarensis*	68.62[a]
Chawia	*Leptonychia usambarensis*	64.95[a]

P-value		0.752
SE		2.82
Ngangao	*Phoenix reclinata*	62.77[a]
Fururu	*Phoenix reclinata*	49.16[a]
Chawia	*Phoenix reclinata*	46.23[a]
P-value		0.639
SE		2.82
Fururu	*Syzigium guineense*	58.61[a]
Ngangao	*Syzigium guineense*	56.94[a]
Chawia	*Syzigium guineense*	54.72[a]
P-value		0.969
SE		5.54
Chawia	*Tabernaemontana stapfiana*	55.24[a]
Ngangao	*Tabernaemontana stapfiana*	52.03[a]
Fururu	*Tabernaemontana stapfiana*	51.94[a]
P-value		0.978
SE		2.82
Fururu	*Pleiocarpa pycnantha*	48.88[a]
Ngangao	*Pleiocarpa pycnantha*	48.88[a]
Chawia	*Pleiocarpa pycnantha*	47.22[a]
P-value		0.978
SE		2.82

Means with the same letter are not significantly different at alpha=0.05

4.2.1 Mycorrhizal features expressed by tree species

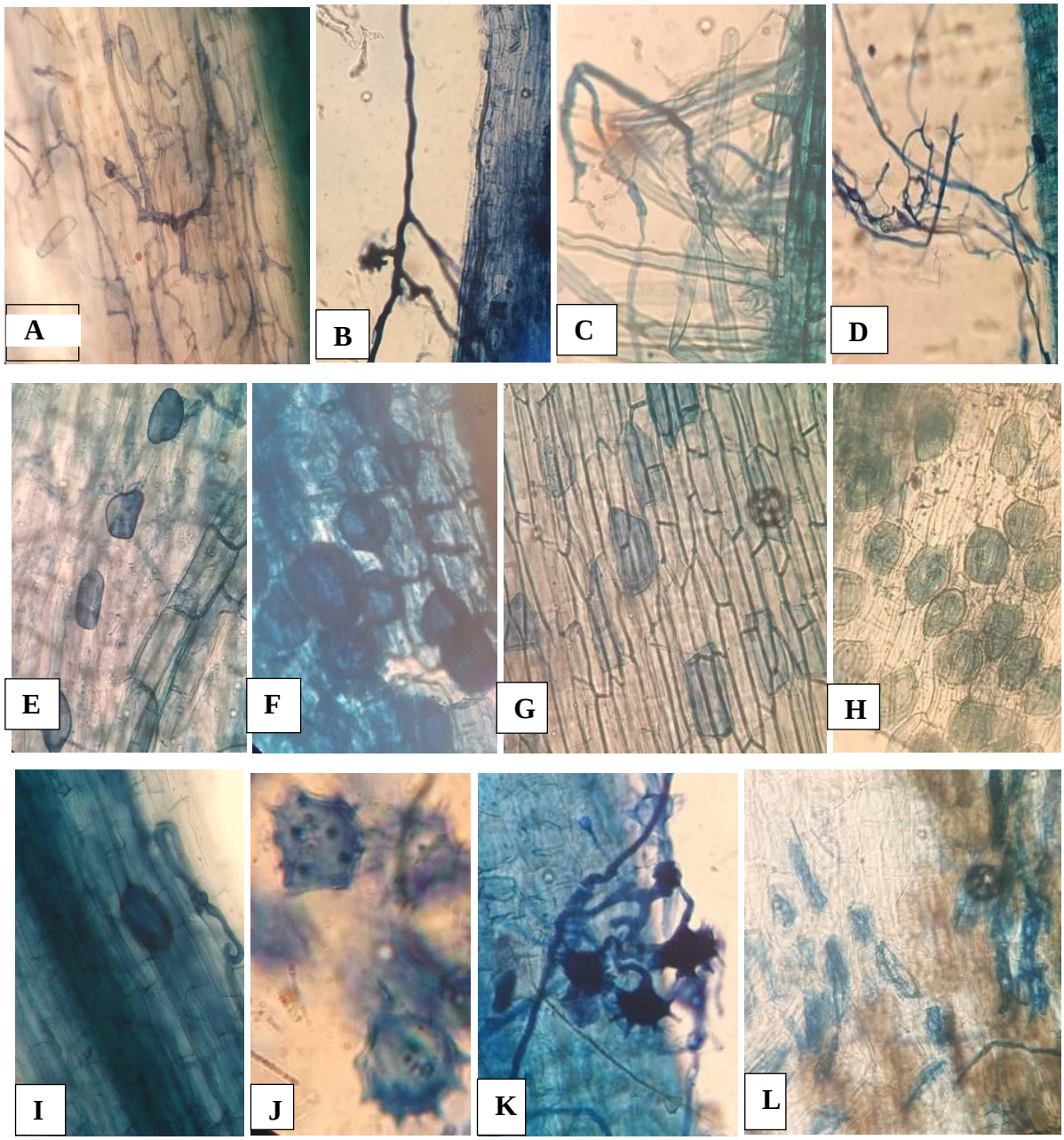

Plate 4.1 Arbuscular mycorrhiza features

Image A: Intraradical hyphae, **Image B-D:** Extraradical hyphae and entry points, **Image E-H:** vesicles,

Image I: hyphal coils, **Image J-K:** spiny auxillary cells, **Image L:** arbuscules

4.3 Molecular characterization of AMF Species colonizing common tree species

4.3.1 PCR amplification

PCR amplification of the total genomic DNA of three of the eight species, *Xymalos monospora(4,9,14)* across fragments, *Pleiocarpa pycnantha(1,20)* in Chawia and Ngangao and; *Phoenix reclinata (11)* in Fururu fragments was successful and yielded amplification products of approximately 1100bp (Plate 1). This was visualized on a 1.0% agarose gel. Bands sizes of ~ 710bp were observed for Genera Acaulospora, Gigaspora, Glomus and Entrophospora (Plate 2). The numbers represents; 4-*Xymalos monospora* in Chawia, 9-*Xymalos monospora* in Fururu, 14- *Xymalos monospora* in Ngangao, 1-*Pleiocarpa pycnantha* in Chawia, 20- *Pleiocarpa pycnantha* in Ngangao and 11- *Phoenix reclinata* in Fururu forest fragment.

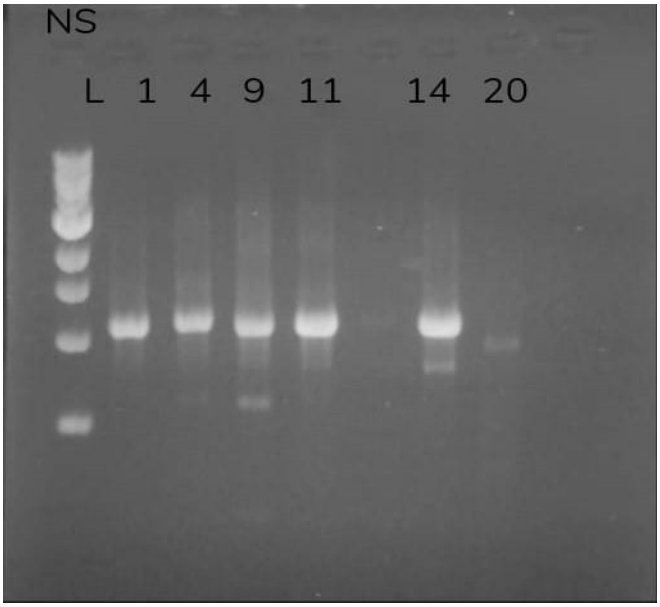

Plate 4.2 1st PCR productNS1/NS4 PRIMER (1100bp)

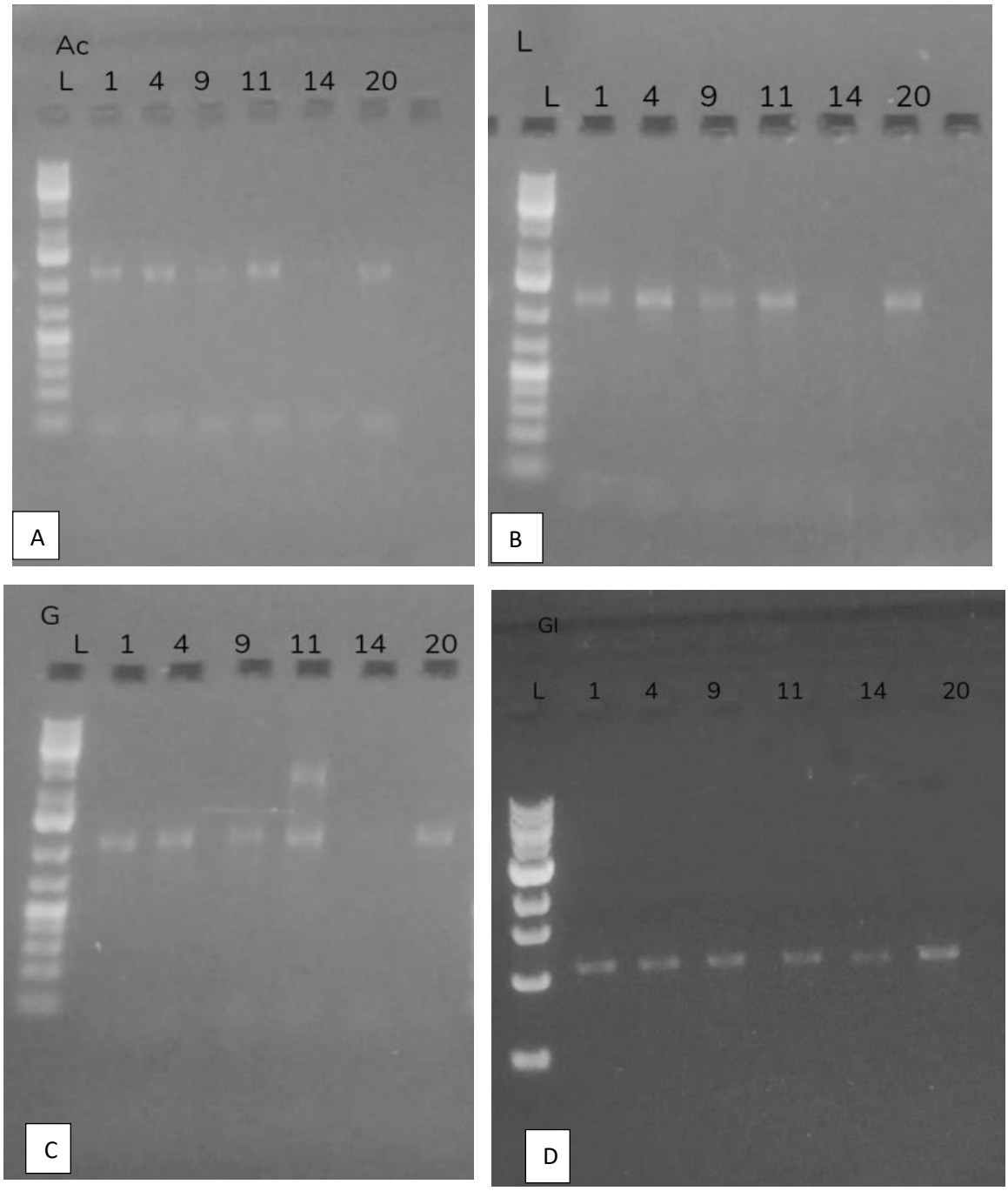

Plate 4.3 2nd PCR product

A-ACAU1660R/ITS1F,B-LECT1670F/ITS4R,C-GIGA5.8R/ITS4R,D GLOM130F/GLOM5.8R

A total of fifteen (15) AMF species were obtained from the phylogenetic tree from the roots of Xymalos monospora and Pleiocarpa pycnantha. Xymalos monospora in Chawia and Fururu forest fragments were colonized by similar AMF species. The AMF species were *Gigaspora margarita*, uncultured Glomus sp1 (AM384970), uncultured Glomus sp2 (AM384968), uncultured Glomus sp3 (AM384971), uncultured Glomeraceae genomic DNA (HF674801) and uncultured Glomeraceae 18S rRNA gene (HE775305). Additionally, the sequence of one sample from GIGA 5.8R/ 1TS1F did not show any similarities with the existing sequences deposited in NCBI Genbank. AMF species colonizing *Pleiocarpa pycnantha* were uncultured Glomus sp1 (EU417649), Glomus sp2 (EU417648), Glomerocycotina species (MG829384), uncultured Glomeraceae genomic DNA sp1 (HF674803), uncultured Glomeraceae genomic DNA sp2 (HF674802) and, three species of uncultured mycorrhizal fungi gene (Figure 4.1). The AMF species colonizing *Phoenix reclinata* had no similarity with the AMF sequences deposited in the NCBI Genbank from Sequence reads for primers GIGA5.8R/1TS1F, GLOM 1310F/GLOM5.8R and LECT1670F/1TS4R. Although bands were observed from the three tree species in ACAU1660F/ITS4R, no sequences were found in Sanger sequencing.

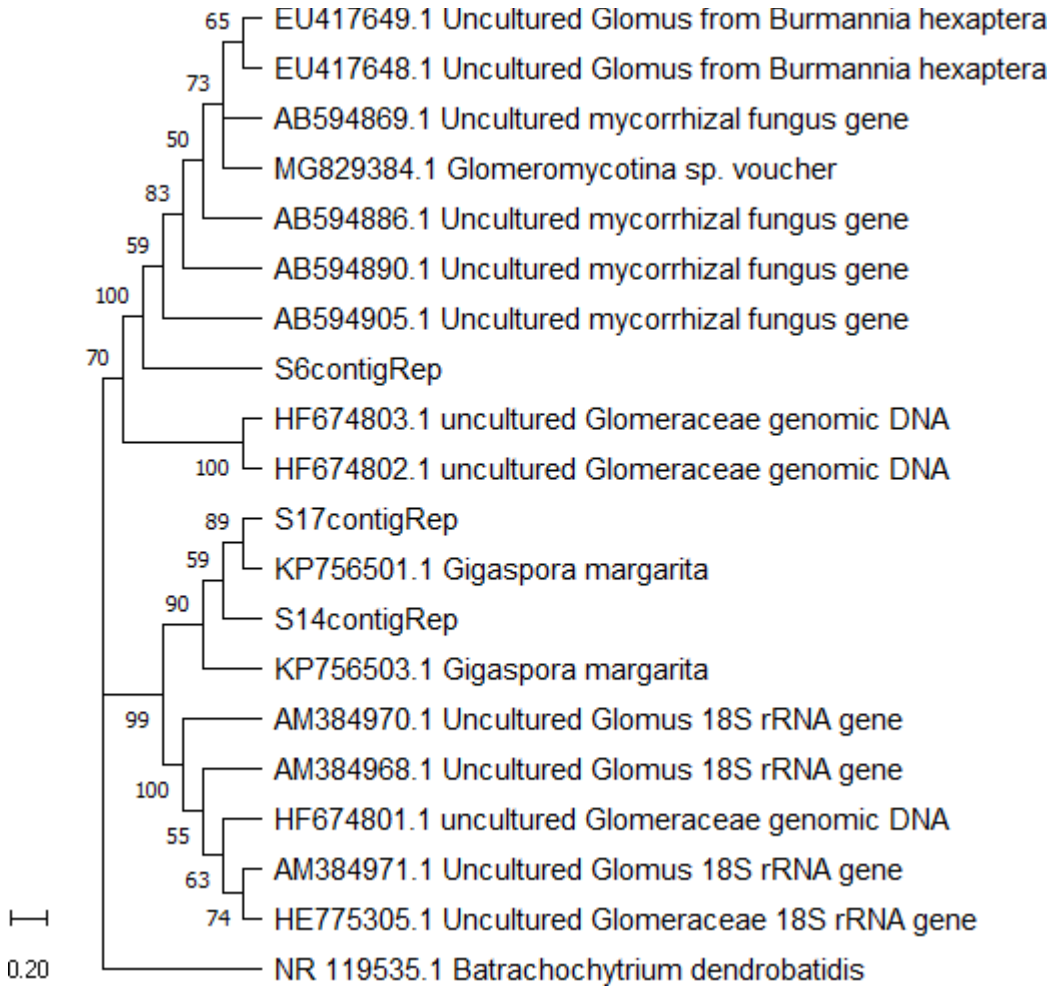

Figure 4.1 Phylogenetic tree generated using MEGA 11 by Neighbor-Joining methodshowing sequences of SSU rDNA of AMF from roots of Xymalos monospora (S14, S17) and Pleiocarpa pycnantha (S6)

4.4 Arbuscular mycorrhiza spores abundance

Spore density decreased with an increase in altitude ranging from 32 to 610 (Table 4.9). The highest mean abundance of AMF spores was recorded in Chawia forest fragments. A significant difference in the spore mean across forest fragmentswas noted ($P < 0.01$).

Table 4.7 Comparison of the log-transformed (log (abundance + 1) spore abundance across forest fragments in Taita hills

Forest fragments	Mean abundance	±SE
Chawia	5.414934[a]	0.577
Fururu	4.523169[ab]	0.592
Ngangao	4.436074[b]	0.849
P-value	0.00752**	

Means with the same letter are not significantly different at alpha=0.05

Actual means are exp(x) – 1, where x is the log transformed mean

4.5 Diversity of AMF species across selected fragments in Taita hills forest

Fururu forest fragment recorded the highest AMF diversity, followed by Chawia and Ngangao forest fragments (Figure 4.8). The AMF diversity across forest fragments was not significantly different ($P > 0.05$).

Table 4.8 Comparison of AMF diversity across forest fragments in Taita hills

Forest fragments	Shannon diversity index
Fururu	1.504[a]
Chawia	1.466[a]
Ngangao	1.376[a]
P-value	0.776*
±SE	0.351

Means with the same letter are not significantly different at alpha=0.05

There was no significant difference in the abundance of AMF species across selected forest fragments Chawia forest fragment recorded the highest mean in AMF species, followed by Fururu and Ngangao forest fragments (Table 4.9).

Table 4.9 Mean Comparison of the log-transformed (log (abundance + 1)) species abundance across forest fragments in Taita hills

Forest fragments	Mean of AMF species	±SD
Chawia	1.28[a]	0.898
Fururu	1.27[a]	0.696
Ngangao	1.06[a]	0.753
P-value	0.379*	

Means with the same letter are not significantly different at alpha=0.05. Actual means are exp(x) – 1, where x isthe log transformed mean

A total of 24 AMF species were recorded during this study with only six (6) assigned species epithet. The AMF were distributed into three classes (Archaeosporomycetes, Glomeromycetes and Paraglomeromycetes), five orders namely (Archaeosporales (1), Diversisporales (11), Gigasporales (6), Glomerales (5) and Paraglomerales (1), seven out

of 16 recognized families (*Archaeosporaceae* (1), *Glomeraceae* (5), *Acaulosporaceae* (8), *Paraglomeaceae* (1), *Gigasporaceae* (3), Scutellosporaceae (2), *Dentiscutataceae* (1) and *Enthrophosporaceae* (1)) and eight genera (*Acaulospora*, *Archaeospora*, *Dentiscutata*, *Gigaspora*, *Paraglomus*, *Entrophospora, Scutellospora and Glomus*) were recorded. Significant differences were recorded in the abundance of AMF species in the Taita Hills forest ($P < 0.001$). *Acaulospora laevis* was the most abundant AMF species regardless of the forest fragments while *Glomus* sp5 was the least (Table 4.10).

Table 4.10 Comparison of the log-transformed (log (abundance + 1) species abundance across forest fragments in Taita hills

AMF species	Mean abundance	±SD
Acaulospora laevis	2.99[a]	0.22
Glomus sp2	1.98[b]	0.23
Glomus sp1	1.91[bc]	0.84
Glomus sp3	1.49[cd]	0.35
Glomus sp4	1.24[cd]	0.15
Paraglomus sp1	1.18[cde]	0.85
Gigaspora sp1	1.09[cde]	0.00
Scutellospora sp1	1.09[cde]	0.00
Acaulospora cavemata	1.07[cde]	0.22
Enthrophospora sp1	1.02[cde]	0.40
Acaulospora foveata	0.96[cde]	0.23
Gigaspora sp2	0.96[cde]	0.23
Scutellospora sp2	0.96[cde]	0.23
Acaulospora scrobiculata	0.82[de]	0.20
Acaulospora sp2	0.82[de]	0.33
Gigaspora margarita	0.82[de]	0.20
Acaulospora sp1	0.76[de]	0.46
Diversisporales sp2	0.69[de]	0.00
Acaulospora denticulata	0.59[de]	0.55
Acaulospora sp3	0.59[de]	0.55
Archaeospora sp1	0.46[e]	0.40
Diversisporales sp1	0.46[e]	0.40
Dentiscutata sp	0.36[e]	0.63
Glomus sp5	0.36[e]	0.63
P-value	< 2e-16 ***	

Means with the same letter are not significantly different at alpha=0.05

Actual means are exp(x) – 1, where x isthe log transformed mean

A total of 16 AMF species belonging to genera Acaulospora (4), Glomus (4), Gigaspora (3), Scutellospora, Diversisporales (2), Entrophospora and Paraglomus were recorded in Chawia forest fragment (Table 4.11). There was significant difference across AMF species. The

species with the most abundant spores were *A. laevis*, *Glomus* sp1, *Glomus* sp2, *Glomus* sp. 3 and *A. cavemata* in descending order.

Table 4.11 Comparison of the log-transformed (log (abundance + 1) species abundance Chawia forest fragments in Taita hills

AMF species	Mean abundance
Acaulospora laevis	3.51[a]
Glomus sp1	3.01[b]
Glomus sp2	2.36[c]
Glomus sp3	1.49[d]
Acaulospora cavemata	1.19[de]
Glomus sp4	1.19[de]
Gigaspora sp1	1.09[e]
Scutellospora sp1	0.96[ef]
Gigaspora sp2	0.89[ef]
Acaulospora scrobiculata	0.82[efg]
Acaulospora sp1	0.82[efg]
Gigaspora margarita	0.82[efg]
Diversisporales sp2	0.69[fg]
Enthrophospora sp1	0.69[fg]
Diversisporales sp1	0.46[g]
Paraglomus sp1	0.46[g]
P-value	<2e-16 ***

Means with the same letter are not significantly different at alpha=0.05

Actual means are exp(x) – 1, where x is the log transformed mean

A total of 14 AMF species belonging to Acaulospora (4), Glomus (4), Entrophospora (1),Scutellospora (1), Gigaspora (1), Paraglomus (1), Archaeospora sp1 and Dentiscutata (1) were recovered) (Table 4.12). Significance difference was observed across AMF species in Ngangao fragment. *Acaulospora laevis*, *Glomus* sp2, *Glomus* sp1, *Entrophospora*, *Glomus* sp4 were themost abundant AMF species.

Table 4.12 Mean comparison of the log-transformed (log (abundance + 1) species abundance Ngangao forest fragments in Taita hills

AMF species	Mean abundance
Acaulospora laevis	2.87^{a}
Glomus sp2	1.89^{b}
Glomus sp1	1.53^{c}
Enthrophospora sp1	1.36^{bcd}
Glomus sp4	1.29^{cde}
Scutellospora sp1	1.09^{cdef}
Acaulospora foveata	0.96^{cdef}
Gigaspora margarita	0.96^{def}
Paraglomus sp1	0.82^{def}
Acaulospora denticulate	0.59^{ef}
Archaeospora sp1	0.46^{f}
Acaulospora sp1	0.36^{f}
Dentiscutata sp1	0.36^{f}
Glomus sp5	0.36^{f}
P-value	1.3e-07 ***

Means with the same letter are not significantly different at alpha=0.05
Actual means are exp(x) – 1, where x is the log transformed mean

A total of 10 AMF species belonging to Acaulospora (6), Paraglomus (1), Glomus (2), and Gigaspora (1) were recovered (Table 4.13). A significant difference was noted ($P < 0.001$). The species with the most abundant spores was *Acaulospora laevis*.

Table 4.13 Comparison of the log-transformed (log(abundance + 1)) species abundance Fururu forest fragments in Taita hills

AMF species	Mean abundance
Acaulospora laevis	2.6[a]
Paraglomus sp1	2.25[a]
Glomus sp2	1.7[b]
Glomus sp1	1.19[c]
Acaulospora sp1	1.09[cd]
Acaulospora cavemata	0.96[cde]
Acaulospora scrobiculata	0.82[cde]
Acaulospora sp2	0.82[cde]
Gigaspora margarita	0.69[de]
Acaulospora sp3	0.59[e]
P-value	1.76e-08 ***

Means with the same letter are not significantly different at alpha=0.05

Actual means are exp(x) – 1, where x is the log transformed mean

Significance difference was recorded in the abundance of AMF species and across forest fragments (P< 0.001) (Table 4.14). The common AMF species across forest fragments were Acaulospora laevis, Gigaspora margarita, Glomus sp2, Paraglomus sp1 and Glomus sp4.The spore abundance of A. laevis was significantly (P<0.05) higher in Chawia compared to Ngangao and Fururu fragments. The spore abundance for Glomus sp2 was only significantly high compared to Fururu. The spore abundance of Paraglomus sp1 was significantly high in Fururu compared to Chawia and Ngangao fragments.

Table 4.14 Comparison of the log-transformed (log (abundance + 1) AMF species abundanceacross forest fragments in Taita hills

Forest fragment	AMF species	Mean abundance
Chawia	*Acaulospora laevis*	3.51[a]
Chawia	*Glomus* sp1	3.01[b]
Ngangao	*Acaulospora laevis*	2.87[b]
Fururu	*Acaulospora laevis*	2.60[bc]
Chawia	*Glomus* sp2	2.36[cd]
Fururu	*Paraglomus* sp1	2.25[cd]
Ngangao	*Glomus* sp2	1.89[de]
Fururu	*Glomus* sp2	1.70[ef]
Chawia	*Glomus* sp3	1.53[efg]
Ngangao	*Glomus* sp1	1.49[efg]
Ngangao	*Enthrophospora* sp1	1.36[fgh]
Ngangao	*Glomus* sp4	1.29[fghi]
Chawia	*Acaulospora cavemata*	1.19[ghi]
Chawia	*Glomus* sp4	1.19[ghi]
Fururu	*Glomus* sp4	1.19[ghi]
Chawia	*Gigaspora* sp1	1.09[ghij]
Fururu	*Acaulospora* sp1	1.09[ghij]
Ngangao	*Scutellospora* sp1	1.09[ghij]
Chawia	*Gigaspora* sp2	0.96[hijk]
Fururu	*Acaulospora cavemata*	0.96[hijk]
Ngangao	*Acaulospora foveata*	0.96hijk
Ngangao	*Gigaspora margarita*	0.96[hijk]
Chawia	*Acaulospora scrobiculata*	0.96[hijk]
Chawia	*Acaulospora* sp1	0.82[ijkl]
Chawia	*Gigaspora margarita*	0.82[ijkl]
Fururu	*Acaulospora scrobiculata*	0.82[ijkl]
Fururu	*Acaulospora* sp2	0.82[ijkl]
Ngangao	*Paraglomus* sp1	0.82[ijkl]
Chawia	*Diversisporales* sp2	0.82[ijkl]
Chawia	*Enthrophospora* sp1	0.69[jkl]
Fururu	*Acaulospora* sp3	0.69[jkl]
Fururu	*Gigaspora margarita*	0.69[jkl]
Ngangao	*Acaulospora denticulata*	0.59[kl]
Chawia	*Diversisporales* sp1	0.59[kl]
Chawia	*Paraglomus* sp1	0.46[l]
Chawia	*Acaulospora* sp2	0.46[l]
Ngangao	*Archaeospora* sp1	0.36[l]
Ngangao	*Dentiscutata* sp1	0.36[l]
Ngangao	*Glomus* sp5	0.36[l]
P-value		< 2e-16 ***
±SE		0.801

Means with the same letter are not significantly different at alpha=0.05

Actual means are exp(x) – 1, where x is the log transformed mean

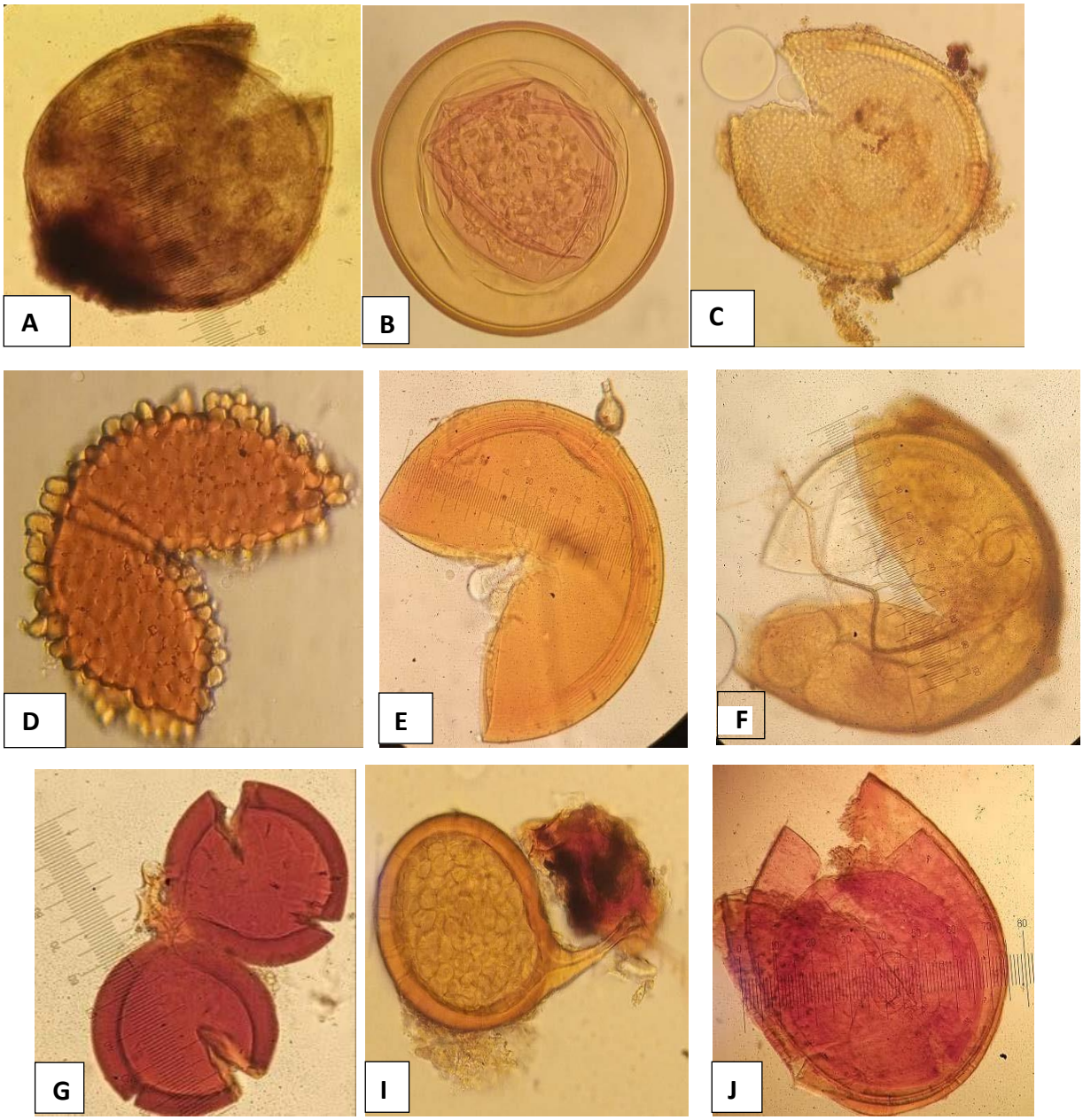

Plate 4.4 Arbuscular mycorrhiza species

A: *Acaulospora cavemata,* B: *Acaulospora laevis,* C: *Acaulospora scrobiculata,* D: *Acaulospora denticulate,* E: *Gigaspora margarita,* F: *Scutellospora sp1,* G: *Glomus sp1,* I: *Glomus sp2* and J: *Archaeospora sp1*

***Identification of AMF species based on morphological characters**

4.6 Correlation among selected variables and AMF diversity across selected fragments in Taita Hills forest

4.6.1 Fururu forest fragment

There was a strong positive correlation (0.75) between AMF and tree diversity and; between altitudes and AMF diversity. However, disturbance levels negatively correlated with AMF diversity (Figure 4.2).

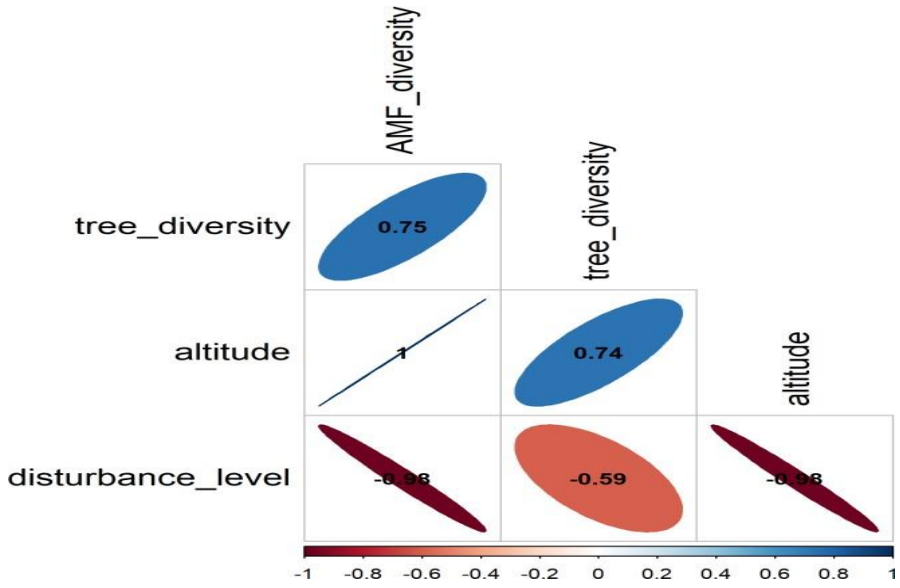

Figure 4.2 Effect of tree diversity, altitudes and disturbances on AMF diversity in Fururu forest fragment

4.6.2 Ngangao forest fragment

A moderate positive correlation of (0.2) was observed between the AMF and tree diversity and;between AMF diversity and disturbance level in Ngangao Forest fragment. Subsequently, altitudes correlated negatively with AMF diversity (Figure 4.3).

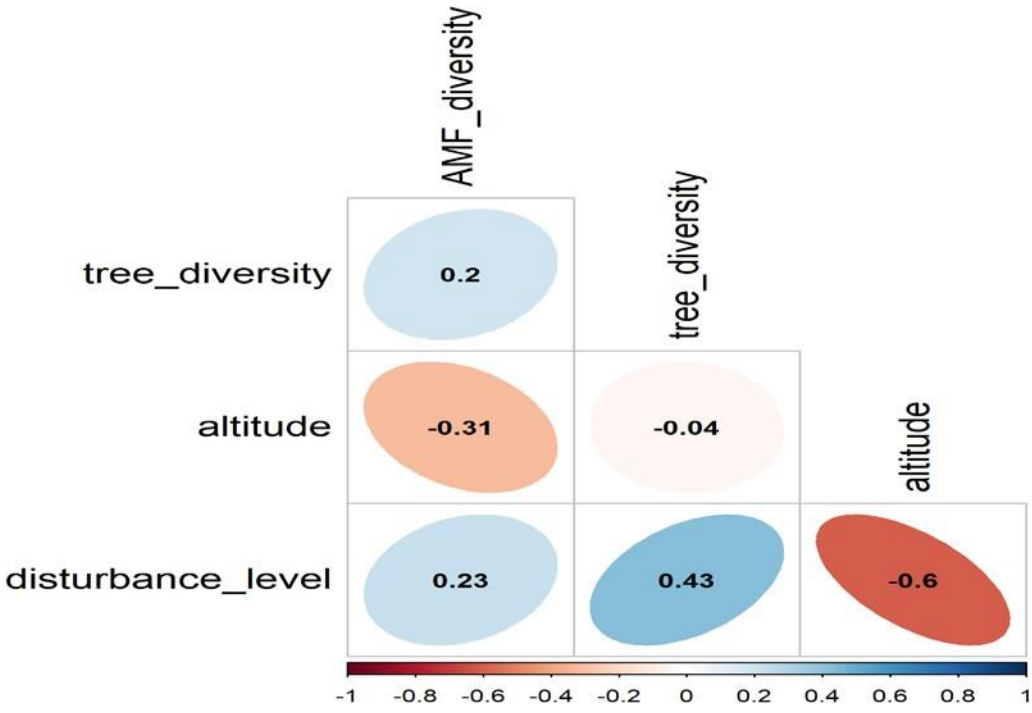

Figure 4.3 Effect of tree diversity, altitudes and disturbances in Ngangao forest fragment

4.6.3 Chawia forest fragment

A negative correlation was evident between AMF and tree diversity (-0.48) in the Chawia forestfragment. Altitudes and disturbances positively correlated with AMF diversity (Figure 4.4).

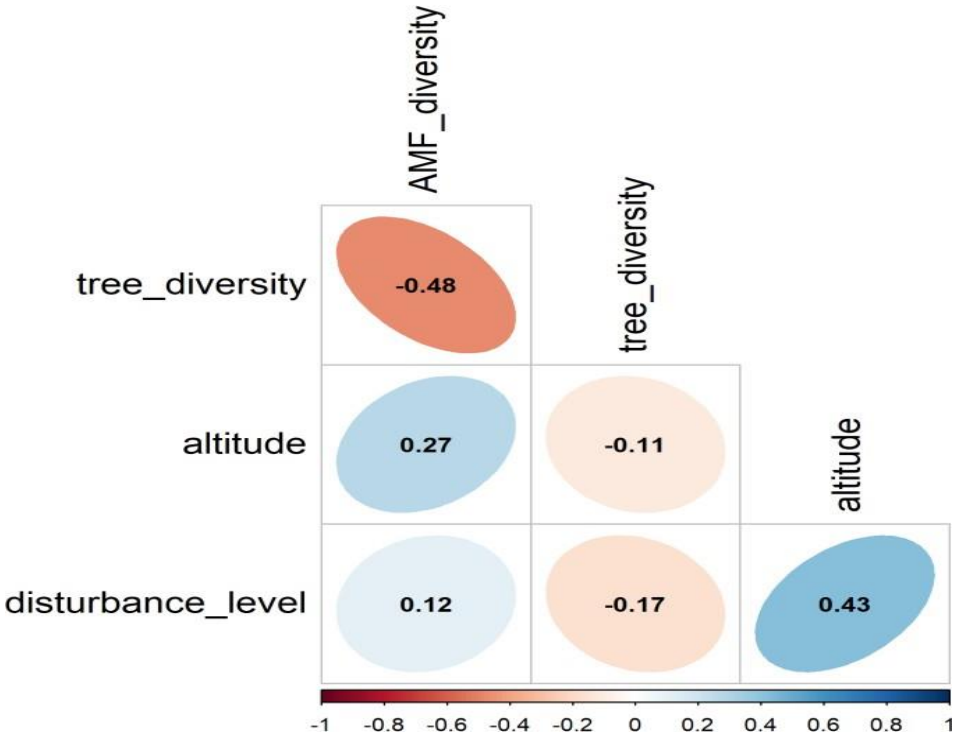

Figure 4.4 Effect of tree diversity, altitudes and disturbances on AMF diversity in Chawiaforest fragment

4.7 Determination of arbuscular mycorrhiza propagules infectivity potential of the forest soils

4.7.1 Assessment of mycorrhizal infective propagules across fragments

The presence of mycorrhizal infective propagules was evident in all fragments. Significant difference in total colonization intensity and colonization of mycorrhizal structures were recorded ($P<0.001$) across the forest fragments. The colonization intensity was high in non-diluted soils (10^0), followed by 10^1 through 10^4 (59.6%, 53.3%, 41.1%, 25.5% and 12.2%) in Fururu forest fragment.The arbuscules and vesicles were most abundant in 10^2 and 10^1, respectively (Table 4.15).

In the Ngangao fragment, significant difference was noted in the effect of dilutions on the colonization intensity (Table 4.16). Mycorrhizal features differed across dilutions. 10^0 recorded the highest percentage of mycorrhizal features. The non-diluted soil (10^0), was highly colonized with hyphae (85.79%) and the highest in dilution (10^4) had 24.84%) colonization. Similarly, a significant difference was evident in the colonization intensity and mycorrhizal features across dilutions except in hyphal coils in the Chawia fragment. 10^0 recorded high colonization intensity, arbuscules, vesicles, entry points and hyphae (Table 4.17). The soils from Ngangao had the highest number of infective propagules, followed by Fururu and the least was Chawia (Figure 4.5). The concentration of infective propagules in Fururu was as high as in Ngangao, however, disturbances caused a drastic decline in Chawia.

Table 4.15 Effects of disturbances on mycorrhizal infectivity propagules in Fururu fragment

Dilution	Arbuscules	Vesicles	Hyphae	Entry point	C/intensity
10^4	0.0c	0.0c	18.6d	10.4c	12.2d
10^3	0.6bc	4.1abc	32.7c	21.3c	25.5c
10^1	2.7ab	8.3a	56.7b	51.6ab	53.5b
10^0	2.9ab	5.5ab	72.2a	58.5a	59.6a
10^2	3.6a	2.3bc	48.1b	42.9b	41.1b
LSD	1.65	3.1	8.59	10.74	7.08
P value	<0.001	<0.001	<0.001	<0.001	<0.001
SE	0.57	1.5	5.13	3.71	2.44

Means with the same letter are not significantly different at alpha=0.05

Table 4.16 Effects of disturbances on mycorrhizal infectivity propagules in Ngangao fragment

Dilution	Arbuscules	Vesicles	hyphae	Entry Point	C/Intensity
10^4	0.86b	0.41c	24.84e	13.58e	22.32e
10^2	2.76b	1.13c	62.73c	41.47c	53.04c
10^1	3.25b	3.82b	72.02b	54.66b	62.21b
10^3	3.83b	1.63bc	48.49d	29.35d	37.67d
10^0	12.05a	8.04a	85.79a	66.38a	73.93a
LSD	2.64	1.7	5.4	7.12	2.91
P value	<0.001	<0.001	<0.001	<0.001	<0.001
SE	0.94	0.6	2.73	2.543	1.03

Means with the same letter are not significantly different at alpha=0.05

Table 4.17 Effects of disturbances on mycorrhizal infective propagules in Chawia fragment

Dilutions	Arbuscules	Vesicles	hyphae	Coils	Appressoria	C/Intensity
10^4	0.0c	0.8d	6.5e	0.5a	7.3e	7.4e
10^2	0.1c	9.5ab	49.5c	0.0a	42.1c	41.7c
10^3	1.12bc	5.3c	19.6d	0.0a	18.0d	17.1d
10^1	1.8b	6.5bc	60.7b	1.9a	58.5b	58.7b
10^0	7.2a	10.7a	76.6a	0.0a	71.3a	73.4a
LSD	0.83	2.35	6.71	2.53	3.62	5.39
P value	<0.001	<0.001	<0.001	0.48	<0.001	<0.001
SE	0.29	0.84	2.39	0.9	1.29	1.92

Means with the same letter are not significantly different at alpha=0.05

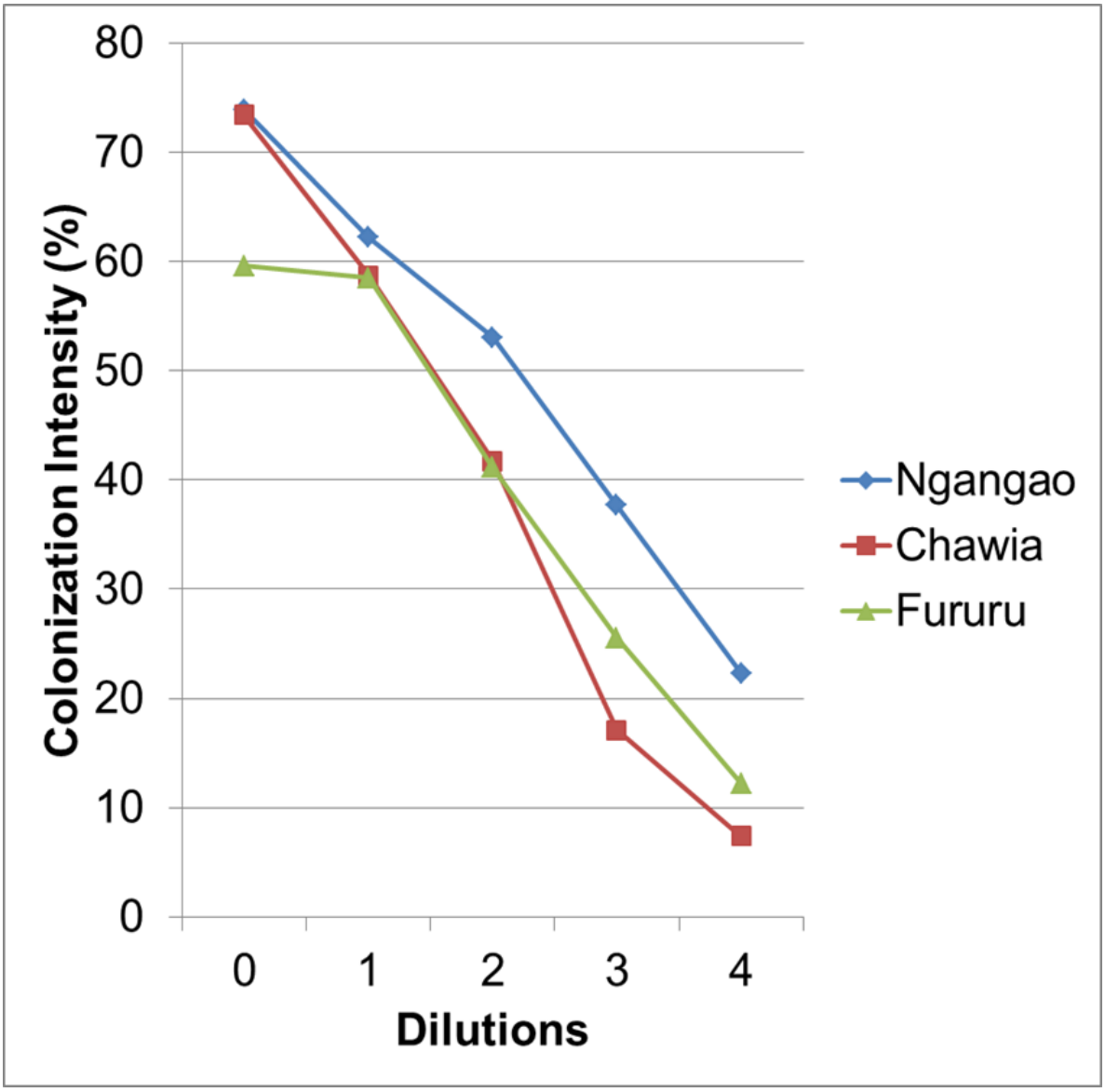

Figure 4.5 Effect of dilutions on MIP across selected forest fragments in Taita hills

4.8 Soil properties across forest fragments

The soil nutrient content was variable in the three forest fragments. Ngangao and Chawia forest fragments were deficient in more minerals than Fururu (Table 4.15). The soils ranged from strongly to extremely acidic 4.87, 4.52 and 4.14 at Fururu, Ngangao and Chawia forest fragments. Except for the Fururu forest fragment that indicated adequate phosphorus

level, the remaining two soil fragments had low phosphorus. Additionally, Potassium, Calcium Magnesium and Zinc were low in Fururu. There was a deficiency in Calcium, Magnesium andZinc in Ngangao soils.

Table 4.18 Soil macro and microelements across forest fragments

Soil Properties	Fururu (TT01)	Ngangao (TT02)	Chawia (TT03)
Soil PH	4.87	4.52	4.14
Exch. Acidity meq %	0.6	0.8	2.1
Total org carbon %	4.78	4.41	4.33
Total Nitrogen %	0.45	0.41	0.40
Phosphorus ppm	20.4	15.00	32.00
Potassium meq %	0.48	0.32	0.16
Sodium meq %	0.24	0.18	0.28
Calcium meq %	0.1	1.2	0.60
Magnesium meq %	1.8	0.84	0.68
Copper ppm	8.39	1.33	1.80
Zinc ppm	6.62	4.39	2.80
Iron ppm	29.8	74.9	78.8

***TT01, TT02 and TT03- codes for soils submitted for analysis from Fururu, Ngangao and Chawia forest fragments, respectively**

CHAPTER FIVE: DISCUSSIONS, CONCLUSION AND RECCOMENDATION

5.1 AMF colonization of tree species

Eight trees species including *Syzigium guineense*, *Pleiocarpa pycnantha*, *Tabernaemontana stapfiana*, *Xymalos monospora*, *Oxyanthus speciosus, Macaranga conglomerata*, *Leptonychia usambarensis* and *Phoenix reclinate* belonging to plant families namely Myrtaceae, Apocynaceae, Monimiaceae, Rubiaceae, Euphorbiaceae, Malvaceae and Arecaceae, respectively were all confirmed to be mycorrhizal.

The tree species' level of colonization intensity varied in selected tree species within a fragment and, not across forest fragments. This could be attributedto the ability of the AMF to colonize and spread in the different species as well as the age of the tree seedling at the time of sampling. The tree species may also differ in their mycorrhizal dependency, an aspect that was not investigated in this study. Various studies conducted in tropical forests have reported 59% of tree species to form AMF (Averill *et al.,* 2014). In Africa, Wubet et *al*., (2003) reported AMF plant taxa from Ethiopia including families of Meliaceae, Sapotaceae, Fabaceae, Myrtaceae, Rosaceae and Euphorbiaceae. Hawley and Dames, (2004) also reported families Rubiaceae, Rutaceae and Myrsinaceae while (Bechem *et al.,* 2018) recording Olacaceae, Apocynaceae, and Sterculiaceae in South Africa.

The family Arecaceae also has been recorded by Soudzilovskaia *et al.* (2020). The Genus Phoenix was reportedmycorrhizal in Tunisia (Chebaane *et al.,* 2020), hence, at the species level, *Phoenix reclinata* is afirst record. *Tambourissa* sp. is the only member reported to be mycorrhizal in the family Monimiaceae (Ducaussol *et al.,* 2008) and therefore the record of Xymalos is the first for the genus in this family. Records exist on species in Euphorbiaceae

being mycorrhizal, with some species in the genus Macaranga (Ahmed *et al.,* 1996), however, the species *Macaranga conglomerata* is the first report. There is evidence of AMF colonization status in the family Rubiaceae (Hawley and Dames, 2004) but no record exists for the Genus Oxyanthus, making the species a first record for the genus. There are records of the family Malvaceae being mycorrhizal with genus Leptonychia mycorrhizal status reported (Soudzilovskaia *et al.,* 2023). However, at the species level, *Leptonychia usambarensis* was the first account. Wubet *et al.,* (2003) reported the AMF status of *Syzigium guineense* from Ethiopia which was in line with our findings on this particular tree species. The family Apocynaceae has also been reported mycorrhizal with the Genus Tabernaemontana reported by (Zangaro *et al.,* 2003) in South Brazil and previous work by Uma *et al.,* (2012) in India. The species *Tabernaemontana stapfiana* is therefore reported as afirst account in members of the Genus Tabernaemontana. Soudzilovskaia *et al.,* (2023), reported the species *Pleiocarpa rostrata* as the only AMF mycorrhizal in the Genus Pleiocarpa (Soudzilovskaia *et al.,* 2023). Our study reports the mycorrhizal status of *Pleiocarpa pycnantha* as the first account.

There was a difference in the colonization intensity of tree species. The variation in the AM colonization intensity in plants informs on the crucial ecosystem functioningsuch as nutrient cycling (Brundrett, 2017). This reflects the different stages of mycorrhizal colonization features depicted in the tree species. AMF status in tree species is limited by seasonality and environmental conditions (Soudzilovskaia *et al.,* 2020). From this study, we cannot conclusively indicate the reasons for the differences in the AMF colonization status of the studied tree species since data was collected in one season.

Mycorrhizal structures including the extraradical and intraradical hyphae, appressoria, hyphal coils, arbuscules and vesicles differed across habitats and tree species with some lacking one or two features. The presence and absence of the mycorrhizal structures in tree species were attributed to variability in age and the nature of the mycorrhiza features of the seedlings at the time of sampling.

The nutrient exchange would be associated with the most active stage of plant growth, hence the variation in species and seasons. Additionally, vesicles and hyphal coils varied across habitats. The vesicles may indicate a decline in the activity, tending towards root senescence and formation of storage features, the vesicles and finally spores, the reproductive and resting stage of the fungus. The tree species were highly colonized with extraradical and intraradical hyphae regardless of habitats, an indication of active nutrient uptake from the soils and flow in the plant. Willis *et al*., (2013) suggest the degeneration of arbuscules 15 days after colonization which was in agreement with the absence of arbuscules in some tree species. Similarly, the observation of different stages of mycorrhizal features reflects different ecosystem functions which are in agreement with findings by Brundrett (2017). Observation by (Birhane *et al.,* 2010) on the variability of mycorrhizal structures due to environmental conditions was in agreement with our results.

The vesicles were predominantly small to medium, sub-globose to elongate and irregular to triangulate in shape suggesting dominance of the genus Glomus. Wubet *et al., (*2003), reported the features of the vesicles as elongate and irregular which are in agreement with our findings. Our findings also on the predominance of the genus *Glomus* over the genus *Acaulospor*a are concurrent with reports by Onguene and Kuyper, (2001) and Marihno *et al.,* (2018) on the dominance of the genus *Glomus* over the genus *Acaulospora* tropical

regions. *Tabernaemontana stapfiana, Xymalos monospora, and Syzigium guinense* had echinulate auxiliary cells suggesting the genus *Gigaspora* while knobbed auxiliary cells were recorded in *Leptonichia usambarensis* indicating the presence of genus *Scutellospora.* Our findings on the type of auxiliary cells to the AMF species colonizing tree species were in agreement with (Wubet *et al.*, 2003).

5.2 AMF species diversity and Mycorrhizal infective propagules (MIP) across forest fragments

Significant difference was observed in the spore abundance across the three forest fragments. The variation in the sporulation is associated with levels of disturbances, altitudes as well as seasonality. Results from Guadarrama *et al.,* (2014) indicate high sporulation of AMF in less disturbed sites hence, contradicting our findings on the abundance of spores in highly disturbed forest fragments. However, our findings are in agreement with Violi *et al. (*2008) and Sturmer and Siqueira *et al.* (2011) who recorded high spore composition in highly degraded forests. Chawia, a disturbed fragment (Teucher *et al.,* 2020) recorded a high number of spores. Birhane *et al.* (2020) also recorded the highest spore abundance in the lowlands of the dry Afromontane forest in Ethiopia. He attributed spore abundance to unfavorable conditions and soil properties experienced with an increase in altitudes. The findings on abundance in low altitudes were in agreement with our results. Shannon Weiner diversity indices presented AMF diversity of 1.50, 1.46 and 1.37 in Fururu, Chawia and Ngangao forest fragments, respectively. However, no significant difference was observed across selected forest fragments. The range in the AMF diversity was in the range for dry forests as documented by (Teixeria-Rios *et al.*, 2013) as between 0.54 and 2.83 which was in agreement with our findings on AMF diversity. Similarity in the

AMF diversity in the three fragments is attributed to the fact that the forest fragments are adjacent and were once a continuous forest. The lack of variation was in agreement with Guadarrama *et al.,* (2014) who suggested similar diversity of AMF in adjacent sites. Twenty four (24) AMF species were documented from the three fragments with the Chawia forest fragment recording AMF species richness of sixteen (16) followed by Ngangao and Fururu forest fragments with fourteen (14) and ten (10) AMF species respectively. Significant difference was recorded in AMF species across the forest fragments. The difference could be due to seasonality as well as environmental conditions. According to de Souza *et al.* (2017), AMF spore richness increase with an increase in altitudes. Our findings indicated otherwise with more AMF species recorded in Chawia, a lowland forest fragment. The predominance of the Genera Glomus and Acaulospora was observed in this study. The two genera have a high sporulation capacity. Marihno *et al. (*2018) suggested high sporulation capacity of the *Glomus* sp. and *Acaulospora* sp. which was similar to our findings. Similarly, de Assis *et al.,* (2018) and Guadarrama *et al.,* (2014) reported dominance of the Genera Acaulospora and Glomus in tropical forests. The rarity of AMF species across fragments was evident with six AMF species restricted to Ngangao, four to Chawia and one AMF species to Fururu forest fragments. The restriction could be due to the level of disturbances. Our results agree with the observation made by Marinho *et al.,* (2019) who attributed the prefence of AMF species to the dispersal barriers of spores caused by disturbances generated by anthropogenic activities.

Correlation analysis revealed a positive association between the tree and AMF diversity in Fururu and Ngangao forest fragments, respectively while the Chawia fragment recorded a negative relationship. The positive association between AMF diversity and plant diversity

is due to the role AMF play in maintaining plant communities while the negative correlation between plant and AMF communities could be attributed to a high level of disturbance in the Chawia forest fragment. Our findings on the positive association between AMF and plant diversity were in agreement with those of Shi *et al.* (2019), who reported an association between plant and AMF diversity. Similarly, Tedersoo *et al.* (2014), reported a positive relationship between relationship between fungal and plant diversity. Our study revealed a positive association between altitudes and AMF diversity in Fururu and Chawia fragments at average altitudes of 1700m and 1550 respectively while Ngangao forest fragments at altitudes of 1825m recorded a negative relationship. Unfavorable conditions in high altitudes could be due to negative association between AMF diversity and altitudes in Ngangao. Our findings coincide with the findings of Lugo *et al.,* (2008) and Birhane *et al.,* (2017) who reported a decline in AMF richness and diversity with an increase in altitudes. The diminishing AMF diversity at high altitudes was associated with unfavourable temperatures (Lugo *et al.,* 2008). Subsequently, positive between disturbances and AMF communities in Ngangao and Chawia forest fragments with low and high levels of disturbance respectively. Fururu fragment with moderate disturbances showed a negative correlation between AMF diversity and disturbances. Similar observations were made by Asmelash *et al.* (2016), who suggested a decline in AMF communities with increased disturbances. However, this is not consistently the case as seen in Bennett *et al.*, (2020) who reported an increase in AMF community to be associated with high plant diversity rather than disturbances.

Our study reported mycorrhizal infective propagules in soils of the three forest fragments at the time of sampling. The significant difference in AMF propagules was recorded across

forest fragments with soil infectivity declining with increase in dilution. The AMF infective propagules were highest in Ngangao soils and least in Chawia soils. The Fururu undiluted soil infectivity was similar to Ngangao. However, diluting the soils displayed a drastic decrease in infective propagules. Decline of soil infectivity in Chawia forest fragment was also observed. This may imply that soils from Fururu and Chawia forest fragments are more sensitive to disturbances. Additionally, the low infectivity in Fururu and inconsistency of AMF propagules in Chawia fragments could be attributed to the two soils displaying less resilience to disturbance. Jasper *et al., (*1991) recorded a drastic decline in AMF colonization when disturbed soils are diluted was concurrent with our findings. Similarly, the disturbance may cause damage to hyphal networks in the soil resulting in a decline in soil infectivity Jasper *et al.,* (1989). Despite the spore abundance in the Chawia forest fragment, it had low infective propagules.

Our study, therefore, possibly attributes hyphae as the main source of infective propagule as was indicated by Bellgard,(1993) who observed unfragmented hyphae as the main source of inoculum. The decline in AMF propagules is associated with soil disturbances caused by anthropogenic activities which decrease the viability (Marihno *et al.,* 2019) leading to decline in concentration of the infective propagules (Schalamuk and Cabello, 2010).

5.3 Soil macro-elements and AMF communities

The soil nutrient content was variable in the three forest fragments with Ngangao and Chawia deficient in more minerals than Fururu (Table 4.20). Soils ranged from strongly to extremely acidic. Except for Fururu which indicated adequate phosphorus level (32ppm), the remaining two soil fragments had low phosphorus. The soil environment is the habitat that greatly affects arbuscular mycorrhizal fungal growth and function. Phosphorus and

soil acidity have for a long time been associated with mycorrhizal functions, hence any changes would have an impact on mycorrhizal activities.

The soil pH was extremely low and the phosphorus levels for two soils were low in two of the forest fragments. The spore abundance and the mycorrhizal soil infectivity did not tally with the acidity and phosphorus levels. Whereas, Fururu had adequate phosphorus and the least acidity, it did not have the least spore abundance and mycorrhizal soil infectivity. The secondary nutrients, magnesium, calcium, and zinc soil nutrients were low, as well as soil acidity, irrespective of the low and adequate status of phosphorus, could still not adequately explain the differences. Our study contradicts many other studies that reported AMF density to decline with elevated levels ofPhosphorus (Camezind *et al.,* 2014).

The soils analysed in this study were composited and may not therefore adequately explain the differences in species diversity, spore abundance, species richness and soil infectivity. In earlier studies, AMF is widely reported to enhance the uptake of Phosphorus, Potassium and Nitrogen in plants (Dhalaria *et al.,* 2020), stimulated plant growthand cushion against environmental stress including salinity, drought and acidity in disturbed ecosystems (Wang *et al.,* 2017).

5.4 Molecular diversity of Arbuscular mycorrhiza species colonizing roots of *Xymalos monospora, Pleiocarpa pycnantha* and *Pheonix reclinata*.

Genetic diversity of AMF was evident from the DNA sequencing results for the *Xymalos monospora*, *Pleiocarpa pycnantha* and *Phoenix reclinata* tree species. Molecular analysis revealed predominance of the genera Glomus in roots of plants which is congruent with several genetic diversity studies in plant species such as *Artemisia umbelliformis* (Binet *et*

al., 2011), *Pericopsis mooniana* (Husna *et al.,* 2015), *Amygdalus scoparia* (Mirzaei and Moradi, 2017), *Ulmus chenmoui* (Song *et al.,* 2021), *Coccothrinax crinita* (Furrazola *et al.,* 2020), *Ferula sinkiangensis* (Luo *et al.,* 2020) and *Carissa edulis* (Ogoma *et al.,* 2021). Additionally, the molecular analysis concurs with the morphological diversity of AMF spores in the site.

The AMF species of the family Glomeraceae are known to have the capability of producing small and numerous spores that are highly adaptative to harsh environmental conditions (Husna *et al.,* 2022). Consequently, Glomeraceae colonize the roots of plant species more rapidly (de Assis *et al.,* 2018). Although the family Acaulosporaceae also showed dominance morphologically, nothing was detected in sequencing even with distinct bands seen during gel electrophoresis. This was also observed in studies by (Wubet *et al.,* 2009; Haug *et al.,* 2021, Redecker, 2000).The reasons for the discrepancy in morphology and molecular of the Acaulosporaceae remain unknown. Haug *et al.* (2021) found no representatives of Acaulosporaceae in either soils or mycorrhizal root disputing his earlier claims that it could have only been found in soil samples during dry seasons due to slow root colonization as compared to the Glomeraeae. Furthermore, Primer sensitivity and methodology are not the reason for the discrepancy since both Wubet *et al.,* (2003, 2009) and Rodríguez-Echeverría *et al.* (2017) used different primers and methods and could not detect the Acaulosporaceae.

In this study, Arbuscular Mycorrhizal fungi showed a preference for the tree species they colonized. *Xymalos monospora* and *Pleiocarpa pycnantha* tree species were majorly colonized by the genus Glomus. Additionally, *Xymalos monospora* was colonized by *Gigaspora margarita*. *Pleiocarpa Pycnantha* was colonized by *Diploceras hypericium*

(AB594805), an ascomycete, uncultured fungus (AB594869, AB594869 and AB594886) and uncultured Glomeromycotina sp. The sequences of AMF species colonizing roots of *Phoenix reclinata* were not found in the NCBI Genbank. These could be new species of AMF. Additionally, the LECT primer did not show any sequence (Wubet, 2003). However, with only two out of the four primers used being successful, unclear bands for sequencing for the unsuccessful primers could underestimate AMF diversity in the roots of the three tree species.

5.5 Conclusion

Mycorrhizal colonization intensity varied across tree species with *Macaranga conglomerata* recording the highest (76.12%) percentage mycorrhizal colonization and least in *Pleiocarpa pycnantha* (48.33%). Genetic diversity revealed predominance of Glomus sp. in roots of selected common tree species. Additionally, mycorrhizal structured differed across tree species indicating different stages of nutrient nycling in the ecosystem. The study showed forest disturbances to have effect on MF species abundance and richness. Anthropogenic factors could explain the differences with disturbance presenting a condition ofadversity and contributing to higher sporulation as was noted in spore abundance. The prefernce of AMF species was attributed to barrier in dispersal which is accelerated by anthropogenic disturbances. Alternatively, the prevailing conditions were not favorable for sporulation of the rare AMF species. Subsequently, levels of disturbances affected AMF diversity and the the increase in tree diversity, translated to increase in AMF diversity, whereas the increase in altitudes translated to a decline in AMF diversity. The most affected infective propagules by disturbance were the hyphae which clearly showed the least disturbed forest, Ngangao with the highest propagules, and Chawia which was the most disturbed displayed the least

mycorrhizal soil infectivity. The mycorrhizal soil infectivity method provided reliable information on AMF species status in tropical forest ecosystem.

5.6 Recommendations

The study recommends;

- Mycorrhizal analysis of tree species should be done before adoption for restoration and reforestration. This will inform forestry sectors on inoculation of tree species with AMF mycorrhiza for better survival and adaptability in the field.
- Adoption of AMF diversity especially AMF richness and abundance as bioindicators of forest disturbances. This would advise on the status of forest ecosystems and serve as a measure to be applied by policy makers to mitigate further biodiversity loss.
- Further studies on mycorrhizal infective propagules hence used as measure of ecosystem resilience. Mycorrhizal propagules are essential in initiation of root colonization. This will guide on whether or not tree species should be inoculated for survival.

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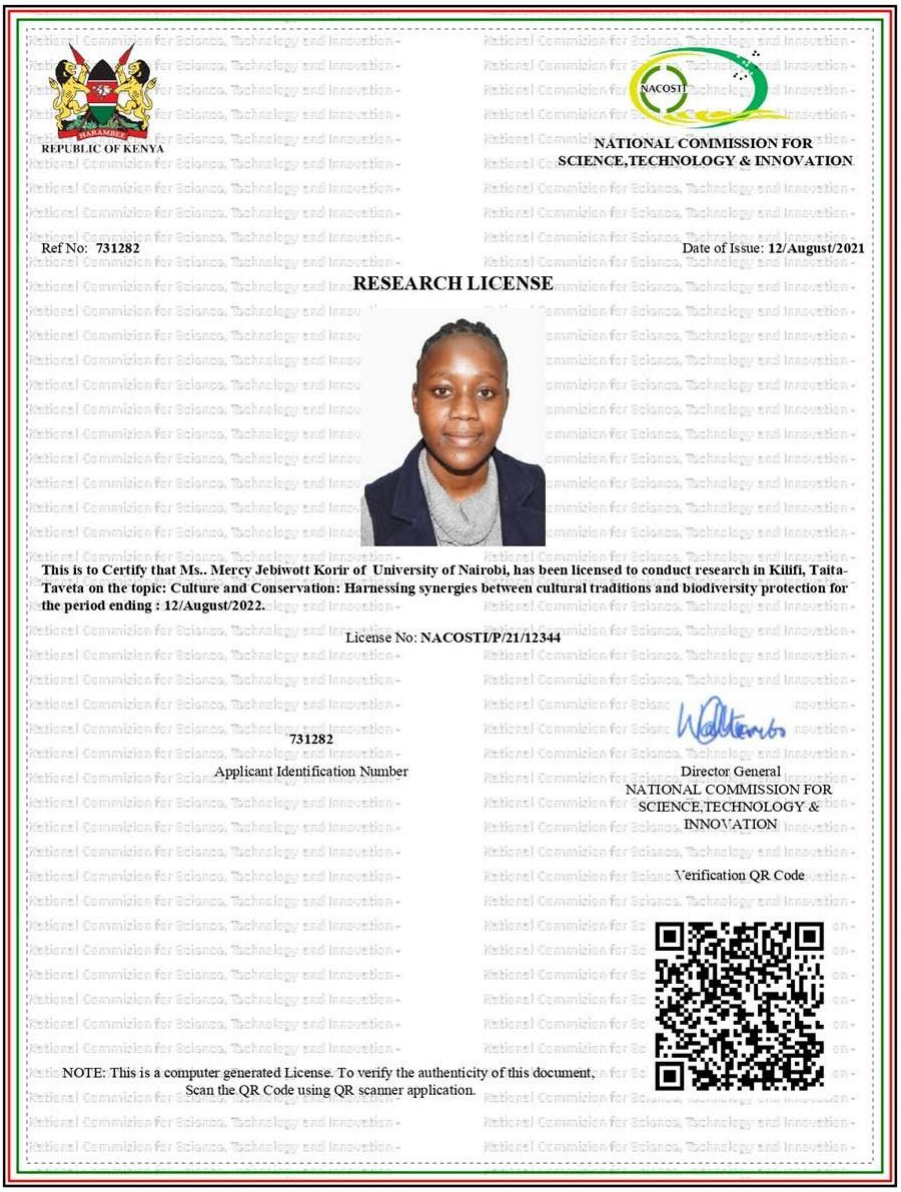
APPENDICES

Appendix I: Research permit



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