FACULTY OF BIOSCIENCE ENGINEERING

OCCURENCE AND ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN SYMBIOSIS WITH *MELIA VOLKENSII* IN KENYAN DRYLANDS

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Master's Dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Science in Bioscience Engineering Technology: Agriculture and Horticulture - main subject Horticulture

Academic year: 2023 – 2024

UNIVERSITEIT GENT

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Ghent, 23/08/2024

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Foreword

Firstly, I would like to express my gratitude to my promoter, Prof. Dr. Ir. Stefaan Werbrouck, for his support and guidance throughout this process, as well as for sharing his knowledge and expertise. Additionally, I thank him for providing this opportunity, connecting me with inspiring individuals, and facilitating this international experience.

Secondly, I would like to thank Better Globe Forestry, and in particular Ir. Jan Vandenabeele, Senior Director and board member, for his time, expertise, and assistance, as well as for the warm welcome in Kenya. Also thanks to the farmers who allowed the collection of soil samples from their fields.

Thirdly, I would like to express my sincere gratitude to my tutor, Prof. Dr. Joyce M. Jefwa, as well as Mercy J. Korir and Felistas Mwania, for their invaluable assistance and expertise in the laboratory at Pwani University, for alerting me to the importance of mycorrhizas and for making me feel at home in Kilifi, Kenya. Additionally, I extend my thanks to Amal Rabaaoui, Jenthe Moyaert, and Stijn van Sprundel for their support with the practical laboratory work at Ghent University.

I would also like to thank Anton Vanhauwere, with whom I have shared this international experience. Furthermore, I express my gratitude to all who have supported me during my thesis research and throughout my entire education. I am especially grateful to my aunt and my parents for their continuous encouragement, patience, and support throughout my education. Thank you for always believing in me and guiding me in the right direction.

I am grateful for the opportunity to conduct this research. Thanks to this dissertation, I have been introduced to African and Kenyan culture and have met wonderful, intriguing, and warm people, including local farmers throughout Kenya. I am thankful for this experience and the wisdom I have gained there.

Asante sana!

Abstract (EN)

Arbuscular mycorrhizal fungi (AMF) are essential partners in plant health, especially in nutrient-poor and drought-prone environments. This study examines the occurrence and abundance of AMF in the rhizosphere of *Melia volkensii*, an indigenous, drought-tolerant hardwood species in Kenya's drylands. This thesis comprises open research conducted at the request of Better Globe Forestry Ltd (Kenya) and completed in collaboration with Pwani University (Kenya) and Ghent University (Belgium). Soil samples were collected from various sites, including farms, forests, nurseries, and plantations, across the counties of Kitui, Makueni, and Kilifi. Both morphological and molecular techniques were employed to identify the AMF species present and to analyze their spore density. In this study, five AMF genera were identified: *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, and *Dentiscutata*. The results showed a significant effect of land use type on spore density, with higher densities observed in forests and plantations compared to farms and nurseries. However, no significant difference was found between planted and naturally occurring *Melia volkensii* trees. Spore density showed a weak negative correlation with annual precipitation and temperature, indicating other environmental factors might also play a critical role. This studie underscores the importance of applying indigenous inoculum or promoting AMF associations with *Melia volkensii* to enhance the trees' resistance and resilience, which is particularly critical in light of current climate changes.

Keywords: Arbuscular Mycorrhizal fungi, *Melia volkensii*, symbiosis, Kenyan drylands, spore density

Abstract (NL)

Arbusculaire mycorrhiza-schimmels (AMF) zijn essentiële partners voor de gezondheid van planten, vooral in omgevingen die arm zijn aan voedingsstoffen en gevoelig zijn voor droogte. Deze studie onderzoekt het voorkomen en de sporendichtheid van AMF in de rhizosfeer van *Melia volkensii*, een inheemse, droogtebestendige hardhoutsoort in de droge gebieden van Kenia. Deze masterproef omvat open onderzoek uitgevoerd op verzoek van Better Globe Forestry Ltd (Kenia) en voltooid in samenwerking met Pwani University (Kenia) en de Universiteit Gent (België). Bodemmonsters werden verzameld op verschillende locaties, waaronder boerderijen, bossen, kwekerijen en plantages, in de provincies Kitui, Makueni en Kilifi. Zowel morfologische als moleculaire technieken werden gebruikt om de aanwezige AMF-soorten te identificeren en hun sporendichtheid te analyseren. In deze studie werden vijf AMF-geslachten geïdentificeerd: *Glomus, Acaulospora, Scutellospora, Gigaspora* en *Dentiscutata*. De resultaten toonden een significant effect van het type landgebruik op de sporendichtheid, waarbij hogere dichtheden werden waargenomen in bossen en plantages in vergelijking met boerderijen en kwekerijen. Er werd echter geen significant verschil gevonden tussen aangeplante en natuurlijk voorkomende *Melia volkensii*-bomen. De sporendichtheid vertoonde een zwakke negatieve correlatie met de jaarlijkse neerslag en temperatuur, wat erop wijst dat andere factoren ook een cruciale rol kunnen spelen. Deze studie benadrukt het belang van het toepassen van inheemse inoculum of het bevorderen van AMF-associaties bij *Melia volkensii* om de weerstand en veerkracht van de bomen te vergroten, wat bijzonder belangrijk is in het licht van de huidige klimaatveranderingen.

Kernwoorden: Arbusculaire mycorrhiza-schimmels, *Melia volkensii*, symbiose, droge gebieden van Kenia, sporendichtheid

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List of abbreviations

AMF - Arbuscular Mycorrhizal Fungi

ASAL - Arid and Semi-Arid Lands

BGF - Better Globe Forestry

DNA - Deoxyribonucleic Acid

EMF - Ectomycorrhizal Fungi

KEFRI - Kenya Forestry Research Institute

KWS - Kenya Wildlife Service

PCR - Polymerase Chain Reaction

PVLG - Polyvinyl-Lactoglycerol

RNA - Ribonucleic Acid

SSU rDNA - Small Subunit Ribosomal DNA

TAE - Tris-Acetate-EDTA (buffer)

Tm - Melting Temperature

Introduction

Climate change is one of the most pressing challenges of our time, affecting the world in multiple ways, such as rising temperatures, changing precipitation patterns, and more frequent extreme weather events. Drylands are particularly vulnerable, given their scarce water resources, delicate ecosystems, and often poor soils. In Kenya, where drylands cover a large part of the country, these climate shifts have led to serious issues like desertification and declining agricultural productivity, which threaten food security and the livelihoods of millions.

In light of this, the preservation and restoration of drylands have become crucial. Trees are central to this effort as they help prevent soil erosion, improve soil fertility, boost biodiversity, and provide vital resources like food and firewood. Indigenous species like *Melia volkensii* are especially important because they are well adapted to the harsh, arid conditions and offer both ecological and economic benefits. However, their growth and survival are increasingly threatened by the changing climate.

A critical yet often overlooked factor that can significantly improve the growth, resilience, and survival of *Melia volkensii* is its symbiotic relationship with arbuscular mycorrhizal fungi (AMF). AMF colonize plant roots, enhancing water and nutrient uptake while also providing protection against diseases and drought. Developing an appropriate indigenous AMF inoculum could therefore be key to bolstering the resilience of *Melia volkensii* in these challenging environments.

Research Questions and Objectives

This research seeks to explore the occurrence and abundance of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of *Melia volkensii* across various regions of Kenya's drylands. The study poses several questions: Which AMF species are present in the rhizosphere of *Melia volkensii*? How does spore density correlate with tree characteristics like age and land use? Additionally, how does spore density vary in relation to climatic factors such as precipitation and temperature?

The primary aim of this study is to gain insights into the diversity and density of AMF in different environments. Moreover, it aims to identify an indigenous AMF inoculum that can enhance the growth and resilience of *Melia volkensii*. This inoculum could be applied in tree nurseries and reforestation projects to improve the survival rates of these trees and boost their resistance to drought and soil-borne diseases

Societal Relevance

This research is socially relevant because it fosters underground microbial life and encourages the growth of AMF cultures. These processes can strengthen vegetation's resistance to extreme climates, increase the resilience of ecosystems, and help combat desertification. Furthermore, a deeper understanding of the relationship between *Melia volkensii* and AMF could improve the productivity and sustainability of agroforestry systems, thereby contributing to food security and creating new economic opportunities for local communities.

Theoretical Framework and Methodology

The research is grounded in the ecology of symbiotic relationships between plants and arbuscular mycorrhizal fungi, with a specific focus on the role of AMF in supporting the growth of *Melia volkensii* in arid environments. The practical aspect of the study, particularly the collection of soil samples, was conducted in collaboration with a fellow student, Anton Vanhauwere. While I focused on analyzing AMF spores in the soil, Anton studied AMF in the roots of *Melia volkensii*. The rest of the research was carried out independently, in close collaboration with Better Globe Forestry and Pwani University in Kenya. Both morphological and molecular techniques were used to identify AMF species and to analyze their diversity and density.

Course of the Study

Soil samples were gathered from 14 different locations across the Kenyan counties of Kitui, Makueni, and Kilifi, where both planted and wild *Melia volkensii* trees were growing. The AMF spores were first identified morphologically based on their characteristics. Following this, DNA was extracted and analyzed using PCR and DNA sequencing to identify the fungi at the molecular level. Finally, the data were statistically analyzed to examine correlations between spore density, tree characteristics, and climatic factors, with the aim of better understanding the interactions between AMF and *Melia volkensii*.

The thesis begins with a literature review that discusses the relevant theoretical frameworks. This is followed by a detailed account of the methodology and results from the field and laboratory research. The findings are then analyzed and discussed in the context of existing literature, leading to conclusions that contribute to a deeper understanding of the role of AMF in supporting the growth of *Melia volkensii* and offering practical solutions to the challenges this tree species faces in the Kenyan drylands.

Literature review

This literature review provides a comprehensive examination of Arbuscular Mycorrhizal Fungi (AMF) and their symbiotic relationship with plants, with a particular emphasis on the interaction between AMF and the tree species *Melia volkensii*. The study begins with an introduction to mycorrhizae and AMF, followed by an in-depth discussion of the life cycle and symbiosis of AMF. It explores the benefits these fungi offer to both plants and ecosystems, as well as the factors that influence AMF colonization. The literature review also highlights the application of AMF in sustainable agriculture. In the subsequent sections, *Melia volkensii* is discussed, including its adaptations to arid regions, the diseases that threaten the species, and its economic and ecological value. Finally, the review delves into the symbiosis between AMF and *Melia volkensii*, emphasizing the benefits of this interaction for the survival and growth of *Melia volkensii* in semi-arid environments.

1. Arbuscular Mycorrhizal Fungi (AMF)

1.1 Introduction

1.1.1 Mycorrhizae

Mycorrhiza represents the symbiotic relationship between plant roots and fungi, where both organisms derive mutual benefits. In this interaction, the fungus gains carbon produced by the plant through photosynthesis, while the plant benefits from enhanced nutrient absorption, particularly phosphorus and nitrogen, facilitated by the fungus (Genre et al., 2020). Additionally, the fungus improves the host plant's ability to absorb water and reduces the risk of pathogenic infections (De Oliveira & De Oliveira, 2010).

Variations in how mycorrhizae colonize plant roots have led to their classification into two primary categories: Endomycorrhizae, associated with the Phylum Glomeromycota, and Ectomycorrhizae, linked to the Phyla Basidiomycota, Ascomycota, and Mucoromycota. Endomycorrhizae are further categorized into arbuscular, orchid, and ericoid types (Kalamulla et al., 2022). Among these, arbuscular mycorrhizal fungi are particularly widespread, colonizing 80-90% of plant species, while only 10% of plants form ectomycorrhizae (Kalamulla et al., 2022).

1.1.2 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are endomycorrhizae belonging to the phylum Glomeromycota. They form a symbiotic relationship with the roots of most terrestrial plants and play a crucial role in the uptake of phosphate, an essential mineral for plant growth, by extending the root absorbing area (Diagne et al., 2020). These fungi are distinguished from other mycorrhizae by their unique intracellular structures, known as arbuscules, which maximize nutrient exchange between the fungus and the plant. AMF are widespread and found in almost all ecosystems, making them one of the most common forms of mycorrhiza. They have a long evolutionary history with land plants and are of great importance in agriculture due to their ability to enhance soil fertility (Smith & Read, 2008; Bonfante & Genre, 2010). Figure 1 illustrates the mechanism behind the symbiosis between the plant and arbuscular mycorrhizal fungi (AMF), highlighting the various morphogenetic features involved.

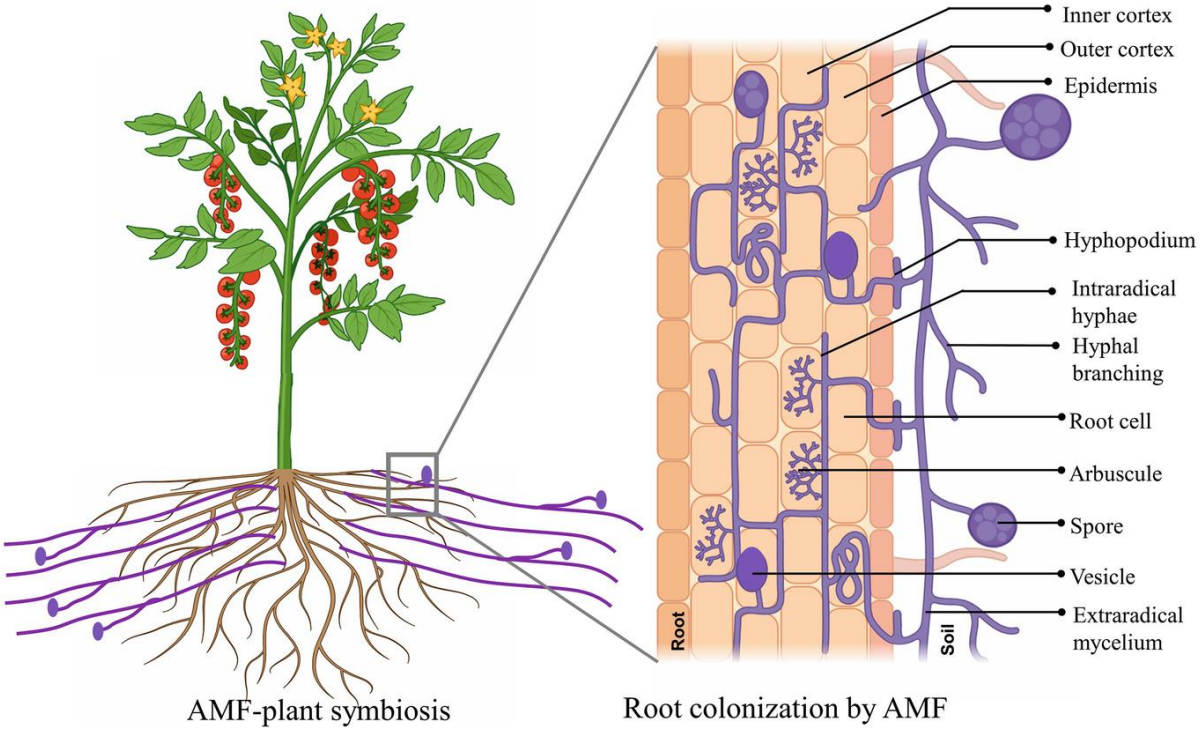

Figure 1: Plant root colonization by arbuscular mycorrhizal fungi (AMF) ((Ahammed & Hajiboland, 2024)

1.2 Life Cycle & Symbiosis

The life cycle of arbuscular mycorrhizal fungi (AMF) is intricately intertwined with their symbiotic relationship with host plants, as AMF rely entirely on their hosts for survival, classifying them as obligate symbionts (Bitterlich et al., 2020). The infection process of host roots by AMF is characterized by a series of distinct stages, each involving complex morphogenetic changes within the fungi, including spore germination, hyphal differentiation, appressorium formation, root penetration, intercellular growth, and arbuscule formation (Giovannetti et al., 1994). This process commences when fungal spores, which serve as the primary inoculum, germinate in the soil.

Spore germination, the initial event in the AMF life cycle, is influenced by spore dormancy and various environmental factors such as pH, temperature, water potential, nutrient content, and soil microbial activities (Giovannetti et al., 1994). These spores detect signaling molecules, particularly strigolactones, exuded by plant roots, which trigger the germination process and direct hyphal growth toward the root (Parniske, 2008). AMF possess the ability not only to recognize potential hosts at an early stage but also to discriminate between host and non-host plants, indicating a degree of host specificity despite their broad host range (Giovannetti et al., 1994). The formation of appressoria, specialized structures used for penetration, signifies the fungus's recognition of the root as a compatible host (Giovannetti et al., 1994).

Upon reaching the root, the fungus penetrates the root epidermis and forms intercellular hyphae and highly branched structures known as arbuscules within the root cortical cells. These arbuscules are vital for the efficient exchange of nutrients between the fungus and the host plant (Smith & Read,

2008). During this symbiotic interaction, the fungus facilitates the uptake of essential nutrients, particularly phosphate, from the soil and transfers them to the plant. In exchange, the plant provides the fungus with carbohydrates derived from photosynthesis, which the fungus utilizes for its growth and reproduction (Genre et al., 2020). This mutualistic relationship is crucial for the plant's nutrient acquisition, especially in nutrient-poor soils, and enhances the plant's tolerance to environmental stresses such as drought and soil-borne pathogens (Diagne et al., 2020).

After the arbuscules have fulfilled their function, they undergo degeneration, while the hyphae continue to grow, eventually leading to the production of new spores in the surrounding soil. Unlike many ectomycorrhizal fungi (EMF), which form aboveground fruiting bodies, AMF complete their life cycle entirely within the soil (Bonfante & Genre, 2010). This life cycle enables the fungus to persist in the soil and colonize new plants. Additionally, AMF spread primarily through root-to-root contact but can also be dispersed by small animals such as ants, grasshoppers, termites, wasps, birds, and rodents, as well as by erosion agents like wind and water (Ricalde & Lucía, 2017).

1.3 Spores

Spores, the reproductive structures of arbuscular mycorrhizal fungi (AMF), are essential for the dispersal and survival of these fungi under varying environmental conditions. The process of sporulation, where a vegetative cell transitions into a spore, typically occurs in response to unfavorable environmental factors, such as nutrient limitations or extreme temperature fluctuations. This process is a survival strategy for the fungi, allowing them to persist through challenging conditions. Sporulation is influenced by a range of factors, including the availability of water and the specific traits of the fungal species involved.

Studies have demonstrated that spore density varies with changes in water availability and precipitation patterns. Seasonal rainfall, for example, stimulates root growth, which in turn enhances the germination of AMF spores and subsequent fungal colonization, often leading to a decrease in spore density in the soil. The observed increase in spore density during dry seasons supports the relationship between AMF sporulation levels and water availability, as suggested by previous research (Bouamri et al., 2014; De Oliveira & De Oliveira, 2010).

However, sporulation patterns are not uniform across all AMF species. For example, species within the *Glomus* genus tend to sporulate consistently throughout the year, whereas species from the *Acaulospora* and *Scutellospora* genera predominantly sporulate during transitional seasons, such as spring and autumn (Bouamri et al., 2014).

Despite the critical role of spores as indicators of AMF presence, spore density is often considered an unreliable metric for assessing mycorrhizal infectivity. This unreliability is partly due to the variability in the methods used to measure spore density, such as wet sieving and decanting, which can differ significantly between studies, making comparisons challenging (Ricalde & Lucía, 2017). Additionally, research has shown that there is no straightforward correlation between spore population density and mycorrhizal infectivity (Hayman & Stovold, 1979). These findings underscore the complexity involved in interpreting spore density as a measure of AMF activity and effectiveness within ecosystems.

1.4 Benefits of Symbiosis

1.4.1 Benefits for plant

Arbuscular mycorrhizal fungi (AMF) exhibit significant diversity, with different species providing distinct benefits and fulfilling various roles in plant health. AMF are instrumental in helping plants cope with a wide range of biotic and abiotic stresses, including salinity, drought, extreme temperatures, heavy metal toxicity, diseases, pests, and pathogens.

In agriculture, AMF contribute to the increased nutraceutical value of crops, enhancing their nutritional quality and overall productivity (Bitterlich et al., 2020). Ecologically, the growth of mycorrhizal networks facilitates nutrient transfer between interconnected plants, which is particularly beneficial for seedlings in competitive environments where light and nutrients are scarce. These networks allow nutrients to be absorbed by the fungus from source roots and efficiently translocated to sink plants without entering the soil solution, thereby optimizing nutrient use (Ricalde & Lucía, 2017).

1.4.2 Benefits for Ecosystems and Soil Health

Arbuscular mycorrhizal fungi (AMF) are essential in land restoration, particularly in degraded areas, by improving soil structure through the production of glomalin, which facilitates soil aggregation and stability (Kalamulla et al., 2022). This enhancement of soil quality supports plant establishment and ecosystem resilience.

AMF also play a key role in maintaining plant diversity and ecosystem functioning. By influencing the structure of plant and bacterial communities, they contribute to the stability and sustainability of ecosystems (Ricalde & Lucía, 2017; Diagne et al., 2020).

1.5 Impact Factors on AMF Colonization

1.5.1 Influence of Soil Properties on AMF

Soil properties play a significant role in the growth and colonization of arbuscular mycorrhizal fungi (AMF). Low phosphorus levels are crucial for regulating AMF spore germination and root colonization, whereas higher phosphorus concentrations inhibit these processes, leading to reduced AMF species richness. Soil organic matter is positively correlated with AM fungal diversity, while factors like soil pH and compaction influence the ability of AMF to infect roots and sporulate. Different AMF species exhibit optimal spore germination at varying pH levels, and soil compaction can severely restrict fungal growth by reducing oxygen availability and altering soil structure (Ricalde & Lucía, 2017; Bouamri et al., 2014; Bitterlich et al., 2020).

1.5.2 Impact of Water Depth and Seasonal Variations on AMF

AMF colonization is significantly influenced by water depth, with a strong negative correlation observed between water levels and AMF activity. Flooding conditions partially inhibit but do not completely prevent AMF colonization, suggesting that while AMF can persist in waterlogged environments, their efficiency is considerably reduced. This is particularly relevant for wetland crops

like rice, where prolonged waterlogging limits AMF root colonization (Kalamulla et al., 2022). Additionally, AMF communities exhibit seasonal variations in density and activity, with distinct differences between rainy and dry seasons, reflecting their adaptability to environmental changes (Ricalde & Lucía, 2017). Furthermore, AMF species richness is positively correlated with temperature and geographic location, favoring diversity in warmer, southern regions (Ricalde & Lucía, 2017).

1.6 Applications of AMF in Sustainable Agriculture

Arbuscular mycorrhizal fungi (AMF) are highly effective bioinoculants in sustainable agriculture, forming symbiotic relationships with a wide range of crop plants. This symbiosis enhances nutrient uptake, particularly phosphorus, promoting overall plant health. AMF propagules, such as spores or colonized root fragments, can be introduced into the soil during sowing or early plant development, facilitating early and effective root colonization (Bitterlich et al., 2020). The use of mycorrhizal inocula has led to a significant reduction in chemical fertilizers and agrochemicals in crops like maize, sorghum, and hemp, lowering production costs and reducing environmental impact (Bitterlich et al., 2020). Moreover, AMF contribute to plant viability, growth, and productivity by alleviating environmental stresses and interacting synergistically with host plants, ensuring higher food production while preserving ecological balance and environmental sustainability (Kalamulla et al., 2022).

2. *Melia volkensii*

2.1 Introduction

Melia volkensii, commonly known as Mukau in Kenya, is a highly valued indigenous tree species. This drought-tolerant, fast-growing, and multipurpose tree is indigenous to the arid and semi-arid lands (ASALs) of East Africa (Mulanda et al., 2012). M. *volkensii* is native to Ethiopia, Kenya, Somalia, and Tanzania (Figure 2) (Kew Science, n.d.). Known for its rapid growth and high-quality hardwood, *Melia volkensii* belongs to the Meliaceae family, which includes the mahogany species (Lugadiru & Wafula, 2016).

Figure 2: Countries where Melia volkensii is native (Kew Science, n.d.)

2.2 Adaption to Drylands

Melia volkensii is well-adapted to the Arid and Semi-Arid Lands (ASAL) of Kenya, primarily due to its unique drought-resistant characteristics. A key adaptation is its deep and extensive root system, which allows the tree to access water from deeper soil layers, a critical survival mechanism during extended dry periods (Mulatya et al., 2002). Additionally, *Melia volkensii* is a deciduous species, shedding its leaves during dry seasons to reduce water loss through transpiration, thereby further enhancing its drought tolerance (Kondoh et al., 2006).

2.3 Diseases

In the drylands of Kenya, *Melia volkensii* is particularly susceptible to fungal diseases caused by species within the *Botryosphaeriaceae* family. A study by Muthama et al. (2017) identified *Lasiodiplodia pseudotheobromae* as the most virulent pathogen, resulting in significant issues such as shoot die-back, cankers, and a 28% mortality rate among affected trees. Other species, including *L. theobromae* and *L. parva*, were also detected, although they exhibited less aggressive behavior.

Soil-borne diseases prevalent in the drylands of Kenya include *Fusarium oxysporum* and *Sclerotium rolfsii*, which can severely affect the growth and survival of various plants and trees (Liamngee et al.,

2015; Momanyi et al., 2021). However, there is limited research on the specific impact of these diseases on *Melia volkensii*.

2.4 Economical and Ecological Value

Melia volkensii has several desirable traits, including coppicing ability, termite-resistant wood, and suitability for dryland agroforestry. Moreover, it holds significant commercial value due to its high-quality timber, which is used in various applications such as furniture making, transport, and energy production in the southeastern drylands of Kenya (Muthike & Githiomi, 2020). This species is particularly esteemed for its diverse range of products, especially its timber, which is comparable in quality to camphor or mahogany wood and is known for its exceptional durability compared to cedar (Lugadiru & Wafula, 2016). The rapid cultivation of *Melia volkensii* for high-quality hardwood has the potential to meet the global demand for mahogany, thereby reducing the need to harvest tropical rainforests for tropical hardwood (Better Globe Forestry, n.d.). Additionally, extracts from the fruits have growth-inhibiting and antifeedant properties against various insect orders (Jaoko et al., 2020).

Melia volkensii is a highly suitable species for agroforestry, integrating effectively with most cereal crops and offering a rotation cycle of ten to twelve years (Lugadiru & Wafula, 2016). Its drought resilience and suitability for agroforestry make it a promising option for promoting sustainable livelihoods in arid and semi-arid regions. Additionally, the economic viability of cultivating *Melia volkensii* in Kenya's drylands for smallholder farmers, particularly when intercropped with green grams and processed into value-added timber products, has been emphasized by previous research (Wekesa et al., 2012).

2.5 Propagation Techniques and Challenges

2.5.1 Seed Propagation

Despite its prolific seed production, the propagation of *Melia volkensii* presents significant challenges due to the complex germination processes that require expertise and stringent measures and the low germination rate (Lugadiru & Wafula, 2016). The process of harvesting seeds is labor-intensive. The removal of the fruit pulp and the extraction of seeds from the nuts is not only time-consuming but also carries the risk of damaging the seeds, contributing to the low germination rates often observed. Furthermore, the fruit pulp contains substances that can cause skin irritation (Dushimimana et al., 2022).

Melia volkensii seeds are classified as orthodox, with their viability preserved when dried to a moisture content of 6% (Njehu et al., 2021). Additionally, these seeds exhibit a form of dormancy. To achieve optimal germination results, it is recommended that *Melia volkensii* seeds be stored in open containers at room temperature (30°C) for up to 6 months. Notably, research has shown that both the germination rate and capacity of *M. volkensii* seeds increase with extended storage time (Njehu et al., 2021).

2.5.2 Vegetative Propagation

In addition to seed propagation, *Melia volkensii* can also be propagated using root-cutting methods. However, obtaining roots from improved trees can be labor-intensive. Stem cutting propagation, however, is not feasible for *Melia volkensii* (Furumoto, 2022). In vitro propagation offers a potential solution, yet it comes with its own set of challenges. After in vitro propagation, the acclimatization of plants to harsh, arid environmental conditions is a challenging process. Plants grown in sterile in vitro conditions must re-establish balance with microbial life during this transition. These challenges significantly contribute to the high mortality rate observed during transplantation (Dushimimana et al., 2022).

2.6 Conservation Efforts and Challenges

Melia volkensii has become increasingly rare in its natural habitat, facing significant threats due to overharvesting. The tree is frequently targeted by poachers for its valuable timber and for use as firewood, leading to a substantial decline in its population in the wild (FAO, 2015). Additionally, the species exhibits poor natural regeneration through seeds, further exacerbating its vulnerability (World Agroforestry Centre, 2013).

In Kenya, efforts are actively being made to conserve this species. Plantations of *Melia volkensii* are relatively common, particularly in semi-arid regions such as Kitui, Mwingi, Machakos, and others. This species is also extensively used in reforestation projects and agroforestry systems. Young plants are typically raised in nurseries and subsequently transplanted either to designated reforestation sites or integrated into farmers' fields to enhance soil fertility, provide shade, and support sustainable agricultural practices (Gatsby Africa, 2023).

2.6.1 Definitions

Agroforestry is a land management practice that integrates trees and shrubs into agricultural landscapes for environmental, economic, and social benefits. This practice involves the deliberate combination of agricultural crops, livestock, and forestry components within the same land area, creating a more diverse, productive, and sustainable land-use system. Agroforestry systems are designed to optimize the interactions between these components, enhancing biodiversity, improving soil health, increasing water retention, and reducing erosion. Additionally, agroforestry can contribute to climate change mitigation by sequestering carbon and providing habitats for wildlife. The World Agroforestry Centre defines agroforestry as a dynamic, ecologically based natural resource management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic, and environmental benefits (World Agroforestry Centre, 2013).

A **tree nursery** is a managed site designed for the production of young trees, shrubs, and other plants. It serves as a controlled environment where plants are grown under optimal conditions to ensure healthy and robust seedlings that can later be transplanted to their permanent locations. Tree nurseries often utilize advanced horticultural techniques to propagate a wide variety of species, providing essential support for forestry, landscaping, and conservation projects. These nurseries play a critical role in the supply chain of afforestation and reforestation efforts by ensuring a steady supply

of high-quality planting material. Nurseries are fundamental for the production of seedlings used in reforestation and agroforestry practices (FAO, 2015).

A **tree plantation** refers to a large-scale, cultivated area where trees are planted and grown primarily for commercial purposes. Plantations are typically characterized by the systematic planting of tree species in rows or blocks, which facilitates efficient management and harvesting. These operations are designed to maximize the production of timber, pulpwood, and other forest products through intensive silvicultural practices. Plantations often involve the planting of fast-growing tree species that are harvested in rotation cycles, contributing significantly to the global supply of wood and wood products. The establishment of tree plantations is an essential component of sustainable forest management and industrial forestry (FAO, 2015).

Figure 3: Farmer Under Melia volkensii Tree Wearing a Shirt Displaying the Inspirational Quote: 'I will leave it better than I found it.'

3. Symbiosis Between AMF and *Melia volkensii*

3.1 AMF Genera associated with *Melia volkensii*

Melia volkensii forms associations with several genera of arbuscular mycorrhizal fungi (AMF) in the soils of arid and semi-arid regions of Kenya, including *Acaulospora* (*Acaulospora scrobiculata*), *Glomus*, *Gigaspora* (*Gigaspora margarita*), *Scuttelospora*, *ClaroideoGlomus*, and *Diversispora* (Dushimimana et al., 2022; Mutabaruka et al., 2002).

3.2 Beneficial Symbiosis between AMF and *Melia volkensii*

Previous research on the symbiosis between *Melia volkensii* and arbuscular mycorrhizal fungi (AMF) in semiarid regions of Kenya has yielded several important findings. First, the study found that *Melia volkensii* plots exhibited a significantly higher number of AMF spores in the soil compared to other tree species and treeless plots. This indicates a strong and beneficial association between *Melia volkensii* and AMF, indicating that the tree creates a favourable environment for these fungi. Second, *Melia volkensii* showed significantly higher root infection percentages by AMF compared to other tree species in the study, including *Leucaena leucocephala*, *Gliricidia sepium*, and *Senna spectabilis*. This high level of colonization reflects a robust symbiotic relationship, wherein AMF play a crucial role in enhancing the tree's root system and overall health. Additionally, the study demonstrated that *Melia volkensii* had significantly higher root water content compared to other tree species. This increased water retention is directly correlated with the level of AMF root infection. The AMF enhance the tree's ability to absorb and retain water, a trait particularly advantageous in semiarid environments where water is scarce. In conclusion, AMF not only enhance nutrient uptake but also significantly improve water retention in the roots of *Melia volkensii*. This relationship supports the tree's survival and thriving under drought conditions, making *Melia volkensii* a resilient species well-suited for agroforestry systems in drylands (Mutabaruka et al., 2002).

3.3 Impact of AMF Inoculation on *Melia volkensii* Plantlets

Another more recent study on the symbiosis between arbuscular mycorrhizal fungi (AMF) and *Melia volkensii*, conducted in the semi-arid region of Kenya, focused on the effects of inoculum application during the acclimatization phase of in vitro-cultivated plantlets. The research examined the impact of both indigenous and commercial inoculums. The study reached several significant conclusions. Firstly, inoculation with native arbuscular mycorrhizal fungi (AMF) significantly improved the survival rate of *Melia volkensii* plantlets during the acclimatization phase. Plantlets treated with AMF exhibited greater plant height, thicker stems, and a higher number of leaves compared to non-inoculated controls. Furthermore, the study found that AMF inoculation resulted in the longest roots among the treated plantlets. This suggests that AMF symbiosis greatly enhances root growth, which is essential for nutrient absorption and overall plant health. Moreover, when the inoculated plantlets were transferred to the field, they demonstrated better growth performance compared to non-inoculated plants. Specifically, AMF-treated plantlets showed larger leaf areas and greater diameter at one decimeter height, indicating a stronger establishment in semi-arid conditions. The native AMF inoculum generally performed well, sometimes even outperforming commercial inoculums. It was

particularly effective in promoting root development and root colonization, which are crucial for the survival and growth of *Melia volkensii* in semi-arid environments. In summary, the symbiosis between *Melia volkensii* and AMF proved to be highly beneficial, especially in enhancing early growth, survival rates, and overall plant quality under semi-arid conditions (Dushimimana et al., 2022).

4. Kenyan Drylands

4.1 Introduction

According to the Köppen-Geiger Climate Classification System, Kenya encompasses several distinct climate zones. Zone A, characterized as a tropical or equatorial zone, is primarily located along the coast. The majority of Kenya falls within Zone B, classified as arid or dry. This includes both hot desert climates (BWh) and hot steppe climates (BSh) (Kahi et al., 2006). Additionally, a small portion of the region around Mount Kenya falls within Zone C, a warm/mild temperate zone (National Geographic Society, n.d.). This diversity in climate zones highlights the wide range of climate types and vegetation found throughout Kenya.

The term "drylands" broadly refers to areas characterized by arid and semi-arid conditions, and in Kenya, these regions are known as "Arid and Semi-Arid Lands" (ASALs). These ASALs account for 89% of Kenya's land area (figure 4) and are home to 36% of the population (Government of the Republic of Kenya, 2012).

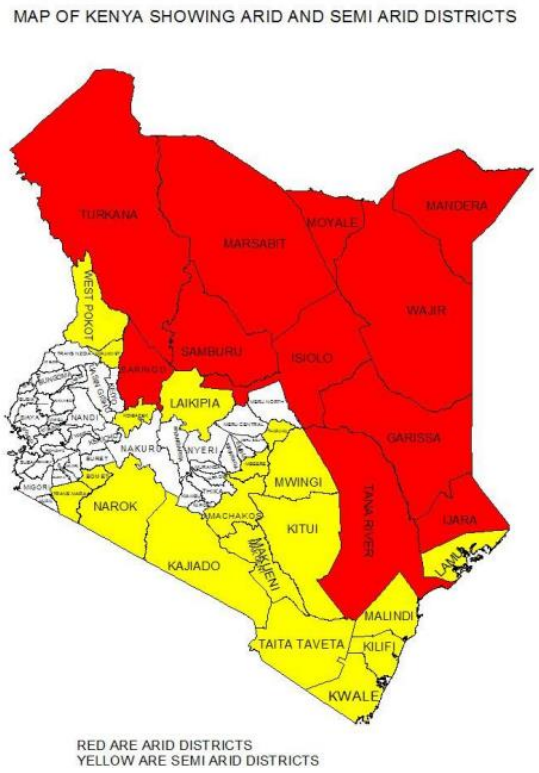

Figure 4: Kenya's Arid (Red) and Semi-Arid (Yellow) Districts (Government of the Republic of Kenya, 2012).

4.2 Climate in the Drylands

The climate in Kenya's arid and semi-arid lands (ASALs) is characterized by a bimodal rainfall pattern, with the long rainy season occurring between March and May, and the short rainy season taking place in November and December. This pattern is illustrated in Figure 5, which shows the monthly

precipitation along with the minimum, maximum, and mean surface air temperatures (Kenya Climatology, 2021).

In the arid regions, annual rainfall ranges between 150 mm and 550 mm, making these areas extremely dry. The soils in arid regions are typically sandy, stony, or rocky, with low organic content, which hinders agricultural activities. Soil fertility is generally low, and both wind and water erosion are significant concerns (Government of the Republic of Kenya, 2012).

In the semi-arid regions, annual rainfall varies between 550 mm and 850 mm. Although these areas receive more rainfall than the arid regions, the amount is still limited and often seasonal. The soils in semi-arid regions tend to be slightly more fertile than those in arid regions, ranging from sandy to clayey. These soils are better suited for limited agricultural activities, particularly when drought-resistant crops or irrigation are employed. Both arid and semi-arid regions experience high temperatures, leading to high evaporation rates, which cause water sources to dry up quickly. These climatic conditions make traditional agriculture particularly challenging in both arid and semi-arid regions (Government of the Republic of Kenya, 2012).

The predominant production system in the arid counties, and in some of the semi-arid counties, is pastoralism. However, in the semi-arid regions, a mixed economic system prevails, incorporating both pastoralism and forms of rain-fed and irrigated agriculture (Government of the Republic of Kenya, 2012).

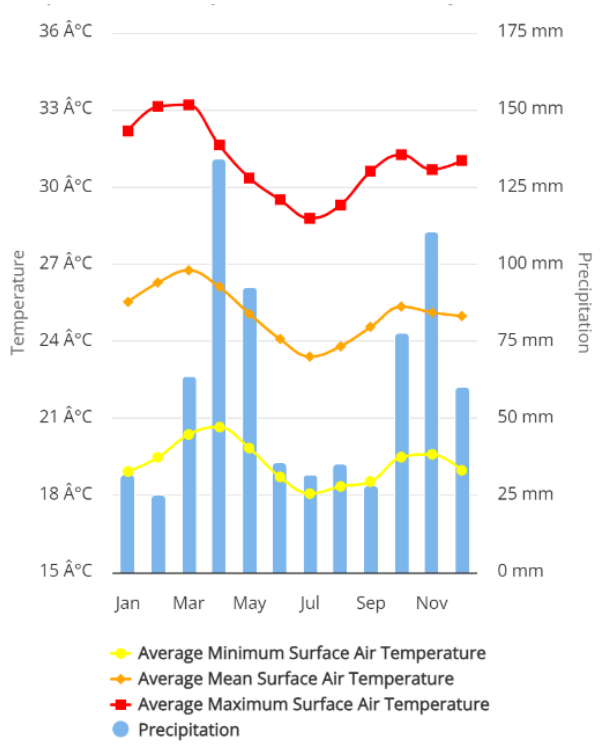

Figure 5: Monthly Climatic Patterns of Surface Air Temperature and Precipitation in Kenya (1991-2020) (Kenya Climatology, 2021)

4.3 The Impact of Climate Change on the Drylands

Climate change exerts a significant impact on drylands, exacerbating the challenges faced by these already vulnerable ecosystems. One of the most critical consequences is the rise in year-round temperatures, which accelerates evaporation and reduces soil moisture. This phenomenon complicates agricultural production and intensifies water scarcity, undermining food security in these regions. Furthermore, the intensification of rainfall during the rainy seasons leads to severe soil erosion. The typically dry soils in these areas are unable to absorb large volumes of water quickly, resulting in runoff that strips the soil of essential nutrients, further degrading land quality. Shifts in the timing of the onset and cessation of the rainy seasons also disrupt agricultural cycles, making it difficult for farmers to determine optimal planting and harvesting periods. These disruptions can lead to shortened growing seasons and reduced crop yields. The increased frequency of extreme weather events, such as heatwaves and prolonged droughts, further exacerbates land degradation, causes crop failures, and leads to livestock mortality. These climatic changes pose a significant threat to the sustainability of livelihoods in dryland regions. As climate change drives the expansion and shifting of drylands, some areas may become unsuitable for intensive agriculture and livestock rearing. This could lead to increased poverty and food insecurity, particularly for populations that rely on these areas for their livelihoods (Government of the Republic of Kenya, 2012).

4.4 The Role of Trees in the Drylands

The planting of trees in the drylands of Kenya can yield significant benefits for both the environment and local communities. Research indicates that trees play a crucial role in improving soil quality and reducing erosion. They contribute to enhanced soil structure, leading to better water retention and decreased soil erosion. Additionally, certain tree species increase organic carbon content and improve soil pH, which enhances soil fertility (Ndlovu et al., 2013). Trees also act as buffers against climate shocks such as droughts, particularly in arid regions. Their deep root systems enable them to access water and nutrients from deeper soil layers, allowing them to survive and even remain productive during dry periods (Place et al., 2016). Moreover, trees contribute to increased food security and income. Trees provide not only timber but also fruits, nuts, and other products that can bolster food security and generate additional income for local populations (Simitu et al., 2009). Integrating trees into agricultural systems, such as agroforestry, helps maintain biodiversity and increases agricultural productivity by optimizing the use of available resources, such as water and nutrients (Rabach et al., 2023).

In conclusion, the planting of trees in the drylands of Kenya offers substantial benefits in terms of soil improvement, climate resilience, food security, and sustainable agriculture. Promoting such initiatives is essential for enhancing the living conditions in these areas. Furthermore, the use of AMF inoculum can further optimize the resilience and growth of these trees.

Research questions and hypothesis

This research examines the occurrence and abundance of arbuscular mycorrhizal spores in the rhizosphere of *Melia volkensii*. The various sampling sites differ in a number of characteristics, including precipitation levels, temperature, purpose of the land, age and the origin of the tree (whether planted or naturally occurring). This leads to the formulation of the following research questions:

Q1: What species of arbuscular mycorrhizal fungi (AMF) occur in the rhizosphere of *Melia volkensii*?

Q2: Does the abundance of the spores correlate with characteristics of the tree, such as age, purpose of the land (e.g., tree in an agroforestry system, a tree in a nursery or plantation, or a tree in native vegetation), and whether the tree is planted or naturally occurring?

Q3: Does the abundance of spores vary in response to climatic features such as precipitation and temperature?

A review of the literature allows the formulation of the following hypotheses:

H1: The rhizosphere of *Melia volkensii* hosts a diverse community of arbuscular mycorrhizal fungi (AMF), including genera such as *Acaulospora*, *Glomus*, *Gigaspora*, *Scutellospora*, *Claroideoglomus*, and *Diversispora*.

H2: The abundance of AMF spores in the rhizosphere of *Melia volkensii* will be influenced by tree characteristics and land use. We expect higher spore abundance in older trees, in trees growing in native vegetation, and in naturally occurring trees compared to planted ones.

H3: The abundance of AMF spores will be positively correlated with precipitation levels and temperature, as these factors can influence both plant growth and AMF activity.

Material and methods

1. Study area

The samples for this study were taken at 14 different locations spread across three counties: Kitui County, Makueni County, and Kilifi County, see figure 6. *Melia volkensii* was found at all sites except for one severely degraded forest area where the species likely existed before. This location was still included in the data. The study locations included agricultural land where *Melia volkensii* either naturally occurred or was planted in agroforestry systems, forests, tree plantations, and Better Globe Forestry nurseries. Most of the trees were planted, with some naturally occurring.

Soil samples were collected in early July 2023, during the dry season. This diverse range of plots provides a comprehensive basis for examining the occurrence and abundance of arbuscular mycorrhizal spores in the rhizosphere of *Melia volkensii*, considering factors such as purpose of the land, tree age, and whether the trees are planted or naturally occurring. Detailed information for each sampling location can be found in the table below.

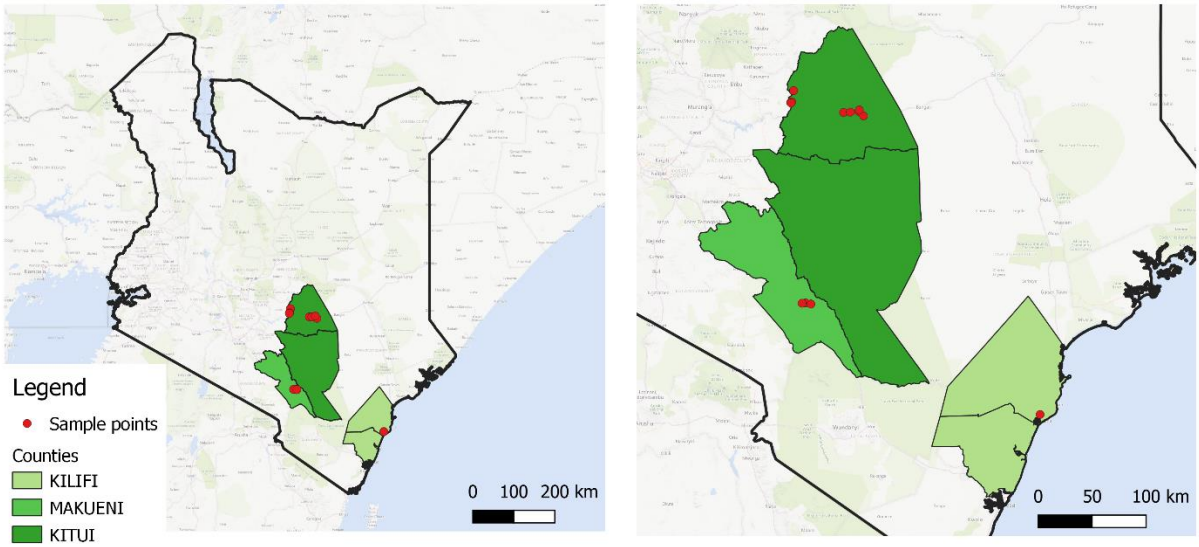

Figure 6: Map with the different sampling points and the three studied counties (QGIS, 2024).

Table 1: For each sampling location, the coordinates, location type, age of Melia volkensii, whether the tree is planted or naturally occurring, and the county are provided.

Plot ID	Location type	Wild/planted	Age *Melia v.* (years)	Coordinates	County (location)
1	Farm	Planted	6	00°35'23.1"S 37°56'06.3"E	Kitui County
2	Forest	Wild	/	00°35'20.4"S 37°56'08.2"E	Kitui County
3	Nursery	Planted	5	00°41'20.8"S 37°54'37.9"E	Kitui County (Kiambere plantation BGF)
4.1	Nursery	Planted	< 1	00°40'57.6"S 37°55'11.5"E	Kitui County (Kiambere plantation BGF)
4.2	Plantation	Planted	13	00°41'34.9"S 37°54'49.3"E	Kitui County (Kiambere plantation BGF)
5	Farm	Wild	25	00°45'55.1"S 38°28'42.1"E	Kitui County
6	Farm	Planted	3	00°48'02.9"S 38°31'04.0"E	Kitui County
7	Farm	Planted	6	00°46'18.3"S 38°21'03.7"E	Kitui County
8	Farm	Wild	10	00°46'09.6"S 38°24'30.1"E	Kitui County
9	Farm	Wild	60	00°45'04.2"S 38°28'56.6"E	Kitui County
10.1	Forest	NO MELIA	/	02°21'59.2"S 38°02'02.7"E	Makueni County
10.2	Forest	NO MELIA	/	02°22'10.4"S 38°00'16.6"E	Makueni County
11	Farm	Planted	12,5	02°22'33.5"S 38°04'43.8"E	Makueni County
12	Forest	Planted	8	03°18'01.9''S 39°59'25.5''E	Kilifi County (KWS Arabuko sokoke forest KEFRI)

Table 2 provides a comparative overview of the climatological data for Kitui, Makueni, and Kilifi counties in Kenya, where the sampling took place. The data are averages for the period 1991-2020 and include annual precipitation, precipitation during the dry season (June-August), average mean surface air temperature, and the Köppen-Geiger climate classification. The precipitation data for June-August is included in the table 2 because this is the period when the samples were collected.

All three Counties belong to semi-arid areas (Government of the Republic of Kenya, 2012). According to the Köppen-Geiger Climate Classification, the northern part of Kitui, where the sample points are located, has a Tropical Savanna Climate (Aw), similar to that of Kilifi. Makueni, on the other hand, has a Hot Semi-Arid Climate (BSh). Kilifi County exhibits the highest annual precipitation (880.33 mm) and the most precipitation during the dry months (191.46 mm). In comparison, Kitui and Makueni receive 696.07 mm and 663.34 mm annually, and 16.23 mm and 11.21 mm during the dry season, respectively. The average mean surface air temperatures of Kitui and Kilifi are similar (25.27°C and 25.56°C), and both are higher than that of Makueni (22.76°C) (Kenya Climatology, 2021).

Table 2: Annual precipitation (mm), precipitation during the dry season from June to August (mm), average mean surface air temperature (°C), and Köppen-Geiger Climate Classification of the three counties Kitui, Makueni, and Kilifi. The data is an average for the period 1991-2020 (Kenya Climatology, 2021).

County	Annual precipitation (mm)	Precipitation JUN-JUL-AUG (mm)	Average mean surface air temperature (°C)	Köppen-Geiger Climate Classification
Kitui County	696.07	16.23	25.27	Tropical savanna climate
Makueni County	663.34	11.21	22.76	Hot semi-arid climate
Kilifi County	880.33	191.46	25.56	Tropical savanna climate

2. Soil sampling procedure

At each location, three soil samples were gathered from the rhizosphere of *Melia volkensii* trees. Where possible, samples were taken from the rhizospheres of three different trees, resulting in three repetitions per location. The sampling was conducted in a triangular configuration with intervals ranging from 5 to 15 meters between sampling points. Soil samples weighing 300 grams each were collected at a depth of 30 cm using a clean mattock (Gerdemann & Nicolson, 1963). The samples were then stored in labelled, sealable paper bags. Relevant information of the site was noted, including for what purpose the land is used, age of the tree and classification as planted or wild.

3. Morphological Identification

The morphological identification of arbuscular mycorrhizal fungi (AMF) plays a crucial role in understanding and distinguishing the various species within the phylum Glomeromycota. This identification relies on specific morphological characteristics of the spores, such as color, shape, size, and the structure of the germination shield and spore wall. By comparing these characteristics with detailed species descriptions from the literature, researchers can accurately determine the taxonomic position of the fungi.

3.1 Extraction of AMF spores from the soil

Spores of Arbuscular Mycorrhizal Fungi (AMF) were isolated from soil samples using the wet sieving method (Gerdemann & Nicolson, 1963) followed by sucrose density gradient centrifugation (Brundrett et al. 1996).

Figure 7 shows the different steps of the protocol. The extraction procedure involved homogenizing soil samples to achieve a uniform composition while also breaking up large aggregates. After accurately weighing 100 grams of the homogenized soil sample, 500 ml of water was added to achieve the desired consistency, and the mixture was subjected to a double wet sieving process. Sieving was performed under a running tap. A stacked sieve system was used, consisting of an upper sieve with a 710 µm pore size and a lower sieve with a 45 µm pore size.

Material retained on the 710 µm sieve, which did not contain relevant spores, was discarded, while the material on the 45 µm sieve, containing the target spores, was retained. The retained material was placed in centrifuge tubes and centrifuged at 1850 rpm for 5 minutes. This procedure resulted in the formation of a sediment or pellet in which the spores were concentrated. After decanting the supernatant from the centrifuge tubes, a 50% sucrose solution (made up of 100 g of granulated sugar and 200 ml water) was added to the tubes and shaken. The tubes were then centrifuged for 1 minute at 1850 rpm to separate the spores into the supernatant.

The enriched supernatant was gently poured through a 45-micron sieve under running water, leaving behind a residue containing concentrated spores, which was then transferred to a petri dish filled with water. The procedure described here ensures the precise extraction and concentration of AMF spores. The process was replicated three times for each sample site.

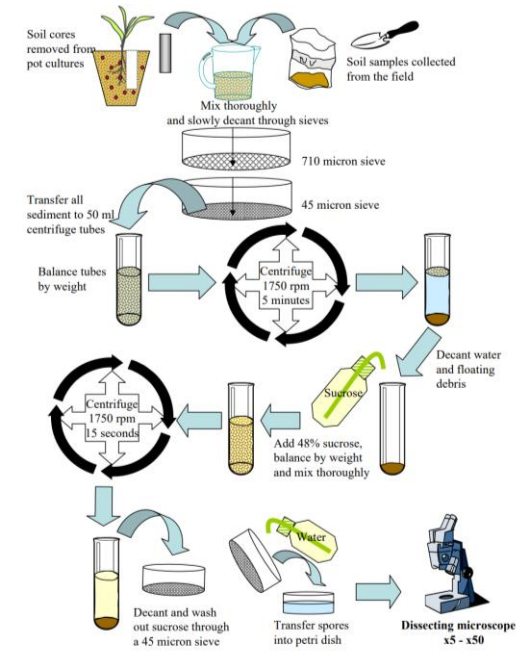

Figure 7: Extraction of AMF spores from the soil (Ingleby, 2007)

3.2 Preparation of diagnostic (microscope) slides for AMF spores

Identification of Arbuscular Mycorrhizal (AM) spores requires the preparation of permanent slides that provide detailed information on the spore wall, hyphae, germinal shield, and other germination characteristics(Brundrett et al., 1996). This process involves a series of steps performed under a dissecting microscope. The Petri dish initially contains AMF spores mixed with soil particles and organic debris. The spores are isolated manually using forceps and sorted by morphotype based on color, then transferred to cavity dishes.

Properties such as color (determined by a color chart [Edinburgh, 1969]), size (small, medium, large), shape (globose, subglobose, ellipsoid), appearance (shiny, dull), presence of hyphae (present, absent), sporocarp (yes, no), and the number of spores per morphotype are recorded for each morphotype (Brundrett et al., 1996). Spore density (SD) is calculated as follows: SD is defined as the number of AMF spores per 100 grams of soil (Krishnamoorthy et al., 2015).

To prepare a diagnostic microscope slide, mount 1-2 spores of each morphotype using a few drops of both polyvinyl-lactoglycerol (PVLG) and Melzer + PVLG mountants. Melzer's reagent can cause a staining reaction in the inner or outer layers of certain species. This staining reaction is crucial for differentiating various species of AMF, as different species can exhibit varying intensities and colors in response to Melzer's reagent, aiding in their identification (Brundrett et al., 1996). Spores on microscope slides must be crushed under the cover slip to reveal inner-wall layers for identification. Label the slide, and allow the mountant to dry (Brundrett et al., 1996).

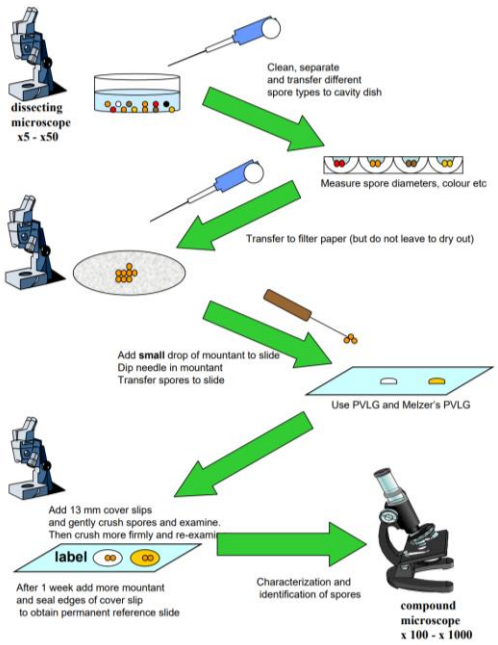

Figure 8: Preparation of diagnostic slides of AMF spores (Ingleby, 2007)

3.3 Identification of spores

Characterization and identification of the spores were conducted under a compound microscope using the 10X to 40X objective. The key morphological characteristics for identifying arbuscular mycorrhizal fungi (AMF) include spore color, shape, and size, germination shield structure, spore wall structure, hyphal structures, and staining reactions in Melzer's solution (Redecker, 2013). The observed morphological characteristics were compared with detailed species descriptions from the papers by Redecker (2013) and Oehl et al. (2011). These studies aim to distinguish the various species within the phylum Glomeromycota based on both morphological and molecular characteristics of the spores. Extensive and up-to-date species descriptions are available on the INVAM website. The AMF spores were identified to the genus level using the INVAM classification system, based on Redecker (2013).

4. Molecular Identification

Molecular identification of arbuscular mycorrhizal fungi (AMF) is a critical technique for accurately distinguishing species within the phylum Glomeromycota, particularly when morphological characteristics are insufficient or ambiguous. This method involves the analysis of genetic markers, such as ribosomal RNA genes, which contain unique sequences that enable the differentiation of closely related species. By employing techniques such as polymerase chain reaction (PCR), DNA sequencing, and phylogenetic analysis, researchers can obtain precise genetic profiles of AMF species. These molecular tools not only complement traditional morphological methods but also provide a more comprehensive and reliable framework for the identification and classification of AMF, thereby enhancing our understanding of their biodiversity and ecological roles.

4.1 DNA extraction

For each soil sample, 300 mg was taken for DNA extraction. Total soil DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following to the manufacturer's protocol (Korir, 2023). The extracted DNA was quantified and its quality was verified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) (Tchiechoua et al., 2022). The DNA was stored at –20° C for further analysis.

4.2 DNA amplification

DNA amplification was performed using the nested PCR protocol described by Ferrol and Lanfranco (2020), which was optimized through iterative trial and error. This protocol enhances the specificity and sensitivity of the amplification process by employing a combination of primer pairs (Green & Sambrook, 2019). The primers used are listed in Table 3. Initially, a broader primer pair, AML1/AML2, was utilized to amplify a large fragment of the target DNA, approximately 800 base pairs in length. This primer pair targets the small subunit rDNA (SSU rDNA, 18S), a highly conserved gene valuable for studying distantly related organisms. It is often selected because the available primer pairs can recover the majority of AMF families (Ferrol & Lanfranco, 2020). Subsequently, the primers AMADF/AMDGR were employed in a second PCR round to achieve a more specific amplification of a smaller fragment, approximately 420 base pairs. This approach improves the accuracy of AMF detection and minimizes the likelihood of non-specific amplifications. The primer pairs AMADF and AMDGR are widely used in AMF research, often in combination with AML1/AML2 (Ferrol & Lanfranco, 2020).

Table 3: Primer Sequences and Their Melting Temperatures (Tm) (Ferrol & Lanfranco, 2020)

Primer	Type	Nucleotide Sequence (5'–3')	Melting temperature (Tm) (°C)
AML1	Forward	5'- ATC AAC TTT CGA TGG TAG GAT AGA - 3'	53.2
AML2	Reverse	5'- GAA CCC AAA CAC TTT GGT TTC C - 3'	54.9
AMADF	Forward	5'-GGG AGG TAG TGA CAA TAA ATA AC -3'	50.8
AMGDR	Reverse	5'- CCC AAC TAT CCC TAT TAA TCA T -3'	49.3

4.2.1 First PCR step

The DNA was amplified in 50 µl reaction volume composed of 10 µl green buffer, 1 µl of 10 mM dNTP, 1 µl of both 10 µM forward primer (AML1) and 10 µM reverse primer (AML2), 0.5 µl Go Taq, 26.5 Milli-Q water and 10 µl of DNA template (Table 4).

Thermocycling conditions for the first PCR step (table 5) were as follows: initial denaturation at 95 ^{0}C for 10 min, followed by 35 cycles at 94 ^{0}C for 1 min, 57 ^{0}C for 1 min, 72 ^{0}C for 1 min, and a final extension step at 72 ^{0}C for 7 min.

Table 4: Composition of reaction mixture used for first PCR step.

Compounds	**µL**
Green buffer	10
10 mM dNTP	1
10 µM forward primer (AML1)	1
10 µM reverse primer (AML2)	1
Go Taq	0.5
Milli-Q water	26.5
DNA (2 ng/µl)	10

Table 5: Thermocycling conditions for first PCR step

Step	**Time**	**Temperature (°C)**	**Repeat**
Denaturation	10 min	95	1 x
Denaturation	1 min	94	35 x
Annealing	1 min	57	35 x
Extension	1 min	72	35 x
Final Extension	7 min	72	1 x

4.2.2 Second PCR step

A second nested PCR step was performed to further enhance the specificity and sensitivity of the amplification (Green & Sambrook, 2019). The PCR products from the first step served as the template for this nested PCR step. The second step of the nested PCR reaction was conducted in a 50 µl reaction mixture, which comprised 10 µl of green buffer, 1 µl of 10 mM dNTP, 1 µl each of 10 µM forward primer (AMADF) and 10 µM reverse primer (AMDGR), 0.125 µl of Go Taq polymerase, 26.875 µl of Milli-Q water, and 10 µl of DNA template (2 ng/µl) (Table 6).

Thermocycling conditions for the second PCR step (Table 7) were as follows: initial denaturation at 95 ^{0}C for 10 min, followed by 30 cycles at 94 ^{0}C for 40 sec, 53 ^{0}C for 40 sec, 72 ^{0}C for 45 sec, and a final extension step at 72 ^{0}C for 7 min.

Table 6: Composition of reaction mixture used for second PCR step.

Compounds	**µL**
Green buffer	10
10 mM dNTP	1
10 µM forward primer (AMADF)	1
10 µM reverse primer (AMGDR)	1
Go Taq	0.125
Milli-Q water	26.875
DNA (2 ng/µl)	10

Table 7: Thermocycling conditions for first PCR step

Step	**Time**	**Temperature (°C)**	**Repeat**
Denaturation	10 min	95	1 x
Denaturation	40 sec	94	30 x
Annealing	40 sec	53	30 x
Extension	45 sec	72	30 x
Final Extension	7 min	72	1 x

4.3 Gelelektroforese

To verify the quality of the PCR reaction, gel electrophoresis was performed. A 1.5% agarose gel was prepared in 1X TAE buffer (Table 8). The gel was created by weighing out 0.84 g of AGAR ISP and dissolving it in 56 ml of 1X TAE. This solution was heated, poured into the mold, and allowed to cool. The samples were prepared by combining 11 µl of PCR product, 2.5 µl of Loading Buffer (Table 9), and 1.5 µl of 10X GelRed. Ladder of 100 bp, used as a reference was prepared, containing 3 µl 100 bp ladder, 3 µl 6X loading dye, 2 µl Gel red and 2 µl of 1X TAE buffer. The samples and the ladder were loaded into the wells of the agarose gel, and the gel was run at 130V for 20 minutes. DNA migration was then visualized under ultraviolet light using a transilluminator in a dark room (Ferrol & Lanfranco, 2020).

Table 8: Composition of 10X TAE Buffer (pH 7.8) (Ferrol & Lanfranco, 2020).

10X TAE Buffer, pH 7.8		
Component	**Concentration**	**Amount**
Tris	400 mM	24.2 g
$Na_2EDTA.2H_2O$	20 mM	3.7 g
$NaAc.3H_2O$	200 mM	13.6 g
Hac	296 mM	8.8 ml
Destilled H_2O		Add to 500 ml

Table 9: Composition of Loading Buffer (Ferrol & Lanfranco, 2020).

Loading Buffer	
Component	Concentration
Sucrose	40%
Glycerol	30%
Bromofenolblauw	0.25%
H_2O	

4.4 PCR Products Purification & Sequencing

DNA purification was performed using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The samples were prepared for Illumina MiSeq sequencing and sent to the extern laboratory to perform the sequencing. The Illumina MiSeq sequencing approach was utilized to identify a broad range of arbuscular mycorrhizal fungi (AMF) and to assess the diversity and structure of rhizosphere AMF communities associated with *Melia volkensii*. Illumina MiSeq sequencing provides a powerful method for addressing AMF diversity and variations within fungal assemblages (Morgan & Egerton-Warburton, 2017). Findings indicate that Illumina MiSeq, supplemented with the appropriate primers, represents the most effective method for AMF identification (Kryukov et al., 2020).

5. Statistical analysis

Statistical analyses were conducted using Jamovi (version 2.3.28), with a consistent significance level of $\alpha = 0.05$. These analyses aimed to investigate the variability and influence of various factors on the spore density of arbuscular mycorrhizal fungi (AMF) in the soil.

First, the normality of the data was evaluated using QQ plots and the Shapiro-Wilk test. The results of this test indicated that the data were not normally distributed, which provided the basis for using non-parametric statistical techniques in subsequent analyses.

To examine the relationship between spore density and various continuous variables, including tree age, annual precipitation (mm), and average mean surface air temperature (°C), the Spearman's Rank Correlation Coefficient (Spearman's rho) test was applied. This non-parametric test was chosen due to the non-normality of the data and provided insight into the strength and direction of the correlations between spore density and these variables.

For the analysis of nominal data, such as location type (Farm, Forest, Nursery, Plantation), the distinction between planted and naturally occurring trees (Planted vs. Naturally Occurring Trees), and different climate types (tropical savanna and hot semi-arid), the Kruskal-Wallis Test (Non-parametric One-Way ANOVA) was employed. This test was used to identify significant differences in spore density between the different groups. When the Kruskal-Wallis test indicated significant differences, the Dwass-Steel-Critchlow-Fligner Pairwise Comparisons test was used as a post-hoc test to further investigate pairwise significant differences between the groups.

Additionally, a linear regression analysis was conducted to assess the influence of various independent variables, such as tree age, location type, the status of the trees (Wild/Planted), annual precipitation, average mean surface air temperature, and climate type, on spore density. This regression analysis provided detailed insights into the contribution of each variable to the prediction of spore density. The overall fit of the model was evaluated using the F-test, which determined the significance of the model as a whole.

6. Artificial Intelligence (AI)

This thesis was composed with the assistance of Artificial Intelligence, specifically ChatGPT (OpenAI, California, USA). The AI was primarily employed to translate and summarize sources and literature, as well as to identify and list key points. Additionally, AI was utilized to enhance and optimize the written text, as English is not my native language.

Results & Discussion

1. Diversity of Arbuscular Mycorrhizal Fungi (AMF) spores

1.1 Morphological Identification

Figure 9 shows the various arbuscular mycorrhizal fungi (AMF) spores, belonging to the phylum *Glomeromycota*, which were isolated from the rhizosphere of *Melia volkensii*. The morphological identification of the isolated spores was conducted at the genus level. The analysis identified several families of AMF based on morphological characteristics: *Glomeraceae*, *Acaulosporaceae*, and *Gigasporaceae*. These families all belong to the order *Glomerales*. Within the family *Glomeraceae*, the genus *Glomus* was identified. Within the family *Acaulosporaceae*, the genus *Acaulospora* was identified. Within the family *Gigasporaceae*, three genera were identified: *Scutellospora*, *Gigaspora*, and *Dentiscutata*. These identifications bring the total number of identified genera to five. Although the specific species within these genera were not identified, Figure 9 clearly shows a differentiation between the spores of different species within these genera: *Glomus* (3 species), *Gigaspora* (2 species), *Dentiscutata* (1 species), *Scutellospora* (3 species), *Acaulospora* (5 species).

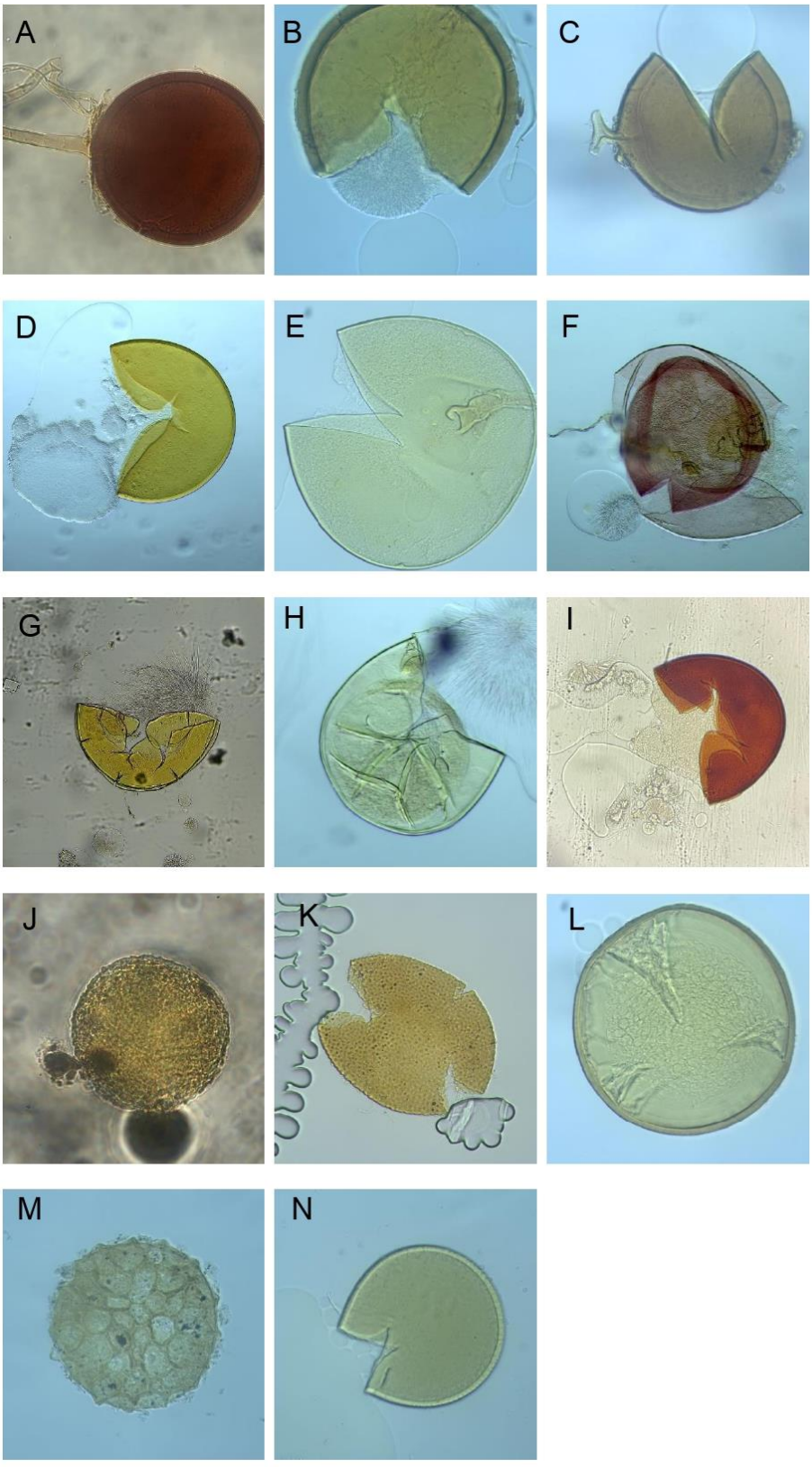

Figure 9: Morphological Diversity of AM Fungi Spores Isolated from the Rhizosphere of Melia volkensii, Identified to Genus Level (Magnification: X40). A, B, C: Glomus sp.; D, E: Gigaspora sp.; F: Dentiscutata sp.; G, H, I: Scutellospora sp.; J, K, L, M, N: Acaulospora sp.

1.2 Molecular Identification

1.2.1 Gel electrophoresis

To assess the quality of PCR amplification from soil samples, gel electrophoresis was conducted. The first PCR, using the primer pair AML1/AML2 (~800 bp), was successful for samples 4 and 8, as indicated by bands matching the position of the positive control, confirming the amplification of the target DNA fragment (~800 bp). No band was observed for sample 9, indicating failed amplification, while sample 12 showed smearing, suggesting DNA degradation or contamination.

In the second PCR step with the primer pair AMADF/AMGDR (~420 bp), multiple bands appeared around the expected position. Despite this, the sample was considered suitable for sequencing. The presence of a prominent band at the expected size (~420 bp) suggests successful amplification of the target DNA, and the specificity of the primers likely ensured correct region targeting. Therefore, the sample was considered suitable for sequencing, especially for gaining a broad understanding of the genetic material present.

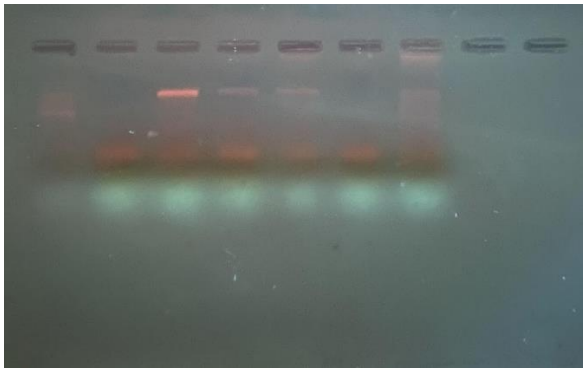

Figure 10: Gel Electrophoresis Results of the First PCR Step. Primers: AML1/AML2. From left to right; ladder, blank control, positive control, sample 4, sample 8, sample 9, sample 12.

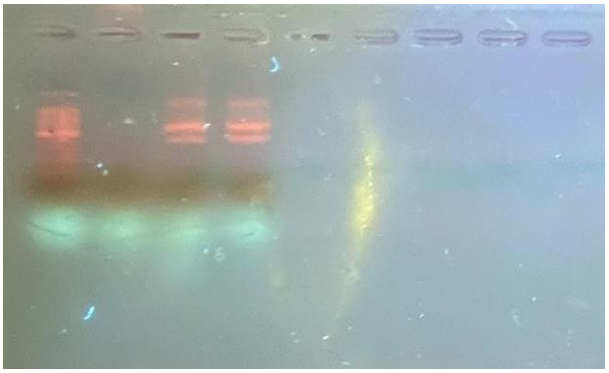

Figure 11: Gel Electrophoresis Results of the Second PCR Step. Primers: AMADF/AMGDR. From left to right; ladder, blank control, sample 4, sample 8.

1.2.2 Sequencing

Due to administrative and technical issues the sequences could not be obtained before the final submission date of this thesis.

Discussion

The findings of this research largely validate existing literature on the associations between *Melia volkensii* and arbuscular mycorrhizal fungi (AMF) in semi-arid regions of Kenya. In this study, five AMF genera were identified: *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, and *Dentiscutata*. This aligns with previous studies, which also recognize *Glomus*, *Acaulospora*, *Gigaspora*, and *Scutellospora* as common genera associated with *Melia volkensii* (Dushimimana et al., 2022; Mutabaruka et al., 2002).

Notably, however, the genus *Dentiscutata* was identified in this research, whereas it is not specifically mentioned in the existing literature. Conversely, the literature reports the presence of the genera *Claroideoglomus* and *Diversispora* (Dushimimana et al., 2022; Mutabaruka et al., 2002), which were not detected in this study. These discrepancies may be attributed to variations in local soil conditions or differences in the methods used for the isolation and identification of AMF spores. Additionally, the timing of sample collection could play a role; the literature suggests that not all species sporulate year-round (Bouamri et al., 2014).

The presence of *Dentiscutata* in this study could also be explained by the surrounding vegetation near the *Melia volkensii* trees. It is possible that AMF spores were extracted from the rhizosphere of other nearby vegetation, rather than being in direct symbiosis with *Melia volkensii*. Therefore, the association between the extracted *Dentiscutata* spores and *Melia volkensii* may not represent a true symbiosis. To confirm this, further research is necessary, including DNA extraction and analysis of *Melia volkensii* root samples to determine if a genuine symbiotic relationship exists.

In summary, the findings from morphological identification support the known symbiotic relationships between *Melia volkensii* and AMF, while also indicating the potential presence of less frequently reported genera like *Dentiscutata*. Comparing these results with molecular identification is crucial, as molecular methods offer a more precise identification of species present in the rhizosphere, providing a clearer and more comprehensive understanding of AMF diversity associated with *Melia volkensii*.

2. Spore Abundance and Tree Characteristics

2.1 Spore Density Across Different Locations

Tables 10 and 11, along with Figure 12, present descriptive statistics and a graphical representation of the spore density of arbuscular mycorrhizal fungi (AMF) in soil samples collected from the rhizosphere of *Melia volkensii*. For each sample site (identified by Plot ID), the average spore density of three replicates was calculated and is recorded in Table 10 and visually depicted in the bar plot (Figure 12).

The spore density of AMF in these soil samples varied from 5 to 169 per 100 g of soil. The data in Table 10 reveal significant variation in the mean spore density across different plots, with values ranging from 9.0 to 101. The highest mean density is observed in Plot 10.1, while the lowest density is recorded in Plot 9.0.

This variability is visually represented in Figure 12, where the bar plot illustrates the average spore density for each Plot ID. The error bars in the graph indicate the range of variation around these means, further confirming the substantial differences between plots. Certain plots, such as 10.1 and 4.2, exhibit noticeably higher spore densities compared to others.

Table 11 provides a summary of the overall descriptive statistics for spore density across all plots. The mean spore density is 29.0, with a median of 21.5. The standard deviation is 29.5, reflecting considerable variability in spore density among the different plots. Spore density ranges from a minimum of 5 to a maximum of 169.

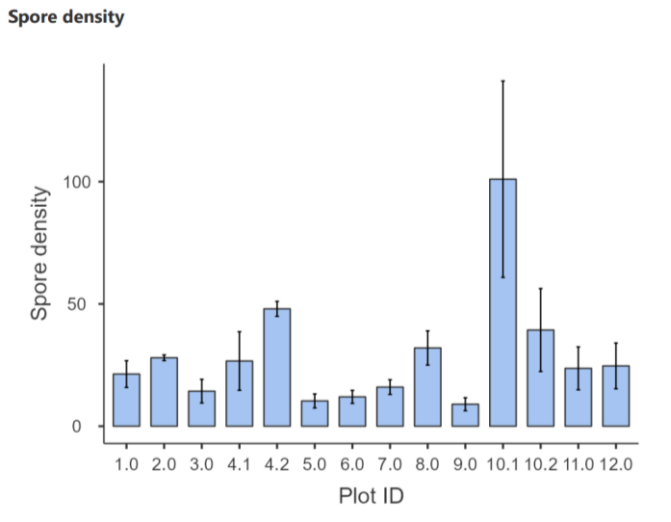

Figure 12: Bar plot representing the average spore density of AMF for each sample location (plot ID)

Table 10: Mean spore density of AMF for each sample location (plot ID)

Descriptives

	Plot ID	Spore density
Mean	1.0	21.3
	2.0	28.0
	3.0	14.3
	4.1	26.7
	4.2	48.0
	5.0	10.3
	6.0	12.0
	7.0	16.0
	8.0	32.0
	9.0	9.00
	10.1	101
	10.2	39.3
	11.0	23.7
	12.0	24.7

Table 11: descriptive statistics for spore density across all plots

Descriptives

	Spore density
Mean	29.0
Median	21.5
Standard deviation	29.5
Variance	871
Minimum	5
Maximum	169

Discussion

A standard deviation of 29.5, as shown in Table 11, reveals significant variability in spore density across different plots, with some areas having notably higher concentrations than others. This finding suggests that local factors, such as the specific location or environmental conditions around a tree, play a key role in influencing fungal spore levels in the soil.

Previous research supports this, showing that spore density can vary widely based on local environmental conditions, including soil moisture and nutrient availability (Bouamri et al., 2014). Moreover, spore density is also influenced by climatic factors, with studies indicating that spore density and mycorrhizal colonization intensity increase with seasonal rainfall and decrease as air temperatures rise (Meddad-Hamza et al., 2017).

However, we cannot directly compare the exact spore densities and mean spore densities with those reported in other studies. This is largely due to the variability in the methods used to measure spore density, such as wet sieving and decanting, which can differ significantly between studies, making direct comparisons challenging (Ricalde & Lucía, 2017).

2.2 Correlation Between Spore Abundance and Tree Characteristics

2.2.1 Age of the Tree

Table 12 presents the correlation matrix between spore density and tree age, analysed using Spearman's rank correlation coefficient (Spearman's rho). Prior to conducting the correlation

analysis, the normality of the continuous data, including spore density and tree age, was assessed using QQ-plots and the Shapiro-Wilk test. The Shapiro-Wilk test results indicated p-values of less than 0.005 for both spore density and tree age, suggesting that the data are not normally distributed. Given the non-parametric nature of the data, Spearman's rank correlation was employed to assess the relationship between the variables.

The analysis reveals a Spearman's rho value of 0.024 for the relationship between spore density and tree age, indicating a very weak positive correlation. However, this correlation is not statistically significant, as evidenced by the p-value of 0.901, which is far above the conventional significance threshold ($p < 0.05$). With 28 degrees of freedom (df), the results suggest that there is no meaningful relationship between spore density in the rhizosphere and the age of the trees in this study.

Table 12: Correlation Matrix Between Spore Density and Tree Age Using Spearman's Rank Correlation

Correlation Matrix

		Spore density	Age tree
Spore density	Spearman's rho	—	
	df	—	
	p-value	—	
Age tree	Spearman's rho	0.024	—
	df	28	—
	p-value	0.901	—

Note. * p < .05, ** p < .01, *** p < .001

Discussion

This study examined whether the number of arbuscular fungal spores around a tree changes as the tree ages. The correlation analysis revealed a very weak and statistically insignificant relationship (Spearman's rho = 0.024, p = 0.901) between spore density and tree age. These results suggest that tree age does not have a significant impact on spore density in the soil. In contrast, previous research, such as a study from northern Ethiopia, reported a significant increase in AMF spore density and root colonization with the age of forest exclusion areas, with older areas (15-20 years) showing higher spore densities compared to younger ones (less than 5 years) (Birhane et al., 2017).

The differences in these findings could be due to variations in ecosystems and environmental factors, such as soil type, climate, and vegetation. The Ethiopian study looked at forest exclusion zones, which might have different ecological characteristics than the single tree environments studied here.

2.2.2 Location type

Table 13, 14 and the accompanying box plot (figure 13) provide an analysis of spore density across different location types (Farm, Forest, Nursery, and Plantation) using non-parametric statistical

methods. Given that the data do not follow a normal distribution, as previously assessed, the Kruskal-Wallis test (Non-parametric One-Way ANOVA) was employed to evaluate differences in spore density among the four location types.

The results of the Kruskal-Wallis test, shown in Table 13, indicate a significant difference in spore density across the location types, with a chi-square (χ^2) value of 13.5 and a p-value of 0.004. This suggests that at least one location type differs significantly from the others in terms of spore density.

Following the significant Kruskal-Wallis test, Dwass-Steel-Critchlow-Fligner pairwise comparisons were conducted to identify which specific pairs of location types show significant differences in spore density. As shown in Table 14, significant differences were observed between Farm and Forest (W = 3.946, p = 0.027) and between Farm and Plantation (W = 3.770, p = 0.039). These results indicate that spore density is significantly higher in Forest and Plantation compared to Farm. Other pairwise comparisons did not reveal statistically significant differences.

Table 13: Kruskal-Wallis Test (Non-parametric One-Way ANOVA) of Spore Density Across Location Types.

Kruskal-Wallis

	χ^2	df	p
Spore density	13.5	3	0.004

Table 14: Dwass-Steel-Critchlow-Fligner Pairwise Comparisons of Spore Density Across Location Types.

Pairwise comparisons - Spore density

		W	p
Farm	Forest	3.946	0.027
Farm	Nursery	0.496	0.985
Farm	Plantation	3.770	0.039
Forest	Nursery	−2.652	0.239
Forest	Plantation	1.840	0.562
Nursery	Plantation	2.921	0.165

The box plot (Figure 13) visually represents the distribution of spore density across the four location types. The plot demonstrates variability in spore density, with Forest and Plantation showing higher median values and greater spread compared to Farm and Nursery. The presence of outliers, particularly in the Forest and Plantation groups, further highlights the variability in spore density within these locations.

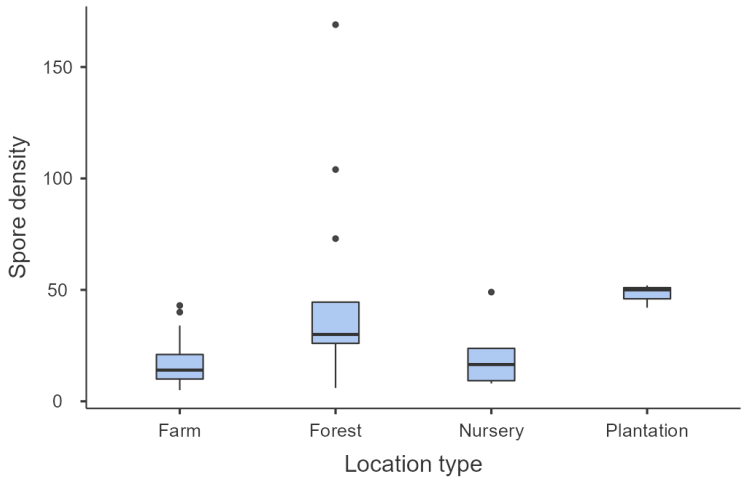

Figure 13: Box Plot of Spore Density Across Different Location Types

Discussion

This analysis looked at whether the number of arbuscular fungal spores was different depending on where the trees were growing; on a farm, in a forest, in a nursery, or in a plantation. They found that the location does matter. Forests and plantations had significantly more spores than farms, while nurseries didn't show a clear difference from any of the other locations.

Forests have mature vegetation, older trees and diverse plant species, providing stable, nutrient-rich conditions that promote AMF spore production. The accumulation of organic matter and minimal soil disturbance further enhance spore density (Birhane et al., 2018). Plantations also support higher spore densities due to controlled management practices, consistent vegetation, and reduced soil disruption. These factors create favorable conditions for AMF colonization (Plenchette et al., 2005). In contrast, farms often experience frequent soil disturbance from plowing and other agricultural activities, which disrupts mycorrhizal networks and reduces spore density. The use of fertilizers and pesticides, along with monoculture practices, further limits AMF populations (Verbruggen et al., 2012).

2.2.3 Planted vs. Naturally Occurring Trees

Table 15 and the accompanying box plot (Figure 14) provide an analysis of spore density in the rhizosphere of *Melia volkensii*, comparing trees that were either planted by humans (Planted) or naturally occurring without human intervention (Wild). Given that the data do not follow a normal distribution, as previously assessed, the Kruskal-Wallis test (Non-parametric One-Way ANOVA) was employed to evaluate differences in spore density between these two groups.

The results of the Kruskal-Wallis test, as shown in Table 15, indicate no significant difference in spore density between the Planted and Wild trees, with a chi-square (χ^2) value of 0.474 and a p-value of 0.491. This suggests that the spore densities in the rhizosphere of *Melia volkensii* trees do not differ significantly based on whether the trees were planted by humans or naturally occurred in the wild.

Table 15: Kruskal-Wallis Test (Non-parametric One-Way ANOVA) of Spore Density Between Planted and Wild Melia volkensii Trees.

Kruskal-Wallis

	χ^2	df	p
Spore density	0.474	1	0.491

The box plot (Figure 14) visually represents the distribution of spore density in the rhizosphere of Planted and Wild *Melia volkensii* trees. The plot shows that both Planted and Wild trees have similar median spore densities, with some variation in the spread of the data. However, consistent with the statistical analysis, there is no substantial difference in spore density between the rhizospheres of trees that were planted by humans and those that occurred naturally.

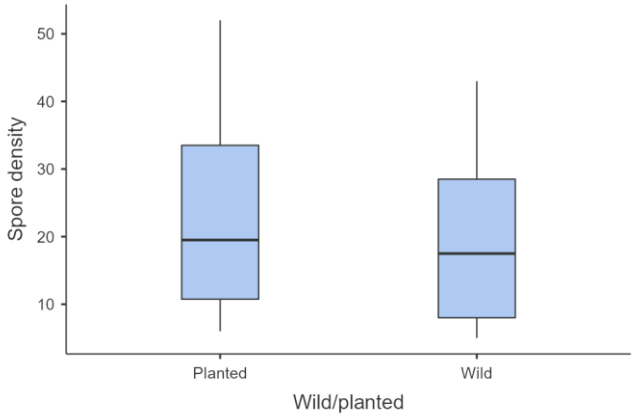

Figure 14: Box Plot of Spore Density in the Rhizosphere of Planted and Wild Melia volkensii Trees.

Discussion

The results suggest that whether a *Melia volkensii* tree was planted or grew naturally does not significantly affect the number of arbuscular fungal spores in its surrounding soil. The Kruskal-Wallis test showed no significant difference in spore density between the two groups ($\chi^2 = 0.474$, $p = 0.491$).

Research shows that the presence of AMF is often more dependent on the overall vegetation structure and soil conditions than on how the trees are established (Smith & Read, 2008). In both human-planted and naturally occurring trees, similar soil conditions can exist that support AMF. These findings have practical implications for reforestation and agroforestry. Planting *Melia volkensii* can effectively replicate the natural conditions necessary to foster beneficial mycorrhizal associations, contributing to improved soil health and ecosystem sustainability.

3. Spore Abundance and Climatic Factors

3.1 Correlation Between Spore Abundance and Climatic Factors

3.1.1 Precipitation and temperature

Table 16 presents a correlation matrix analysing the relationships between spore density, annual precipitation (mm), and average mean surface air temperature (°C). Prior to conducting the correlation analysis, the normality of these continuous variables was assessed using QQ-plots and the Shapiro-Wilk test. The Shapiro-Wilk test results indicated p-values of less than 0.005 for all variables, suggesting that the data are not normally distributed. Consequently, Spearman's rank correlation coefficient (Spearman's rho), a non-parametric measure, was employed to assess the relationships between these variables.

The correlation matrix shows that there is a negative correlation between spore density and both annual precipitation and average mean surface air temperature, with Spearman's rho values of -0.265 in both cases. However, the p-values for these correlations are 0.090, indicating that they are not statistically significant at the conventional alpha level of 0.05. This means there's a slight tendency for fewer spores to be present in areas with more rain or higher temperatures. However, this trend isn't strong enough to be considered statistically significant, meaning we can't confidently say it's a true pattern rather than just random chance.

There is a perfect positive correlation (Spearman's rho = 1.000) between annual precipitation and average mean surface air temperature, with a highly significant p-value of less than 0.001. This suggests that these two variables are perfectly correlated in this dataset, which might be due to the specific environmental conditions.

Table 16: Correlation Matrix Between Spore Density and Annual precipitation (mm), Average mean surface air temperature (°C) Using Spearman's Rank Correlation

Correlation Matrix

		Spore density	Annual precipitation (mm)	Average mean surface air temperature (°C)
Spore density	Spearman's rho	—		
	df	—		
	p-value	—		
Annual precipitation (mm)	Spearman's rho	−0.265	—	
	df	40	—	
	p-value	0.090	—	
Average mean surface air temperature (°C)	Spearman's rho	−0.265	1.000 ***	—
	df	40	40	—
	p-value	0.090	< .001	—

Note. * p < .05, ** p < .01, *** p < .001

Discussion

The analysis explored the correlation between spore density, annual precipitation, and average mean surface air temperature, revealing a slight negative correlation with both climatic factors (Spearman's rho = -0.265). Although this suggests that spore density may decrease in areas with higher rainfall and temperatures, the lack of statistical significance (p = 0.090) indicates that this trend is not strong enough to confirm a definite relationship.

The absence of a strong, statistically significant correlation is somewhat surprising, as previous research has shown that climatic factors often have a clear influence on spore density. Studies indicate that spore density fluctuates with water availability and precipitation patterns. Seasonal rainfall can stimulate root growth, promoting the germination of AMF spores and fungal colonization, often resulting in reduced spore density in the soil due to increased colonization activity. More spores are found in drier, harsher environments (Bouamri et al., 2014; De Oliveira & De Oliveira, 2010). This partially aligns with the observed trend in this study, where increased precipitation was associated with lower spore density, although this was not statistically significant.

Furthermore, AMF species richness has been found to correlate positively with temperature and geographic location, favoring diversity in warmer, southern regions (Ricalde & Lucía, 2017). This suggests that while temperature may influence AMF diversity, it does not necessarily result in higher spore density, as observed in this study.

The perfect positive correlation between annual precipitation and average mean surface air temperature (Spearman's rho = 1.000) could indicate that in this particular dataset, precipitation and temperature are not independent variables but are instead strongly linked, possibly due to seasonal patterns or geographic factors.

In conclusion, while there appears to be a slight tendency for spore density to decrease with higher precipitation and temperature, this study does not provide strong evidence to support this as a definitive pattern. This may be due to the small sample size. Further research with larger sample sizes or different environmental conditions may be necessary to clarify the relationship between climatic factors and spore abundance.

3.1.2 Climate

The analysis presented in table 17 and figure 15 examines spore density in relation to different climate types, as classified by the Köppen-Geiger Climate Classification. Specifically, the study compares spore density between two climate types: the tropical savanna climate, which is found in Kitui and Kilifi Counties, and the hot semi-arid climate, characteristic of Makueni County.

To evaluate the differences in spore density between these climates, a Kruskal-Wallis test (Non-parametric One-Way ANOVA) was conducted, given that the data did not meet the assumptions for normal distribution. The results indicate a significant difference in spore density across the climate types, with a chi-square (χ^2) value of 4.29 and a p-value of 0.038. This suggests that the type of climate

has a statistically significant effect on spore density and that spore density is notably higher in the hot semi-arid climate compared to the tropical savanna climate.

Table 17: Kruskal-Wallis Test (Non-parametric One-Way ANOVA) of Spore Density Between Hot Semi-Arid Climate and Tropical Savana Climate

Kruskal-Wallis

	χ^2	df	p
Spore density	4.29	1	0.038

The box plot provides a visual representation of the spore density distribution across these two climate types, further illustrating the higher spore density observed in the hot semi-arid climate compared to the tropical savanna climate. This analysis underscores the influence of climate on spore density, with significant differences observed between the regions studied.

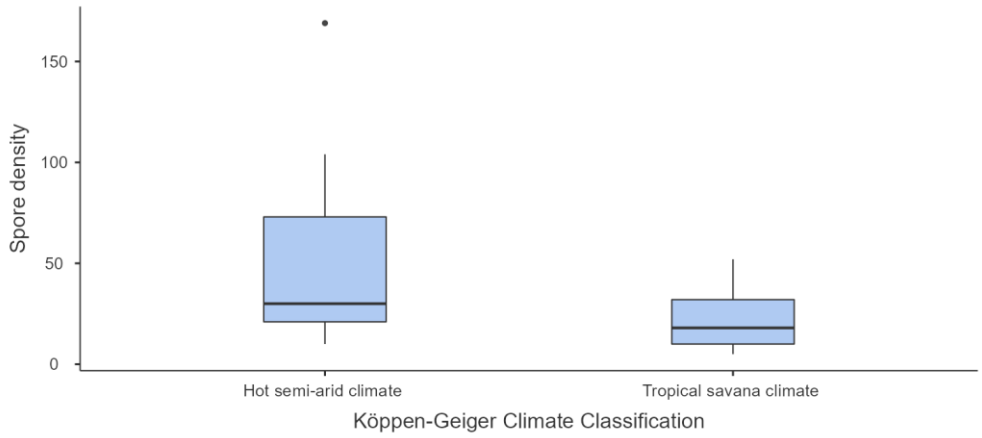

Figure 15: Box plot of Spore Density Between Tropical Savanna and Hot Semi-Arid Climates.

Discussion

This analysis specifically compared spore density in two climate types: the tropical savanna climate found in Kitui and Kilifi Counties, and the hot semi-arid climate characteristic of Makueni County. The results indicate that climate significantly influences spore density, with higher densities observed in the hot semi-arid climate. The Kruskal-Wallis test (χ^2 = 4.29, p = 0.038) confirms a statistically significant difference between these climates.

The higher spore density in the hot semi-arid climate can be attributed to the harsher environmental conditions, which may favor the sporulation of arbuscular mycorrhizal fungi (AMF). This may be a survival strategy of fungi to protect themselves against unfavorable conditions (Allen, 2007). The literature also confirms that climatic factors, such as temperature and precipitation, play a crucial role in regulating AMF spore density. Previous research indicates that, generally, mycorrhization levels are higher in subhumid and humid climates, like Tropical Savana climate, compared to semi-arid and desert climates, like Hot Semi-Arid climate. The effect this has on spore density is not mentioned (Meddad-Hamza et al., 2017).

Conclusion

This thesis underscores the vital role of arbuscular mycorrhizal fungi (AMF) in the resilience and growth of *Melia volkensii* in Kenya's drylands. Through comprehensive field and laboratory analyses, the study identified five AMF genera—*Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, and *Dentiscutata*—within the rhizosphere of *Melia volkensii*, revealing both expected and novel fungal associations. The findings indicate that spore density is significantly influenced by environmental factors such as climate type, with higher densities observed in hot semi-arid climates. This suggests that AMF may adopt a survival strategy by producing more spores under harsher conditions, enhancing their resilience against environmental stresses.

The study also found that spore density varied across different land use types, with forests and plantations showing higher spore densities than farms. Surprisingly, no significant difference in spore density was found between planted and naturally occurring *Melia volkensii* trees, suggesting that both conditions can foster beneficial mycorrhizal associations if other environmental factors are favorable. Furthermore, the analysis showed no statistically significant correlation between spore density and factors like precipitation and temperature, indicating that other local environmental factors might also play crucial roles in regulating spore density.

These results have important theoretical and practical implications. Theoretically, they contribute to our understanding of AMF ecology in arid and semi-arid environments, highlighting the complex interactions between fungi, host plants, and environmental conditions. Practically, the findings can inform reforestation and agroforestry strategies by emphasizing the importance of promoting AMF associations to enhance tree survival and growth in challenging climates. By integrating AMF inocula into nursery practices and reforestation projects, the resilience of *Melia volkensii* and other native tree species can be improved, thereby supporting ecosystem sustainability and enhancing the livelihoods of local communities.

However, the study has some limitations. The reliance on spore density as a measure of AMF activity may not fully capture the complexity of AMF-plant interactions. The lack of correlation between spore density and mycorrhizal colonization confirmed that spore counting is not a reliable indicator of mycorrhizal infectivity. Additionally, the limited sample size may affect the generalizability of the findings. Future research should focus on larger-scale studies and incorporate more detailed molecular analyses of AMF communities. We have identified which AMF species are present in the rhizosphere of *Melia volkensii*, but further research is needed to determine whether these species have positive effects on the tree's resilience to climate change, drought resistance, and soil-borne diseases. Moreover, future studies should investigate how to best formulate and apply these AMF inoculums to *Melia volkensii*. Examining the long-term effects of AMF inoculation on *Melia volkensii* growth and survival across different environmental conditions would provide valuable insights for optimizing reforestation and agroforestry practices in dryland regions.

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