DIVERSITY AND COMPOSITION OF FUNGAL COMMUNITIES IN SOILS OF PROTECTED AND NON-PROTECTED AREAS IN SINGIDA REGION, TANZANIA

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ABSTRACT

Soil is a crucial natural resource that provides vital services for ecosystem sustainability (Yadav *et al.,* 2021). However, human activity and climate change are causing the health of the soil to decline (Devi *et al.*, 2020; Kirui, 2021). In Tanzania, especially in areas like Singida where human activity worsens the condition, 37% of the soil exhibits low degradation, 54% is moderately declined, and 9% is severely degraded (URT, 2018; Kirui, 2021; Hake *et al.*, 2024). Beneficial soil microorganisms, which are essential for plant growth, uptake of nutrients, and retaining water, are under threat due to this deterioration, underscoring the necessity of researching how land use affects fungal communities and soil health (Basset, 2024).

This study focused on assessing soil fungal diversity and composition in the soils of protected and non-protected areas in Itigi District, Singida Region, Tanzania. The isolated *Penicillium chrysogenum* and *Trichoderma harzianum* were tested in pot experiment for their ability to promote plant growth and enable soil restoration. A cross-sectional study design provided a snapshot of community awareness regarding soil fungi conservation from both site types. A structured questionnaire was administered to 150 randomly selected household for insights. Soil samples were collected using systematic and purposive sampling techniques, resulting in 60 composite samples across the study area.

Descriptive statistical analysis revealed that male respondents averaged 67.35%, while female respondents averaged 32.65%. Furthermore, 81.8% of respondents were farmers, with those aged 41 and above (54.55%) being predominant. Fungal diversity was higher in protected areas, with species like *Trichoderma viride* (37.5%), *Penicilium chrysogenum* (35%), *Trichordema koningiopsis* (32.2%), and *Trichoderma harzianum* (29.65%) thriving in stable conditions, while non-protected areas exhibited resilient fungi such as *Fusarium soloni* (29.1%), *Aspergillus terrus and Aspergilus niger,* with 28.97% and 27.14%, respectively indicating adaption to disturbed environments. Inoculation with *Penicillium chrysogenum* significantly enhanced wild finger millet (*Eleusine indica)* growth, underscoring its potential to improve soil health and overall land productivity.

DECLARATION

I, Regina Jacob, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it neither has been submitted nor is concurrently submitted elsewhere for degree award in any other institution.

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CERTIFICATION

The individual below confirm that they have reviewed and endorse the examination of the dissertation titled "*Diversity and composition of fungal communities in soils from protected and non-protected areas in Itigi District, Singida region, Tanzania*". This is submitted as part of the requirements for obtaining a Master of Science degree in Biodiversity and Ecosystem at Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This work is dedicated to my cherished Sisters of Charity of St. Vincent de Paul, my beloved siblings, and my late parents, Jacob Ighembe and Veronica Ng'imba. May their souls rest in peace for eternity Amen.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
CV	Coefficient of Variation
DMRT	Duncan's Multiple Range Test
GPS	Global Positioning System
LSD	Least Significant Difference
NCA	*Amaranthus spinosus* + Negative Control treatment
NCE	*Eleusine indica* + Negative Control treatment
NM-AIST	Nelson Mandela African Institution of Science and Technology
Ns	Not significant
PAB	*Amaranthus spinosus* + *Penicillium spp*. with maize bran treatment
PAM	*Amaranthus spinosus* + *Penicillium* spp. with millet seed treatment
PCA	*Amaranthus spinosus* + Positive Control
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PEB	*Eleusine indica* + *Penicillium chrysogenum* with maize bran treatment
PEM	*Eleusine indica* + *Penicillium chrysogenum* with millet seed treatment
SD	Standard Deviation
TAB	*Amaranthus spinosus* + *Trichoderma spp*. with maize bran treatment
TEB	*Eleusine indica* + *Trichoderma spp*. with maize bran treatment
TEM	*Eleusine indica* + *Trichoderma spp*. with millet seed treatment
TN	Total Nitrogen
TOC	Total Organic Carbon
URT	United Republic of Tanzania

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Soil, the skin of the Earth is one of the essential natural resource and significant constituent that contributes to the ecosystem sustainability (Yadav *et al.,* 2021). Soil is essential for life, water filtration, climate regulation, food, fiber, and fuel resources (Telo da Gama, 2023; Basset, 2024). It forms the foundation for terrestrial ecosystems, providing nutrients and water for plants, protecting rivers and groundwater from pollutants, and regulating water cycles (Timmis & Ramos, 2021; Detheridge *et al.,* 2016). Soil ecosystem amenities are vital for agronomic production, biodiversity preservation, and nutrient cycling, contributing to the globular bio-economy's benefits like resource use competence, reduced waste, and economic chances (Stavi *et al.,* 2016; Pereira *et al.,* 2018). Soil provides numerous services for human well-being, counting security, protection from environmental shocks, access to balanced diets, purifies water and air, and energy for temperature control (Anikwe & Ife, 2023; McMichael *et al.,* 2005). However, the contribution of soils in ecosystem services is declining due to increasing anthropogenic pressures and climate change that lead to soil degradation, threatening global well-being (Basset, 2024).

Soil degradation is a global crisis affecting about 40% of the Earth's land, with Africa experiencing severe degradation of over 46% of its territory, costing $9.3 billion annually (Kirui, 2021). Major contributors to soil degradation include land use changes such as unsustainable agricultural practices deforestation, and climate which negatively impact ecosystem services and biodiversity change (Nkonya *et al.,* 2016). Annually, 12 million hectares of agricultural soil are lost due to degradation, affecting beneficial soil microbes, including fungi essential for soil health and optimum plant growth (Ziadat *et al.,* 2022; Wang, 2022). Agriculture and deforestation, driven by the increasing global food demand, contribute to 80% of deforestation and biodiversity loss, further harming beneficial soil fungi and the overall ecosystem health (Barlow *et al.,* 2016; Pete *et al.,* 2019). Further, about 23% of the world's land is unproductive, necessitating rehabilitation, with contributing factors including land use changes, unsustainable agricultural production system and climate change (Berchoux *et al.,* 2019; Blanko & Lal, 2023). Soil microbes such as fungi, bacteria, oomycetes, are vital for soil health and life, fixing atmospheric nitrogen, producing plant growth hormones,

degrading contaminants, and breaking down organic matter to create stable soil organic carbon (Wang *et al.,* 2024).

Soil fungi are crucial microorganisms that significantly contribute to the health and function of terrestrial ecosystems, with a varied range of species performing diverse ecological roles (Tedersoo *et al.,* 2014; Essene *et al.,* 2017). Beneficial fungi, which comprise the majority of soil microbial communities, are important for maintaining soil health, helping plant growth, and improving ecosystem resilience to environmental stressors (Devi *et al.,* 2020; Jagadesh *et al.,* 2024). Soil fungi including mycorrhizal, saprotrophic, and bio-control fungi, work together to improve plant health and land productivity by enabling water and nutrient uptake, decomposing organic matter, and suppressing disease-causing pathogens (Mehta *et al.,* 2016). According to the study done by Gayathri *et al.* (2021), stated that the soil fungi biomass, particularly hyphae and fruiting bodies, increase soil organic carbon accumulation to 71%, aiding in humus production, potentially mitigating climate change. Mycelia networks of fungi in soils improve water infiltration and retention, reduce soil erosion, and form extensive associations with plant roots (Barrios *et al.,* 2023). They also regulate greenhouse gases by sequestering or transforming gases in soil, and contribute to air purification through bio-filtration (Giltrap *et al.,* 2021). In the bio fertilizer market, mycorrhizae accounted for 36.3% in 2022, valued at USD 995.3 million, while rhizobia, the second most common bio-fertilizer, held a 24.8% market share in North America in 2022 (Wei *et al.,* 2024).

Sustainable soil conservation practices promote an increase in microbial richness in the ecosystem (Mondaca *et al.,* 2024). In contrast, unsustainable land use causes soil degradation negatively impacting beneficial microbial fungi population and encourages pathogenic fungi, which make up a smaller percentage (1-5%) of the overall soil fungal community worldwide (Ayangbenro *et al.,* 2022) ; Mohammadiani, 2024). Pathogenic fungi can cause numerous plant diseases, reducing crop yields and compromising ecosystem stability (Pandey *et al.,* 2017). Soil-borne plant pathogenic fungi cause diseases like root rot, stem rot, crown rot, damping-off, and vascular wilts, causing significant economic losses in the ecosystem worldwide (González *et al.,* 2020). Examples include *Fusarium* spp., which causes root rot wilt and tomato and potato early blight in various crops, and *Phytophthora* spp, responsible for sudden oak death and potato blight (Desprez-Loustau, 2009).

In Tanzania, the Local Government Authorities reports varying levels of soil degradation across different regions. According to the findings by URT-LDNT (2018) and URT (2018),

9% of the areas are extremely degraded, 54% are categorized as medium degraded, and the remaining 37% had low levels of degradation. In Singida region, soil degradation is reported to be extremely high due to various anthropogenic activities (URT, 2018; Hake *et al.,* 2024). The region provides a unique opportunity to examine the effects of various land use regime on soil fungal communities.

Approximately 80 to 90% of all plants form symbiotic relationships with fungi forming hyphae networks (Yuvaraj & Ramasamy, 2020). Many plants prefer a fungus-to-bacteria ratio of approximately 10:1 in forested soils, which helps trees grow better (Li *et al.,* 2021). However, areas such as Itigi in Singida region, limited studies on the abundance and distribution of fungi hinder soil conservation efforts. Understanding fungal distribution in these areas is crucial for effective ecosystem management, sustainable agricultural practices, and environmental conservation in both protected and non-protected zones. Therefore, this study explored fungal diversity and composition in the soils of protected and non-protected areas in Itigi District, Singida Region, Tanzania by: (a) Evaluating the influence of community knowledge, land use practices on soil fungi; (b) Assessing the diversity of fungal communities in soils from both protected and non-protected areas, and (c) Identifying and testing potentials of beneficial fungi isolates in promoting plant growth. The findings provide insights for conservation of soil fungi and sustainable land management strategies, fostering more informed decision-making in land use and ecosystem protection.

1.2 Statement of the problem

Soil microorganisms like fungi are crucial for ecosystem health, playing key roles in nutrient cycling, decomposition, and plant health, including disease suppression, plant growth promotion as well as nutrient uptake enhancement. However, many soils in semi-arid regions, such as those in Singida region, have been reported to experience a significant decline in soil microbial populations (URT-LDNT, 2018; URT, 2018). The decline in soil microbial populations is described to be a major contributor of soil degradation, since 80 to 90% of plants depend on soil microbial communities particularly the soil fungi (Hoorman, 2009; Bogati & Walczak, 2022; Zhang *et al.,* 2023; Wang *et al.,* 2023). Soil degradation affects 40 to 46% of land productivity and costing $9.3 billion annually, causing poverty in agrarian communities (Nkonya *et al.,* 2016).

Despite the decline of soil microbial populations, particularly fungi, in semi-arid regions like Singida, which is largely assumed to be driven by human activities, with serious implications for land productivity, the environment, and the livelihoods of farming communities little has been done to address this challenge. Therefore, this study aimed to address fungal diversity and composition in the soils of protected and non-protected areas in Itigi District, Singida Region, Tanzania by: (a) examining the impact of community knowledge, land use practices on soil fungi; (b) assessing fungal diversity in soils of protected and non-protected areas; (c) identifying and testing potentials beneficial soil fungi. The findings offer valuable insights to guide conservation efforts and sustainable land management, supporting better decisions for land use and ecosystem protection

1.3 Rationale of the study

Understanding the diversity of soil fungal communities in the Itigi district, particularly in protected and non-protected areas, is crucial for effective ecosystem management. Soil fungi, which make up to 90% of soil organisms and are vital for plant growth and survival, play essential roles in nutrient cycling, organic matter decomposition, and plant-fungal symbioses (Van der Heijden *et al.,* 2008; Tedersoo *et al.,* 2014; Fall *et al.,* 2022). Fungal communities also contribute significantly to biogeochemical cycles, such as organic matter turnover and nitrogen fixation (Ramond *et al.,* 2022; Lavergne *et al.,* 2023). Despite their importance, research on soil fungal diversity in regions like Singida, Tanzania, is limited, with most studies focusing on soil fertility. Investigating and contrasting fungal populations in protected and non-protected regions may yield important information about their ecological advantages, supporting ecosystem resilience and soil health (Prober *et al.,* 2015; Coletta *et al.,* 2023). This research informs better land-use strategies in semi-arid areas like Singida and contributes to the broader understanding of land use, soil biodiversity, and ecosystem services in Tanzania's semi-arid regions, supporting informed decision-making for conservation and sustainable land management.

1.4 Research objectives

1.4.1 General objective

To assess the diversity of fungal communities in soils from protected and non-protected areas in Itigi district, Singida region.

1.4.2 Specific objective

(i) To assess the effect of community-based knowledge on land use practices on soil fungi distribution in protected and non-protected areas.

(ii) To determine the diversity of fungal communities in soils from protected and non-protected areas.

(iii) To identify and test potential beneficial soil fungi isolates from -protected and non-protected areas.

1.5 Research questions

(i) How do community-based knowledge on land use practices affect soil fungi distribution in protected and non-protected areas?

(ii) How does the fungal community diversity differ in soils from protected and non-protected areas?

(iii) What are the potential beneficial soils fungi present in soils from protected and non-protected areas?

1.6 Significance of the study

This study provides valuable insights into the diversity of fungal communities in the soils of the Itigi district, for effective soil and land management planning. The findings identify potential beneficial fungi that can be harnessed for sustainable agricultural practices, revegetation, and ecological restoration efforts in the region. Furthermore, the study offers comprehensive information that can assist policymakers and conservation strategists in maintaining ecosystem integrity. The results contribute to the scientific understanding of the complex relationships between land use, soil microbial communities, and ecosystem functioning, particularly in semi-arid environments.

1.7 Delineation of the study showing solutions and outcomes

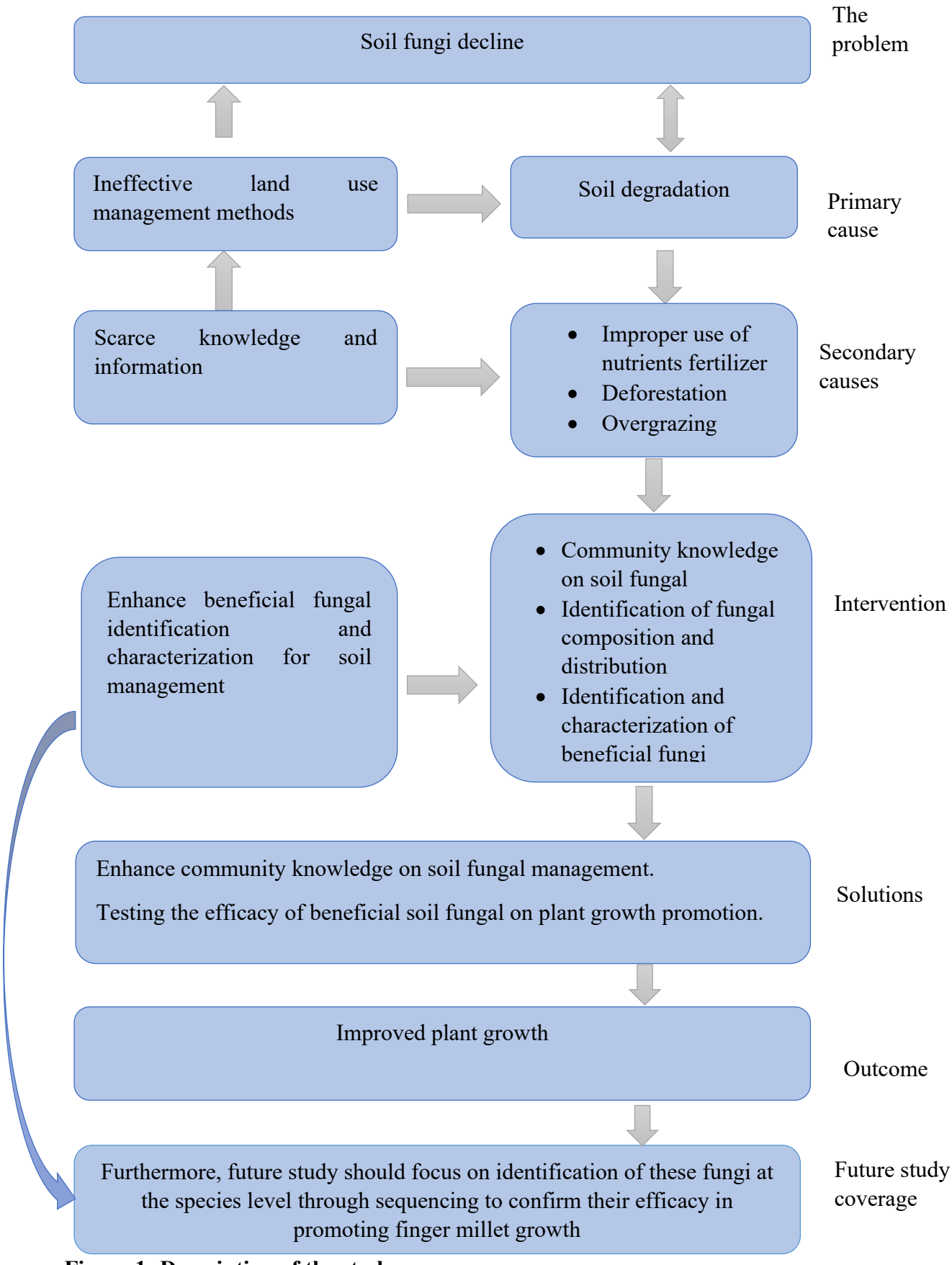


Figure 1: Description of the study

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of microbes

Microbes, including bacteria, archaea, viruses, fungi, protozoa, and algae, are microscopic and are the earliest forms of life on earth, found in various environments and their presence significantly impacts their environment (Maraz & Khan, 2021). The majority of the planet's microbial mass is found in oceans, comprising between 50 and 90 percent of the ocean's biomass (Gasol & Kirchman, 2018). Microbes colonize all living things on dry land, with 1 g of superficial soil containing more than 10^9 bacterial and archaeal cells, trillions of viruses, tens of thousands of protists and 200 million of fungal hyphae (Rappuoli *et al.,* 2023). The airborne microbes that commonly originate from soil and water through the liquid air and soil air interface form bio aerosols contributing up to 25% to atmospheric aerosols (Chen *et al.,* 2020).

Throughout Earth's long history, microorganisms have played a unique role in the movement of materials and energy, fostering the formation and advancement of human civilization (Zhao *et al.,* 2020). The most significant effect of the microbes on earth is their ability to recycle the primary elements that make up all living systems, especially carbon, oxygen, and nitrogen (Gupta *et al.,* 2017; Prasad *et al.,* 2021). Microbes also support many industrial activities, starting from traditional fermentation of bread, cheese, beer, wine, to the production of chemicals, energy, enzymes, pharmaceuticals, and to waste treatment and pollution control where humans use their ability of microbes to degrade materials including fossil oils and plastics (Rappuoli *et al.,* 2023).

In the soil ecosystems, of all the microorganisms, fungi form up to 90% whereas, 80-90% of plants rely on soil microbial communities, particularly soil fungi for growth (Hoorman, 2009; Tedersoo *et al.,* 2014; Větrovský *et al.,* 2020; Lavergne *et al.,* 2023; Jagadesh *et al.,* 2024). Fungi, play significant role in decomposing organic matter, forming symbiotic relationships with plant roots, and enhancing soil structure, complete the complex network that sustains soil health and productivity (Liu *et al.,* 2020; Zhang *et al.,* 2023; Hartmann & Six, 2023). They enhance soil fertility; fix nitrogen even after death, contributing to carbon sequestration and climate change reduction by forming 30-50% of soil organic matter (Luo *et al.,* 2020; Hemkemeyer *et al.,* 2021). Fungi are the most important for soil formation and decomposition of organic matter (Van der Heijden *et al.,* 2008; Tedersoo *et al.,* 2014; Husain *et al.,* 2023).

Despite the important role played by soil fungal communities in the ecosystems, over recent decades, increased human activities and climate change on the planet have affected population of this important microbe on the earth, and not much has been done to address the challenge.

2.2 Soil fungal community composition and diversity

Soil fungi constitute one of the most diverse groups of organisms on Earth, second to insects in terms of known diversity (Pérez-Moreno *et al.,* 2021; Access, 2024). Soil ecology and management influence their diversity and composition (Luo *et al.,* 2021; Rakić *et al.,* 2022). For example, in forest soils, fungi are hyper-diverse, naturally entailing a few dominant and many less abundant species that encompass some of the major fungal phyla, Basidiomycota, Ascomycota, Glomeromycota, Chytridiomycota, and Zygomycota (Othman *et al.,* 2022; Pérez-Izquierdo *et al.,* 2022). Protected forests support important fungal diversity serving as a key indicator of terrestrial biodiversity and ecosystem health, which differs significantly with that of non-protected areas due to environmental and human-induced factors (Avigliano *et al.,* 2019; Rakić *et al.,* 2020). Protected areas often have higher fungal diversity and richness because they experience fewer disturbances like land conversion and overgrazing (Rakić *et al.,* 2020).

Earlier studies e.g., by Pereira *et al.* (2017) found that more than 85% of the soil fungal species were present in protected as compared to non-protected areas. In agricultural soils, fungi account for more than half of the total microbial biomass, which is a substantial contribution to the overall microbial biomass of the environment (Moreno *et al.,* 2021). The fungi are the link in the production due to their potential pathogenicity on crops chains and the capacity to fix nutrients (Moreno *et al.,* 2021). Conversely, non-protected areas (disturbed) exhibit reduced fungal diversity due to human activities that lead to soil degradation (Scramoncin *et al.,* 2024). Non-protected (disturbed) areas are better inhabited with opportunistic fungi that thrives in disturbed conditions but lack the ecological roles of specialized fungi found in protected areas (Tomao *et al.,* 2020). Functional groups such as mycorrhizal fungi, which help plants absorb nutrients, are typically more prevalent in protected areas, contributing to healthier plant communities (Latgé,a & Chamilos, 2019). In non-protected areas, human impacts can lead to increased pathogen presence, which can harm plant health and ecosystem stability (Coletta *et al.,* 2023). However, there is still limited information about soil fungal community composition and diversity in some areas including Itigi District.

2.3 Ecological role of soil fungi

Soil fungi are indispensable for maintaining soil health, fertility, and overall ecosystem stability due to their multifaceted roles (Fall *et al.,* 2022). Classification of soil fungi based on whether they are beneficial or non-beneficial, helps in understanding their diverse roles and contributions to soil health, fertility. Ecosystem stability, providing insights for ecological research and sustainable land management practices (Rodriguez-Sanchez *et al.,* 2021).

2.4 Beneficial soil fungi

The majority of fungi that live in soil generally have direct and indirect benefits to human (Tedersoo *et al.,* 2014; Frąc *et al.,* 2018; Zanne *et al.,* 2018). Soil fungi are very successful inhabitants of soil, due to their high flexibility and their capacity to adapt various environment in response to adverse or unfavorable conditions (Coleine *et al.,* 2022). They have key roles in soil health and fertility, which is considered one of the most important characteristics of soil ecosystems (Frąc *et al.,* 2018). Considering that soil is a living system and that many processes and features interact to produce soil health, the integrated approach to soil health has a significant impact on the activity of soil microbiota (Rinot *et al.,* 2019). For example, most soil fungi are saprotrophs that play roles in the decomposition and stabilization of organic materials in soil (Zanne *et al.,* 2018; Shourie & Vijayalakshmi, 2022). By promoting nutrient cycling, enhancing soil structure, decreasing erosion, and preserving nutrients and water in plant root zones, this has significant effects on natural production. (McKenna *et al.,* 2020). The following are some important key complex roles played by soil fungi that contribute to overall ecosystem stability:

2.4.1 Nutrient cycling

Fungi break down complex organic matter into simpler chemical compounds through decomposition process (Shourie & Vijayalakshmi, 2022). This process releases essential soil nutrients like nitrogen, phosphorus, and potassium back into the soil, making them available for plant uptake (Prakash *et al.,* 2014; Tunlid *et al.,* 2022). By facilitating nutrient cycling, fungi help sustain plant growth and land productivity (Prakash *et al.,* 2014; Bhattacharyya & Furtak, 2022). Examinations of the ectomycorrhiza fungus Paxillus involutus and Laccaria bicolor have revealed links between the breakdown of organic materials and the release of organic nutrients, as well as how these processes are controlled by nutritional signals (Tunlid *et al.,* 2022; Chauhan *et al.,* 2023). *Aspergillus niger* a common soil fungus often found in

decaying plant material decomposes organic matter and contributes to the breakdown of complex carbohydrates (Mousav *et al.,* 2016). *Mucor spp.* a genus of fast-growing molds commonly found in soil that decomposes organic substrates, playing a role in nutrient cycling (Li & González, 2008). Others include *Penicillium spp*., *Trichoderma spp*., *Coprinus comatus*, *Pleurotus ostreatus*, and *Fusarium spp*. are all effective decomposers that promote plant growth, suppress pathogens, and decompose organic matter in soil (Li & González, 2008; Srinivasan *et al.,* 2020; Thaha *et al.,* 2020; Ocimati *et al.,* 2021).

2.4.2 Soil structure and stability

Microorganisms like fungi produce extracellular polymeric substances that are crucial for soil structure, health, and fertility (Ali *et al.,* 2024; Costa *et al.,* 2018; Shar *et al.,* 2019). The fungi body, and the mycelium helps in binding soil particles together, creating aggregates (Rashid *et al.,* 2016; Costa *et al.,* 2018; Shar *et al.,* 2019). This aggregation improves soil structure, enhancing aeration and water infiltration (Costa *et al.,* 2018; Yang *et al.,* 2024). A well-structured soil is less prone to erosion and compaction, promoting a healthier environment for plant roots and microorganisms. Fungi, including Mycorrhizal Fungi like *Glomus* spp*., Rhizophagus* spp., and *Laccaria* spp., play a crucial role in soil structure and stability (Li & González, 2008; Li *et al.,* 2022). They form symbiotic relationships with plant roots, enhancing nutrient uptake and soil structure (Zhang *et al.,* 2021; Li *et al.,* 2022; Wang *et al.,* 2022). Saprophytic Fungi like *Trichoderma* spp. and *Pleurotus* spp. *Aspergillus* spp., *Fusarium* spp., decompose organic matter, contributing to soil organic carbon and stability (Li & González, 2008; Ty´skiewicz *et al.,* 2022). Ectomycorrhizal Fungi like *Amanita* spp. and *Boletus* spp. associate with tree roots, improving nutrient cycling and forming a dense mycelia network (Liu *et al.,* 2020; Dyshko *et al.,* 2024).

2.4.3 Mycorrhizal associations

The most important type of mycorrhizae is arbuscular mycorrhizae, which form symbiotic relationships with terrestrial plant roots and are found in over 90% of terrestrial plants (Wahab *et al.,* 2023; Coque *et al.,* 2020). Many plants form symbiotic relationships with mycorrhizal fungi (Ty´skiewicz *et al.,* 2022). These fungi extend the root system's capacity to absorb water and nutrients beyond the root zone, which in return, they derive carbohydrates from the plants (Liu *et al.,* 2020; Dyshko *et al.,* 2024). This relationship enhances plant resilience to drought and nutrient-poor conditions, contributing to ecosystem stability (Liu *et al.,* 2020; Dyshko *et*

al., 2024). Mycorrhizal associations are essential for plant health and soil ecology (Dyshko *et al.,* 2024). Examples of plants that form symbiotic relationships with mycorrhizal fungi include pine trees, oak trees, birch trees, arbuscular mycorrhizae, fungi like *Glomus spp*., and *Rhizophagus irregularis* (Heklau *et al.,* 2021). For field crops like corn, wheat, and soybeans form an association with ericoid mycorrhizae, fungi like *Hartigia* spp., and *Oidiodendron* spp. (Brody *et al.,* 2019), whereas in orchards, blueberries form an association with orchid mycorrhizae, fungi like *Epulorhiza spp*., and *Rhizoctonia spp*. (Formica, 2014; Ding *et al.,* 2014).

2.4.4 Disease suppression

Certain soil fungi can suppress plant pathogens by outcompeting them for resources or producing antimicrobial compounds (Prakash *et al.,* 2014; Wang & Kasyanov, 2024). This biological control helps maintain plant health and reduces the need for chemical pesticides, promoting a more sustainable agricultural system. Biocontrol fungi, such as Trichoderma spp., help suppress soil-borne plant pathogens, reducing plant disease incidence and boosting agricultural productivity (Sharma *et al.,* 2014). *Beauveria bassiana* is an entomopathogenic fungus that targets insect pests but can also suppress some soil-borne pathogens, directly and or indirectly by reducing their impact on plants (Lida *et al.,* 2023). *Fusarium oxysporum* sp. *radicis-lycopersici*, a strain of *Fusarium* that can suppress certain soil-borne diseases in tomatoes crop by competing with pathogenic fungi for nutrients and space (Zhang *et al.,* 2023). Other includes, *Penicillium spp*., *Gliocladium spp*., and *Myrothecium verrucaria* are fungi known for producing antibiotics, inhibiting plant pathogen growth, and producing enzymes to degrade pathogen cell walls (Srinivasan *et al.,* 2020; Ocimati *et al.,* 2021; Hassine *et al.,* 2022). They also have bio control properties, suppressing diseases through competition and toxic compounds (Castillo *et al.,* 2015; Hassine *et al.,* 2022).

2.4.5 Carbon sequestration

Fungi play a role in carbon cycling by decomposing organic matter and storing carbon in the soil (Gougoulias *et al.,* 2014; Omokaro & Ogechi, 2014). This process can help mitigate climate change by reducing the amount of carbon dioxide in the atmosphere (Gougoulias *et al.,* 2014). Fungi involved in carbon sequestration include white-rot fungi like Trametes versicolor and Ganoderma lucidum, which decompose lignin in wood, releasing carbon stored in plant biomass back into the soil (Abdel-Hamid *et al.,* 2013; Kijpornyongpan *et al.,* 2022). Brown-

rot fungi like *Serpula lacrymans* and *Gloeophyllum trabeum* contribute to carbon cycling and soil stabilization (Abdel-Hamid *et al.,* 2013). Mycorrhizal fungi like *Glomus* spp. and *Rhizophagus irregularis* facilitate carbon transfer and improve nutrient uptake (Wahab *et al.,* 2023). Saprophytic Fungi, examples: *Pleurotus ostreatus* (oyster mushroom), *Fusarium spp* are involved in the decomposition of organic matter, contributing to soil organic carbon and enhancing soil structure (Omokaro & Ogechi, 2014;Anthony *et al.*, 2024).

2.4.6 Biodiversity support

Fungi contribute to the overall biodiversity of soil ecosystems, by providing habitats and food for various soil organisms, including bacteria, nematodes, and protozoa (Coleman *et al.,* 2024). This biodiversity is crucial for ecosystem resilience, enabling the soil community to adapt to environmental changes and disturbances (Philippot *et al.,* 2024). Fungi play a crucial ecological role in the soil ecosystem by providing habitats, food sources and biogeochemical cycling of carbon by acting as primary decomposers of organic matter (Walker & White, 2018; Srinivasan *et al.,* 2020; Anthony *et al.,* 2024). Mycorrhizal fungi, such as *Glomus* spp. and *Rhizophagus* irregularis, form symbiotic relationships with plant roots, enhancing nutrient exchange and supporting diverse plant species (Kariman *et al.,* 2018; Liu *et al.,* 2020; Dyshko *et al.,* 2024). Decomposer fungi, like *Trametes versicolor* and *Pleurotus ostreatus*, break down organic materials, releasing nutrients back into the soil (Omokaro & Ogechi, 2014). Endophytic fungi, like *Neotyphodium* spp., live within plant tissues without harm, enhancing plant growth and stress tolerance (Nisa *et al.,* 2015; Baron & Rigobelo, 2022). Pathogenic fungi, like *Fusarium spp.* and *Rhizoctonia solani*, regulate plant populations and create ecological balances (Peng *et al.,* 2021; Yiallouris *et al.,* 2024). Lichenized fungi, like *Cladonia* spp. and *Usnea* spp., contribute to soil formation and stability, providing habitats for small soil organisms (Lumbsch, 2016).

2.4.7 Indicators of soil health

The presence and diversity of soil fungi serve as indicators of soil health (Ghosh & Dutta, 2024). Soil health is influenced by the presence of a diverse community of fungi, which contribute to the balance and functionality of the ecosystem (Devi *et al.,* 2020; Ghosh & Dutta, 2024). A diverse community of fungi, including mycorrhizal species, can indicate specific soil conditions, such as healthy, nutrient-rich soils conducive to plant growth (Wahab *et al.,* 2023; Ghosh & Dutta, 2024). The total biomass of fungi in the soil can be quantified using methods

like soil sampling and molecular techniques (Tauro *et al.,* 2021). A balanced ratio of fungal to bacterial biomass can indicate soil health, reflecting stable organic matter and nutrient cycling (Nielsen & Winding, 2002; Frąc *et al.,* 2018; Tauro *et al.,* 2021). The percentage of root colonization by mycorrhizal fungi can also indicate soil health, with higher rates suggesting beneficial interactions that enhance nutrient uptake (Nielsen & Winding, 2002; Frąc *et al.,* 2018; Odriozola *et al.,* 2024). The functional traits of fungi, such as decomposers *viz*. pathogens, and their enzyme activity, can provide insights into soil health (Nielsen & Winding, 2002). Extensive mycelial networks improve soil structure by binding soil particles, enhancing aeration and water infiltration (Nielsen & Winding, 2002). Fungi also contribute to the formation of soil aggregates, which are essential for soil health, enhancing water retention and reducing compaction (Frąc *et al.,* 2018; Nielsen & Winding, 2002).

2.5 Non-Beneficial soil fungi

Many plants pathogenic fungi are well-known for destructive or eliminating plants from nature by inducing disease and damages on them (Agrios, 2009). Fungi are the primary pathogens in plants, causing approximately 70% of all plant diseases, with over 10,000 out of the 70,000 known fungal species causing plant diseases (Agrios, 2009). Fungal pathogens pose a significant threat to agriculture, causing reduced crop yield and lower product quality by attacking cultivated plants and their products, including seeds, fruits, and grains (Gladieux *et al.,* 2017).

Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts (Bardin *et al.*, 2015; Köhl *et al.*, 2019). Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics or enzymes, competition for nutrients and the induction of plant host defenses (Kumar *et al.*, 2024). While effective in the control of plant diseases, these mechanisms may pose risks to non-target species including mycorrhizal and saprophytic fungi, soil bacteria, plants, insects, aquatic and terrestrial animals, and humans (Brimner *et al.*, 2003; El-Baky & Amara, 2021). Fungal biological control agents like Trichoderma spp. have been linked to non-target effects like mycoparasitism, reduced plant root colonisation, mushroom disorders, Rhizobium nodulation, and changes in plant growth (Jangir *et al.*, 2019; Ferreira & Musumeci, 2021).

In addition, the genera Trichoderma and Gliocladium have been linked to respiratory disorders and shellfish toxicity in humans, respectively (Jangir *et al.*, 2019). Biological control agents, such as Pythium oligandrum, Talaromyces flavus, Coniothyrium minitans and Ampelomyces quisqualis have modes of actions that may pose risks to non-target fungi, bacteria, plants and animals (Punja, 1997). Future studies on the ecological implications of any biological agent release and techniques for identifying potential non-target effects are necessary. To investigate and lessen adverse biological effects, sufficient monitoring and the application of molecular tools to detect and track the movement of biological control agents are required. (Brimner & Boland, 2003).

Understanding species and their effects on soils is vital for effective management and mitigation strategies in agriculture and ecosystem conservation (Agrios, 2009; Gladieux *et al.,* 2017). Non-beneficial soil fungi, such as *Fusarium* spp, *Rhizoctonia solani*, *Pythium* spp, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria* spp, and *Colletotrichum* spp, can significantly impact plant health, agricultural productivity, and soil quality (Coque *et al.,* 2020). *Fusarium* spp causing wilt, root rot, and produce harmful mycotoxins (Bodah, 2017; Coque *et al.,* 2020). *Rhizoctonia solani* causes damping-off in seedlings and root rot in legumes and vegetables. *Pythium* spp cause damping-off and root decay, while *Sclerotinia sclerotiorum* causes white mold and Sclerotinia blight in beans, sunflowers, and tomatoes. *Botrytis cinerea* causes rot in fruits and flowers, leading to wine quality issues. *Alternaria* spp. cause leaf spots, blights, and produce harmful mycotoxins (Coque *et al.,* 2020). *Colletotrichum* spp. causes fruit rot and significant yield losses.

However, a wide range of novel fungal illnesses has surfaced over the 20th century (Bodah, 2017; Gladieux *et al.,* 2017). This is most likely a result of human actions that have drastically altered the planet's ecosystems on a worldwide scale (e.g., global trade, extensive deforestation, fragmentation of habitats, urbanization, and changes in agricultural techniques) (Bodah, 2017; Gladieux *et al.,* 2017).

2.6 Drivers of differences in fungal communities' composition and distribution

Fungal communities in nature are influenced by ecology, environment, and biology that contribute to their diversity in an ecosystem (Bahram & Netherway, 2022; Lumibao *et al.,* 2024). Fungi are ubiquitous and form diverse communities temporally and spatially spanning multiple scales across many ecosystems (Bahram & Netherway, 2022). Their distribution and

composition are also affected by organic matter, nutrient availability, competition with other organisms, environmental factors like moisture, temperature, and soil pH, and biological interactions (Bahram & Netherway, 2022; Lumibao *et al.,* 2024). The spread and varied makeup (distribution) of fungal communities is influenced by variables that are discussed below:

2.6.1 Climate and weather patterns

Temperature and precipitation directly or indirectly influence fungal growth, reproduction and their distribution pattern. Seasonal variations, such as the colder temperatures of winter, can reduce microbial activity and cause fluctuations in microbial populations (Vargas-Gast´elum *et al.,* 2015; Hu *et al.,* 2024). This shift could negatively affect the diversity and functioning of fungal communities in these ecosystems, where water is an essential factor. Fungal activity and diversity are heavily influenced by temperature and precipitation patterns, with even minor rainfall enhancing primary productivity (Vargas-Gast´elum *et al.,* 2015; Hu *et al.,* 2024). Adequate soil moisture is crucial for microbial activity and nutrient cycling, as waterlogged conditions can create anaerobic environments that favor different types of soil fungi microbes, while prolonged drought can decrease their diversity and activity (Luo *et al.,* 2020).

Different fungi have adapted to specific climatic conditions, for example, tropical fungi thrive in warm, humid environments, while cold-adapted fungi are found in Polar Regions (Vargas-Gast´elum *et al.,* 2015). However, climate change models predict that arid and semi-arid regions will see temperature increases of 0.5–2 °C and fewer precipitation events in the next century (Vargas-Gast´elum *et al.,* 2015; Hu *et al.,* 2024). This situation is likely to cause challenges to soil fungal communities' composition and distribution.

2.6.2 Plant diversity and composition

Fungal communities are tightly connected to their host plants, with specific plant species fostering particular mycorrhizal fungi that establish symbiotic relationships with plant roots (Toju *et al.,* 2013). Plant diversity and composition significantly influence fungal communities through various mechanisms (Toju *et al.,* 2013). Comprising over a million species of mycorrhizal, endophytes, saprophytes and pathogens worldwide (Bollmann-Giolai *et al.,* 2022). Some plant releases specific organic compounds via root exudates, which can attract particular fungal species (Bardgett *et al.,* 2014).

Additionally, the physical structure of plant communities such as leaf litter and root systems creates diverse microhabitats that support varied fungal assemblages (Hättenschwiler & Vitousek, 2000). The relationships between plants and mycorrhizal fungi further illustrate this influence, as certain plants associate with specific fungal partners; affect fungal community composition (Smith & Read, 2008). Moreover, diverse plant systems can modify competitive dynamics among fungi, fostering a rich community where different fungi can thrive (Wang *et al.,* 2018). The overall, interplay between plant diversity and fungal communities is complex and dynamic, with high plant diversity typically leading to more robust and varied fungal communities (Zuo *et al.,* 2021).

2.6.3 Land use and disturbance

Ecological disturbances like wildfires, floods, or storms significantly influence both the diversity and abundance of species within ecosystem (García de León *et al.,* 2018). The specific effects of these disturbances are influenced by the characteristics of the disturbance itself and the traits of the affected ecosystem (García de León *et al.,* 2018). Human-driven land-use changes, such as agriculture, grazing, farmland preparation, and charcoal-making, are major disturbances that significantly affect soil fungal communities worldwide (Makero & Kashaigili, 2016; Gupta & Richardson, 2021; Ksentini *et al.,* 2022; Atala *et al.,* 2023). A study by Peay *et al.* (2022), estimated that globally, anthropogenic activities have degraded or influenced around 30-40% of soil fungal diversity.

Furthermore, intensive agriculture practices, including the use of land preparation, burning, pesticides application, fertilizers, and tillage, can reduce 18 to 28% fungal communities and reduce soil fungal diversity by 25-35% (Bastida *et al.,* 2020; Hartmann *et al.,* 2021; Ksentini *et al.,* 2022; Atala *et al.,* 2023). Additionally, soil erosion due to improper farmland preparation can degrade 20-30% of soil fungal communities (Vimal *et al.,* 2022). Disturbed environments often lead to a decline in native fungal species and an increase in opportunistic species that can thrive in altered conditions (Clavel *et al.,* 2024).

2.6.4 Microbial interactions

Ecological interactions play a crucial role in shaping fungal communities (Niego *et al.,* 2023). Fungi interact with various microorganisms, including bacteria and other fungi, through competitive, mutualistic, or antagonistic relationships, which can significantly influence the composition of microbial communities (Pawlowska, 2024). For instance, competition for

resources between bacterium *Pseudomonas fluorescens* can inhibit the growth of the fungal pathogen *Fusarium oxysporum* spp. (De Vries *et al.,* 2018). Additionally, some bacteria produce antimicrobial compounds that suppress fungal pathogens; for example, *Bacillus subtilis* releases lipopeptides that inhibit fungi such as *Botrytis cinerea*, a common plant pathogen (Kloepper *et al.,* 2004). Mutualistic relationships also play a role, as certain bacteria enhance nutrient uptake for fungi (Frey-Klett *et al.,* 2011). Rhizobacteria, like *Bacillus* spp. and *Pseudomonas* spp. can improve phosphorus availability for mycorrhizal fungi, such as *Glomus* species, thereby benefiting both partners (Hodge & Campbell, 2009).

Furthermore, bacterial activity can alter soil structure and chemistry; for instance, actinobacteria like Streptomyces can decompose organic matter, affecting soil pH and moisture levels, which in turn influences fungal community composition, favoring species like Trichoderma (Schlatter *et al.,* 2017). Finally, a diverse microbial community can enhance the stability and resilience of fungal communities; for example, higher bacterial diversity can promote stability in ecosystems dominated by fungi such as *Mortierella* spp. and *Aspergillus* spp. allowing them to coexist and thrive (Allison & Martiny, 2008). In summary, microbial interactions are essential drivers of fungal community dynamics, influencing which fungi dominate specific environments.

2.6.5 Soil type

Soil type and its properties, including texture and structure such as sandy, clay, and loamy soils; significantly influence fungal community distribution by affecting water retention and aeration (Wang *et al.,* 2021). Sandy soils drain quickly and may not retain enough moisture for fungal growth, while clayey soils hold water better, supporting different fungal species (Wang *et al.,* 2021). Moreover, different soil textures can lead to different soil microbial species (Yan, 2011), loam soils have the highest microbial activity while clay soil has the lowest (Cai *et al.,* 2003).

Nutrient content is also crucial, as nutrient-rich soils provide essential resources like nitrogen and phosphorus, fostering greater fungal diversity (Janowski & Leski, 2022). Soil pH significantly influences which fungal species thrive, as many prefer slightly acidic conditions, thereby affecting nutrient availability (Xia *et al.,* 2024). Moisture retention varies among soil types, with soils that retain moisture supporting more robust fungal communities compared to well-drained soils (Janowski &Leski, 2022; Xia *et al.,* 2024).

The amount of organic matter in the soil further impacts fungal distribution, as organic-rich soils offer nutrients and habitats (Ali *et al.,* 2021). The organic matter content of the soil significantly influences fungal distribution by providing essential nutrients, creating suitable habitats, and affecting decomposition rates (Janowski &Leski, 2022). Areas rich in organic material support diverse fungal communities, as fungi rely on these resources for growth (Ali *et al.,* 2021; Janowski & Leski, 2022). Additionally, organic matter impacts soil pH and moisture, which are crucial for fungal development. High organic levels can lead to increased competition and create microhabitats with distinct conditions, further shaping fungal community composition (Ali *et al.,* 2021). The distribution of microorganisms in soils is affected by habitat characteristics such as organic matter content, aggregate size, water content, and temperature, which influence their survival and movement, especially in terrestrial ecosystems (Vargas-Gast *et al.,* 2015).

Lastly, microbial interactions within soil types create unique conditions that can be competitive or synergistic, shaping the overall composition of fungal populations. Together, these factors illustrate the complexity of soil characteristics and their vital role in determining the growth, diversity, and distribution of fungal communities across different environments.

2.6.6 Symbiotic relationships

Fungi such as mycorrhizal species form symbiotic relationships with plants, and the composition of fungal communities is influenced by the types of plants present (Khaliq *et al.,* 2022; Wang *et al.,* 2022). These mutualistic relationships are generally more successful in diverse and balanced plant communities, which support greater fungal diversity (Barkman *et al.,* 2020). Beyond mycorrhizal associations, fungi engage in various symbiotic relationships with other organisms, such as lichens (with algae) and endophytes (with plants). These associations can affect fungal diversity and distribution by facilitating colonization of specific habitats. However, shifts in plant communities, particularly in areas not protected from poor land management, can disrupt these relationships and lead to changes in fungal community structure (Graham *et al.,* 2017). Conversely, changes in plant communities specifically in non-protected areas, often driven by invasive species or land management practices, can disrupt these interactions and lead to shifts in fungal community structure (Graham *et al.,* 2017).

2.6.7 Pollution

Pollution from chemicals, heavy metals are environmental pollutants, most notably cadmium, lead, arsenic, mercury, and chromium, and other contaminants can alter fungal communities by selecting for tolerant species while harming sensitive ones (Sun *et al.,* 2022; Rashid *et al.,* 2023). This can lead to reduced diversity and shifts in community structure. It is reported that some heavy metals can stimulate microbial growth at low concentrations; elevated levels severely inhibit the growth, proliferation, and diversity of soil microbial populations (Azarbad *et al.,* 2013; Abdu *et al.,* 2017). This inhibition negatively impacts the beneficial activities of these microbes, ultimately affecting crop health and productivity (Rashid *et al.,* 2023). Previous studies have shown that microorganisms are generally more sensitive to heavy metal (HM) toxicity than other living organisms, including plants in the same soil environment (Giller *et al.,* 1998; Gikas *et al.,* 2007).

However, the toxicity levels of heavy metals can vary among different microbial groups, depending on the inherent toxicity of the specific metal and its bioavailability in the soil (Giller *et al.,* 1998; Abdu *et al.,* 2017). The consequence of heavy metal pollution on soil microbial communities is usually adverse, resulting in the decrease of microbial biomass and activity, the disturbance of specific taxa, and shifts in the composition of the microbiome (Azarbad *et al.,* 2013; Sun *et al.,* 2022).

2.7 Commercial application of soil fungi in biodiversity ecosystem management

2.7.1 Soil health and fertility

Fungi are essential for soil health they also play a number of other roles including aiding in nutrient cycling, disease suppression, and water dynamics, which enhances plant growth (Daynes *et al.,* 2013; Nwakanma & Unachukwu, 2017). Fungi decompose dead organic matter into biomass and organic acids, making nutrients more accessible, while their hyphae bind soil particles into stable aggregates that improve soil structure and enhance water retention and drainage (Daynes *et al.,* 2013). A study by Błaszkowski *et al.* (2021) demonstrated that arbuscular mycorrhizal fungi (AMF) can lead to substantial increases in crop yields and improve soil structure by stabilizing aggregates and promoting beneficial microbial communities.

In addition, fungi like *Trichoderma harzianum* are increasingly used in biological fertilizers to foster plant growth and control soil-borne pathogens. Research by Sharma *et al.* (2022) highlighted the effectiveness of *Trichoderma harzianum* in improving soil health indicators, such as microbial activity and nutrient cycling, while also boosting the growth rates of various crops. The study found that crops treated with *Trichoderma harzianum* exhibited enhanced root development and increased biomass, underscoring its potential as a sustainable agricultural tool (Sharma *et al.,* 2022). Aspergillus niger can be viewed as beneficial; however, in agricultural settings, its presence poses risks due to mycotoxin production.

In contrast, in controlled industrial applications, it serves beneficial purposes (Perrone *et al.,* 2007). *Aspergillus niger* is widely used in industrial applications for producing enzymes and organic acids, while also playing key role in biodegradation and nutrient cycling in soil (Perrone *et al.,* 2007). Another important fungus is *Penicillium;* many species of *Penicillium* are ubiquitous and can be found in various habitats worldwide (Moretti & Sarrocco, 2016). As saprotrophic fungi, they thrive on decaying organic matter, playing a crucial role in nutrient cycling, contributing to soil health and ecosystem functioning and promoting plant growth (Babu *et al.,* 2015; Moretti & Sarrocco, 2016).

2.7.2 Environmental monitoring

Soil fungi play a crucial role in environmental monitoring, serving as both biodiversity indicators and tools for pollution bioremediation (Daynes *et al.,* 2013; Nwakanma, & Unachukwu, 2017). Their diversity and abundance can reflect ecosystem health and stability, making them valuable indicators of environmental changes such as pollution and climate impacts (Gange *et al.,* 2023). For instance, Gange *et al.* (2023) examined fungal diversity in urban soils and advocated for the use of soil fungi in urban planning and environmental monitoring. Additionally, certain soil fungi, like *Phanerochaete chrysosporium*, can be utilized to detect and degrade heavy metals and organic pollutants, enhancing their role of bioremediatory (Zhang *et al.,* 2022). Additionally, fungi like *Ganoderma lucidum* have proven effective in reducing heavy metal concentrations in industrial wastewater, as shown in a study by Ali *et al.* (2021), with its impactful applications of soil fungi in biotechnology.

2.7.3 Bio-control agents

Several commercial fungal bio control agents are used in biodiversity and ecosystem management. For example, *Trichoderma spp*. help control soil-borne pathogens and enhance

crop yields, while *Beauveria bassiana* and *Metarhizium anisopliae* target insect pests, providing natural pest control options (Sharma *et al.,* 2022; Mesquita *et al.,* 2023). Additionally, endophytic fungi like *Penicillium sp.* and *Hypocrea sp*. used as plant growth promoters and biological control agents against various pathogens, including *Fusarium oxysporum f.sp. cucumerinum*, which causes wilt in cucumbers (Abro *et al.,* 2019). Abro *et al.,*2019; Mesquita *et al.,* 2023), showed that, all isolates of *Penicillium sp.* and *Hypocrea sp* significantly inhibited the mycelial growth of the pathogen.

Given the above, soil fungi are essential for soil health, contributing to nutrient cycling, disease suppression, and improved water dynamics, which enhance plant growth (Daynes *et al.,* 2013; Nwakanma & Unachukwu, 2017). They decompose organic matter, making nutrients more accessible and improving soil structure through the formation of stable aggregates (Błaszkowski *et al.,* 2021). Notably, *Trichoderma harzianum* is used in biological fertilizers to promote plant growth and manage soil-borne pathogens (Sharma *et al.,* 2022), while *Aspergillus niger* and *Penicillium* species play significant roles in biodegradation and nutrient cycling (Perrone *et al.,* 2007; Moretti & Sarrocco, 2016). Additionally, soil fungi serve as biodiversity indicators and tools for pollution bioremediation, with species such as *Phanerochaete chrysosporium* and *Ganoderma lucidum* effectively degrading pollutants and heavy metals (Ali *et al.,* 2021; Zhang *et al.,* 2022).

This research therefore, aimed to assess the factors affecting the distribution and abundance of beneficial fungal communities in soils from protected and non-protected areas in the Itigi District, focusing on community knowledge, land use practices, and species diversity. Additionally, the study aimed to determine the soil fungi diversity and identify potential beneficial fungi in soils from non-protected and protected areas in the Itigi District.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site description

The study was conducted at Mitundu and Mgandu wards located in Itigi district, Singida region, central Tanzania (Fig. 2). Itigi district lies between latitudes 5.70°S and 6.00°S and longitudes 34.50°E and 35.00°E (Kiondo *et al.,* 2019). The main ethnic groups in the study area are the Nyaturu, Kimbu, Sukuma, and Nyamwezi. The population of the Mgandu ward is 26 887, with 4436 households, while that of Mitundu wards is 32 370 and with 6044 households (URT, 2022). Itigi district borders Manyoni district to the east and the Ikungi district to the north border in the south, Itigi district shares a boundary with the Chunya district of the Mbeya region, and to the west, it shares boundaries with the Uyui and Sikonge districts of the Tabora region (Isinika *et al.,* 2021). The district climate is characterized by semi-arid dry land with an average temperature of 21.6 °C and receives an average annual rainfall of 632 millimeters, with an elevation of 1306 meters above sea level (Nyaombo, 2021). The predominantly soil texture include sand, clay loam and sandy loam, which favor optimum vegetation and fungal growth (Nyaombo, 2021). The dominant vegetation in the study area include savanna woodlands and semi-arid glass land, with scattered trees, such as miombo woodlands, Ambrella thorn acacia (*Acacia tortilis*), Red acacia (*Acacia seyal*), African myrrh (*Commiphora africana*) plant trees, and dense shrubs. The ground layer covered with grasses like Bermuda grass (*Cynodon dactylon*) and Red oat grass (*Themeda triandra*), Indian goose grass (*Eleusine indica*) (Makero, 2017).

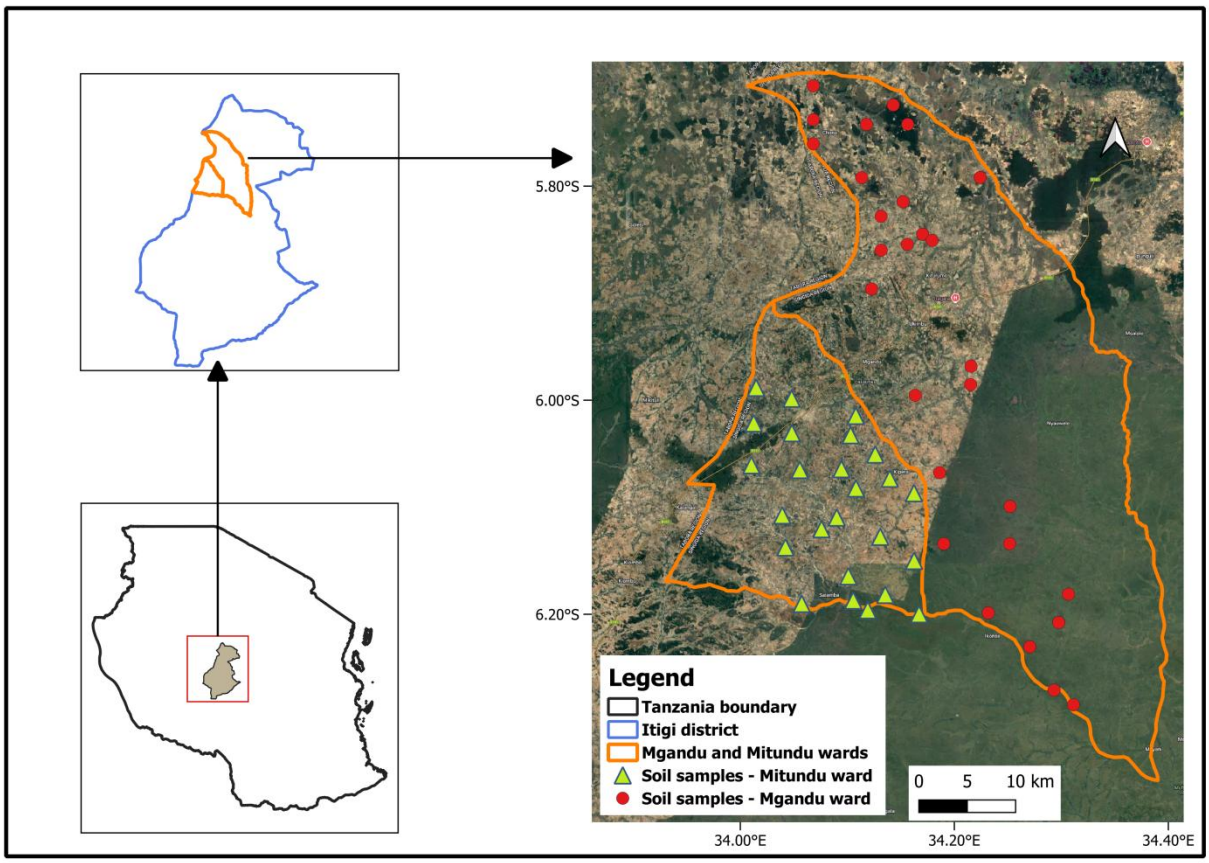


Figure 2: The map showing the study area with soil sampling points

3.2 Assessment of community-based knowledge on land use practices and their influence on soil fungi in protected and non-protected areas

(i) Study design

A cross-sectional study design was adopted (Levin, 2006) to provide a snapshot on current land use practices, and their influence on soil fungi distribution in protected and non-protected areas in the study sites.

(ii) Data collection and sampling procedures of respondents

The surveys addressed agro-economic activities and factors contributing to soil degradation that led to soil fungi degradation. Using a structured questionnaire, 150 household heads were randomly selected from two wards to ensure comprehensive representation of community perspectives. Specifically, eighty-three (83) households were chosen from Mgandu ward and sixty-six (66) from Mitundu ward. The variation in the number of respondents between these two wards was due to the greater community involvement and willingness to participate in Mgandu, where many residents from Mitundu also partake in agricultural activities, making

them more active in Mgandu than in their own area. This selection method aimed to offer a broad impression of community insights and practices regarding soil management.

The sample size for questionnaire respondents was determined using equation 1 below (Fisher, 1991). Combination of random and purposive sampling techniques was used to gather data (Singh & Masuku, 2014). Random sampling technique was employed to select households (community members), ensuring a representative sample of the general population. On the other hand, purposive sampling was used to select ward leaders game reserve and agriculture extension, staff that termed government employees (Singh & Masuku, 2014). The sample size calculated by using Fisher's equation shows below:-

n=Z^2.p.(1-p))/d^2 …….....Equation 1

Where:

n = required sample size

Z = z-value, representing the standard normal deviate at the desired confidence level (e.g., 1.96 for 95% confidence),

p = estimated proportion of the population with the desired characteristic (if unknown, 0.5 is used for maximum variability)

1−p = proportion of the population without the characteristic

d = margin of error or precision (e.g., 0.05 for ±5% error)

Due to due to financial constraints and time, instead of 50% which is equal to 192 respondents of the calculated sample size, only 150 households (Fernald *et al.*,2017) with minor modifications, representing 1.43% rate of the total population of 10,480 houses (Angelsen *et al.*, 2015).

(iii) Data collection and processing

Survey data was collected using KoboToolbox platform (version 2023.2.4) on a Lenovo tablet (KoboToolbox, 2023). Responses were recorded directly on the tablet using the KoboToolbox tool, ensuring real-time data entry. The tool automatically coded and organized the data into an Excel sheet for analysis.

(iv) Data analysis

The collected demographic data was subsequently imported into Statistical Package for the Social Sciences (SPSS version 20) for descriptive and multinomial regression analysis. Respondent's demographic characteristics were analyzed using Descriptive statistics (chi-square) to determine the influence of demographic characteristics on land use practices and the influence of demographic factors on land use practices and management. The regression model assessed variables on gender, age, education level, and occupation with reference group being one of the demographic characteristics in each category. The analysis provided coefficient estimates, standard errors, z value, and p-value to identify significant predictors for the land practices such as choice of fertilizer, land preparation methods, and causes of soil degradation. In addition, descriptive statistics employed to determine the influence of gender on farmland preparation methods and the impact of education on causes of soil degradation in protected and non-protected areas in the study sites.

3.3 Determining the diversity of fungal communities in soils samples collected from protected and non-protected areas

3.3.1 Soil sampling in protected and non-protected areas in the study sites

(i) Soil Sampling procedures at protected and non-protected areas

In soil sampling, sixty (60) soil samples were collected from both wards. In each ward, 15 composite soil samples were collected from each land use type, with 15 samples collected from the non-protected area and 15 from the protected area. This resulted in 30 soil samples per ward, representing all land use types. A representative soil sample from non-protected areas were collected using a quadrat of 2m x 2m at the depth of 30 cm using soil Augar, whereby five samples were taken from each quadrat and then mixed by halfing and quatering to obtain a single representative sample of 0.5 kg per sites (Fernández-Ugalde *et al.*, 2020). The GPS coordinates were recorded using a handheld Germin eTrex 10 device at the place where the soil sample was collected. The representative samples were packed in well-labeled zipped bags that were kept in cool box to maintain proper soil quality before processing it.

In the protected area, systematic sampling was employed whereby transects of 100 m was employed, and the distance from one transect to another was 50 m (Fransson *et al.,* 2016) with little modification. The present roads were used as base/line transects to construct transects and

spot quadrats in each transect (Shayo, 2022). The four quadrats were constructed and the distance from one quadrat to another was 20 m. A walk of 20 m from the transect road to the interior was adapted to avoid bias (Shayo, 2022). The soil samples from each sampling point were collected for fungal isolation (Fernández-Ugalde *et al.,* 2020). The soil was collected from five points from each quadrat and mixed to make composite soil by halfing conning and quartering it (Naveen & Madhukar, 2022). The 0.5 kg of soil sample was then packed in labeled zipped bags and kept into a cool box until arrival at the NM-AIST laboratory then transferred to a refrigerator (4°C for fungi isolation, and species characterization).

(ii) Soil samples preparation

Before isolating soil fungi, the samples were dried to reduce moisture content, which helps inhibit the growth of unwanted microorganisms and minimizes competition with the target fungi (plate 1). This process also aids in reducing contaminants, ensuring a cleaner environment for effective isolation (Carter & Gregorich, 2007; RARC & JICA, 2014). Then soil samples were ground and sieved through a 2 mm mesh. Sieving is a common practice in soil sample preparation, ensuring that only fine particles are retained for further analysis (Walkley, 1935; Tan, 2010).

Plate 1: Air drying of soil process

3.3.2 Data collection

(i) Media preparation

A weight of 39 g of Potato Dextrose Agar (PDA) granulated, manufactured in India by HiMedia Laboratories Pvt. Ltd, was weighed using analytical balance model PA214 made in China and put into sterile laboratory bottle that accommodated 1L to make full strength PDA. Sterilization was done using autoclave model Cl-40 M manufactured by ALP Co., Ltd in Tokyo, Japan at 121 °C for 15-20 minutes to sterilize and eliminate any contaminants. Cooling up to 45-50℃ and mixed with streptomycin sulfate (100 µgmL-1) then pouring of PDA into 90 mm size petri-dishes was done under sterile Biosafety Cabinet model LCB-1203B-B2 to allow the agar to solidify. Then all the petri-dishes that were left unused were stored in 4℃ refrigerators for future use.

(ii) Fungal isolation and incubation

A 1g soil sample from a 0.5 kg composite was suspended in 9ml of autoclaved distilled water in a 15ml-Falcon tube, then thoroughly mixed to create a solution that was serially diluted from 10^{-1} to 10^{-7} (Aziz & Zainol, 2018; Rosas-Moreno *et al.,* 2023). Using micropipettes, 0.1ml samples from each dilution of 10^{-4}, 10^{-5}, and 10^{-6} plated onto Potato Dextrose Agar and spread using silver round beats (Gomez *et al.,* 2014). These plates were then incubated at 25ºC (Aziz & Zainol, 2018; Rosas-Moreno *et al.,* 2023).

(iii) Data collection for colony forming unit (CFUs) counting

Observation of colonies forming unit was done from 2nd day from incubation, a 10^{-4} dilution was selected for colon count using colony counter (model Motic type S/N 1546552 made from China) and recorded in excel spreadsheet (Aziz & Zainol, 2018). Selection was based on macro-morphological characteristics such as color, shape, structure, and texture (Gomez *et al.* (2014; Rosas-Moreno *et al.,* 2023). The count of viable fungi was indicated as colony-forming units per milliliter from diluted 1g of dry soil, using Equation 2 (Alabbad, 2016).

$$CFU\ per\ mL = \frac{(No.of\ colonies\ \times DF)}{VP\ (mL)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots equation\ 2$$

CFU/mL = Colony Forming Units, DF = Dilution factor, VP =Volume plated, mL = milliliters.

Plate 2: Illustrates serial dilution method, fungal isolation, pot experiment and drying of plants for biomass measurement

(A-B), isolated fungal growth, colony forming Unity (CFU(s)) and types (C-D), pure cultures of beneficial fungal tested in plants growth in pot experiment (E), Maize bran and finger millet fungi carrier materials (F), Eleusine indica and Amaranthus spinosus plant species treated by Penicillium chrysogenum and Trichoderma harzianum (g), Dried wild finger millet (*E. indica*) and Amaranthus spinosus for biomass measurement

(iv) Data collection on fungi diversity assessment

Using a stereomicroscope differentiation of colonies grown in a petri-dish was done and colonies displaying different macro-morphological characteristics were used to aid in visual culture evaluations. Fungal composition was counted and recorded in excel spreadsheet to group them differently (Wang *et al.,* 2022). A pure culture was obtained from each distinct colony and transferred to fresh Potato Dextrose Agar (PDA) for micro-morphological

identification. This was carried out using a trinocular and optika Stereomicroscope at TARI-Tengeru pathology laboratory, with the aim of differentiating fungal mycelia and spores.

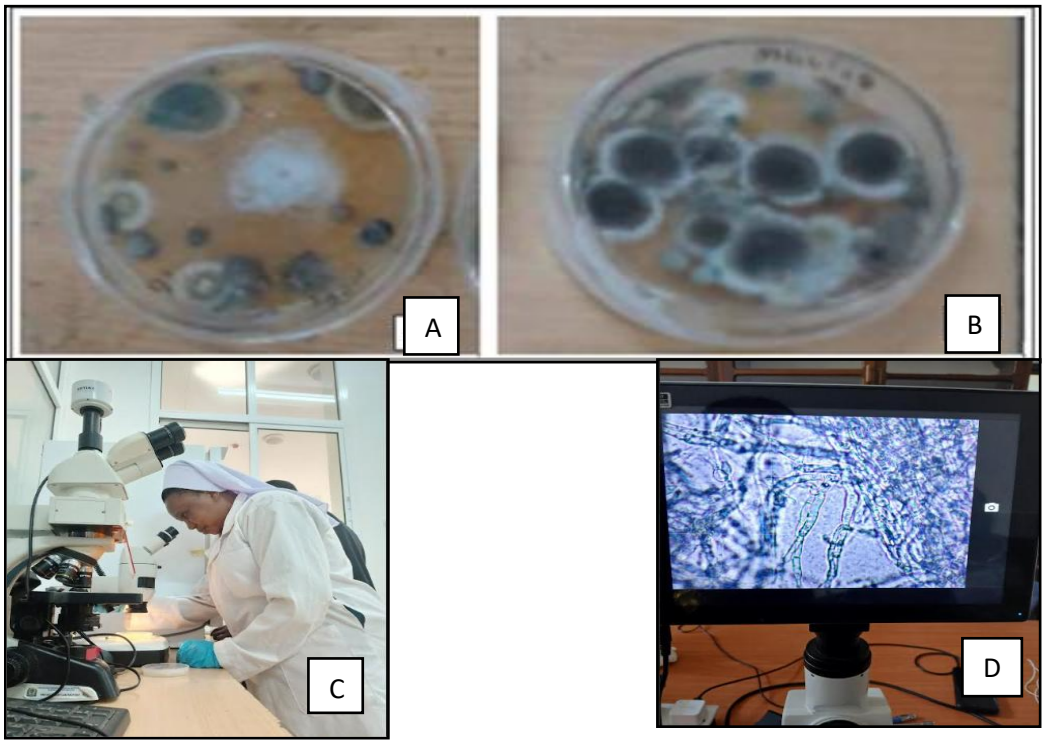


Plate 3: Fungal colon cultures from soils of (A) non-protected areas soil samples (B) protected areas soil samples (C) colony counting and macro-morphology identification (D) Micro-morphology identification

3.3.3 Data analysis

Quantitative data on colony forming unit and fungal composition were subjected to Chi-square and one way analysis of variance (one-way ANOVA) and mean separation test, using Jupyter notebook version 7.2.2 and GENSTAT Program version 21 respectively. Correlation between colony forming unit and species composition was made to assess the relationship between them. The qualitative data from colony forming unit and species composition were used for comparisons within the google search engine on MYCOBANK Database https://www.mycobank.org/, https://fungalgenera.org/, reviewed and published research articles to assist in identifying the possible beneficial fungi. For fungal composition, abundance and distribution a statistical analysis of the data was conducted using Chi-square and one-way analysis of variance (ANOVA). To assess significant differences in composition, abundance, and distribution, mean separation was conducted using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$.

3.4 Identification and efficacy of beneficial soil fungi isolates from protected and non-protected areas in promoting plant growth

3.4.1 Data collection

(i) Molecular identification of potential beneficial soil fungi isolates

Among the 15 soil fungi the two suspected beneficial fungi species (three-suspected *Penicillium* and four *Trichoderma*) fungal species, one *Penicillium* and one T*richoderm*a were selected and identified to species level using molecular techniques. Pure colonies of these fungal species were obtained through sub culturing. The DNA extraction was done from the pure cultures of suspected beneficial fungi using a method used by Srivastava *et al.* (2024), and the CTAB (Cetyl Tris-methyl Ammonium Bromide) method (https:// static. igem. org/ mediawiki/ 2021/ 2/23/ T--IISER_TVM--docs--Fungal_Protocols.pdf) with a few modifications.

A portion of the fungal colony was mixed with sterile distilled water in a 2 mL Eppendorf tube to collect the fungal spores, with 500 µL of this suspension utilized for genomic DNA extraction. To the fungal suspension, 400 µL of CTAB buffer was added and homogenized for 3 minutes using a homogenizer to lyse the fungal spores, followed by incubation at 65 °C for 30 minutes. The mixture was allowed to cool, and an equal volume of 24:1 (Chloroform Isoamyl alcohol) was added and well mixed. The mixture was centrifuged at 13 000 rpm for 10 minutes at 4 °C. The aqueous phase was transferred into a new eppendorf tube, and 2/3rd volume of ice-cold isopropanol was added and mixed gently. The mixture was incubated in the freezer at -20 °C for 2 hours, and then centrifuged at 13 000 rpm for 20 minutes at 4 °C. The genomic DNA pellet was washed with 1 mL of 70% ethanol and allowed to air dry for 20 minutes. The DNA pellet was eluted with 50 µL of TE buffer and stored at -20 °C freezer before using for PCR. The Quick load Ladder of 1Kb DNA ladder (#NO0468S) BioLabs was used. The reasons for selecting these fungi were abundantly in many petri-dish cultures and its history wide use in many researches for biodiversity and ecosystem management.

(ii) Efficacy of beneficial soil fungi isolates in promoting plant growth

A pot experiment was conducted at the Nelson Mandela African Institution of Science and Technology screen house, to assess the efficacy of *Penicillium* and *Trichoderma* spp. isolates in promoting wild finger millet (*Eleucine indica*) and wild *amarants* (*Amaranthus spinosus*)

spp plant growth. The fungi were first grown in two sterilized media i.e. maize bran and finger millet seeds. The experiment was set in a completely randomized design with three replications and two observations per plot (Fig. 3).

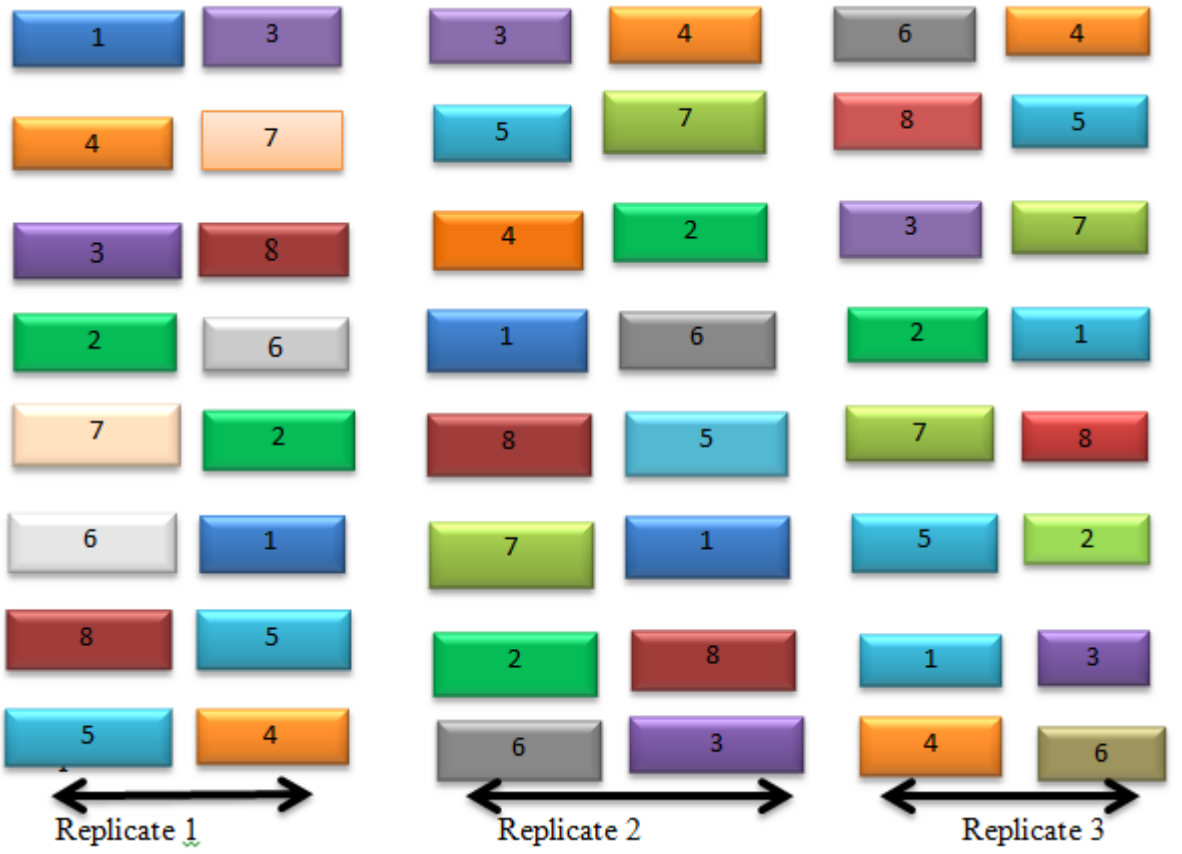


Figure 3: Pot experiment layout with three replicates and two observations per treatment plot (pot)

A total of 48 pots each with a capacity of 2L were filled with about 2 kg of sterilized soil collected from the study area. Experiment treatments included PEB (*Penicillium* sp + *Eleucine indica*+ Maize bran), PEM *(Penicillium* sp + *Eleucine indica*+Millet seed), PAB (*Penicillium* sp + *Amaranthus* spp +Maize bran), PAM (*Penicillium* sp + *Amaranthus* spp + millet seed), PCE (Positive control (commercial inoculant)) + *Eleucine indica*), NCE (Negative control + *Eleucine indica*), NCA (Negative control + *Amaranthus* spp), PCA (Positive control (commercial inoculant + *Amaranthus* spp), NCE (Negative control + *Eleucine indica*), NCE (Negative control+ *Eleleusine indica* and NCA (Negative control +*Amaranthus* spp), NCA (Negative control +*Amaranthus* spp).

Seeds were collected by locally purchased from Singida local market and collecting from field. Planting process was done by placing five seeds per hole then after germination the seedlings

thinned to two seedlings. Watering was done regularly using tape water to maintain adequate soil moisture in the soil. Spraying of insecticides the screen house was done to kill insects' pests and leaf miners that could affect plant growth before and after seeds germination. The plants (*E. indica* and *Amaranthus* species) were inoculated with *Penicillium* and *Trichoderma* spp spore suspensions (10^7 spores/mL) prepared from 1g of maize bran and finger millet seeds as the career materials (Plate 4). Data on, growth parameters such as plant height, root length, number of leaves, number of branches (in *Amaranthus*), number of tillers (in *E. indica*), fresh and dry plant biomass to assess the treatments, were collected between 4-6 weeks after planting.

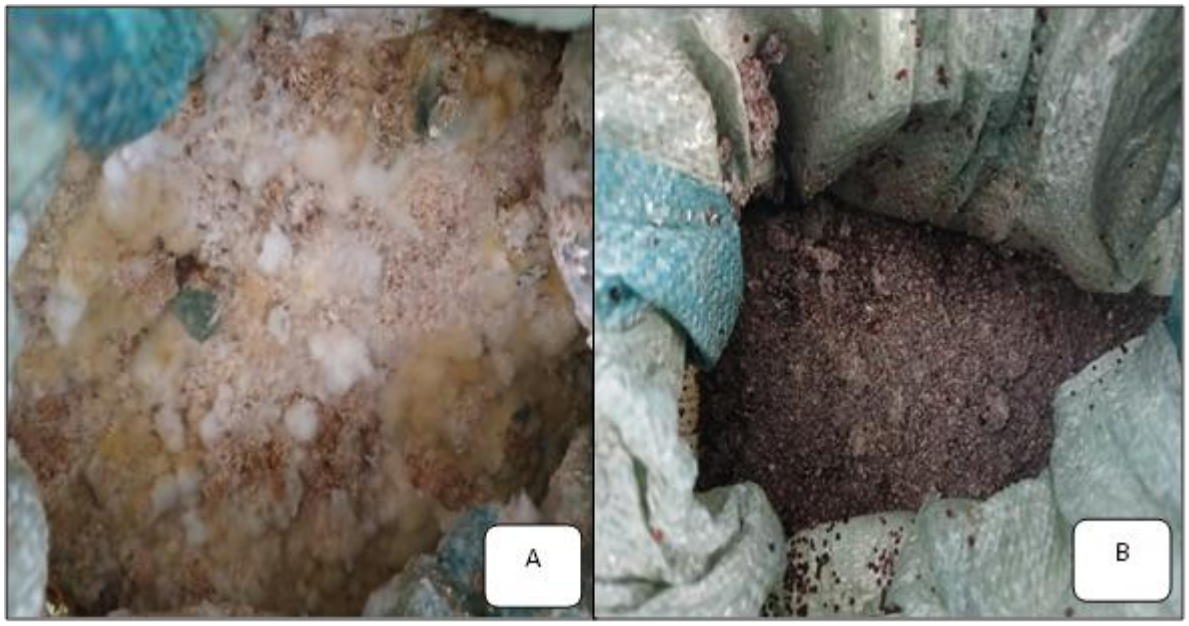


Plate 4: Fungal carrier materials: (A) Maize bran and (B) Finger millet seeds fungal carrier materials

3.4.2 Data analysis

Gel-electrophoresis was used to identify beneficial soil fungi at species level using a ladder of 100 ng. The *Penicillium* spp and *Trichoderma* spp were confirmed by band at 520ng and 345 ng, respectively (Rahman *et al.,* 2021). Data on plant height, number of leaves tillers and leaf height for *E. indica,* above ground biomass and number of branches for *Amaranthus spp.* were subjected to One-way (ANOVA) and mean separation test to analyze the data statistically. For parameters with significant differences, mean separation was conducted using Duncan's Multiple Range Test (DMRT) at a significance level of ($p \leq 0.05$) to observe the differences of the experimental treatments. Correlation was conducted to assess the relationship between experimental treatments and growth parameters.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Overview

Results from the assessment of effects of community-based knowledge on land use practices and their influence on soil fungal communities indicated significant differences across the study sites. The results on fungal community diversity in soils of both non-protected and protected areas in the study areas helped to identify and evaluate the efficacy of potential beneficial fungi present in these environments in promoting plant growth. The results for specific objectives have been presented and discussed as here under:

4.2 Assessment of community-based knowledge on land use practices and their influence on soil fungi in protected and non-protected areas

This section aimed to investigate the interplay between community knowledge on land use practices on soil fungi. Central to this study is the question on how to improve soil health, ecosystem understanding and promote sustainable practices that stimulate soil health and fungal diversity.

4.2.1 Demographic characteristics of respondents across the study site

The gender demographic respondents in Mitundu and Mgandu wards showed low female and high males' number of respondents (Fig. 4). The average gender distribution analysis (67.35 male and 32.65 female) in both wards revealed a significant difference in gender distribution ($p = 0.01$), with males comprising 64.5% and females 35.2% at Mitundu ward and 69.9% and 30.1% male and female, respectively at Mgandu. The observed significant gender imbalance across the study area suggests that social norms may play a crucial role in determining who represents families in discussions or decision-making processes. This could indicate a need for more inclusive practices that consider female perspectives. Similar studies by Nkhoma *et al.* (2019), Zamanian *et al.* (2016) and Miller *et al.* (2023) have documented gender imbalances in various contexts, indicating that societal norms often dictate the representation of genders in community engagement and decision-making.

The age of respondents showed statistical significance difference ($p = 0.02$), with the majority of respondents aged 41 years old and above, while younger respondents (under 20) were

excluded due to limited knowledge of local activities (Fig. 4). The predominance of respondents aged 41 and older may reflect the greater experience and knowledge of older individuals regarding local activities. Earlier study by Schlenk *et al.* (2009) have highlighted the importance of including various age groups in research to capture a holistic view of community needs. While older adults possess extensive knowledge of local resources, their predominance can skew community needs representation, highlighting the necessity of including younger voices to develop inclusive programs for all age groups (Leach *et al.,* 2024).

The education level distribution among respondents revealed that majority of respondents (58.8%) attended primary education, while 8.0% of respondents have completed secondary education, 26.5% have non-formal education and, 6.9% have attained tertiary education indicating statistical significance difference among the group with a p-value of less than 0.01. The statistical significance observed findings in the ($p < 0.01$) suggests that educational disparities are not random hence reflecting the low knowledge on the land use practices and soil microbes. Additionally, the low percentages of secondary (8.0%) and tertiary education (6.9%) indicate critical skill gaps that hinder social knowledge and awareness on land use practices and overall community well-being, leading to reduced civic engagement and improper land practices. Research in environmental management emphasizes the critical role of education in promoting environmental awareness and sustainable practices (Essomba *et al.,* 2022). A lack of advanced education can result in insufficient understanding of sustainable land use practices, which are vital for community resilience and environmental sustainability (Ardoin *et al.,* 2023; Essomba *et al.,* 2022). Overall, educational attainment is closely linked to effective environmental management and public participation (Ardoin *et al.,* 2020).

Occupationally, most of the respondents engaged in farming activities making this occupation having high respondents about 81.7% (at Mitundu ward) and 81.9% (at Mgand ward). Business men/women comprised only 9.9% (at Mitundu) and 2.4% (at Mgandu) and government employee 8.5% and 16.9 % at Mitundu and Mgandu, respectively. These results imply that the agriculture is the primary occupation in these areas, suggesting a strong reliance on agricultural practices for livelihoods (Porfirio *et al.,* 2018). Policymakers should prioritize agricultural support by, addressing diverse needs, and promoting economic diversification. Implementing sustainable land use practices is crucial to ensure environmental health. The age distribution indicates that older people dominate, possibly undermining the viewpoints and requirements of younger people. Additionally, low educational attainment highlights critical skill gaps that hinder local engagement and sustainable land use practices.

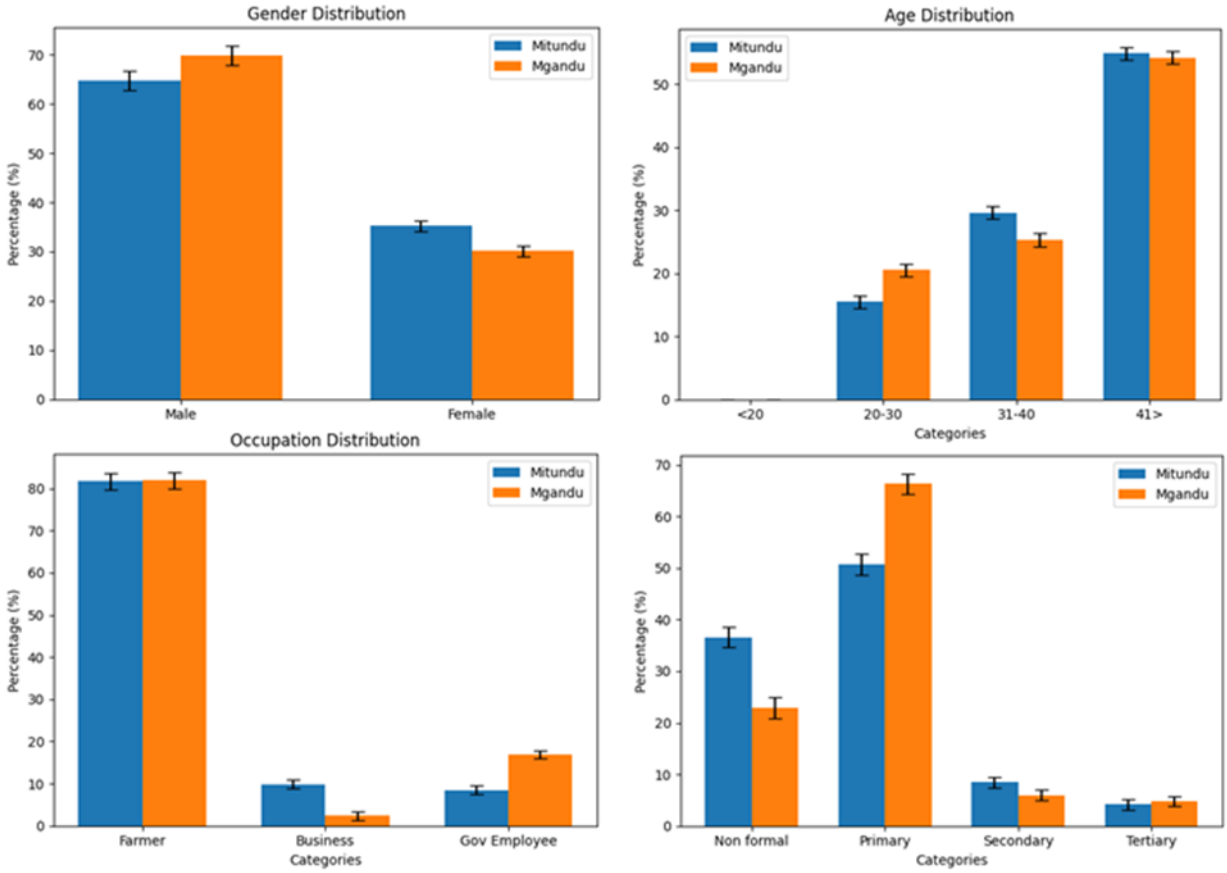


Figure 4: Bar graphs showing relationship between demographic characteristics of respondents and land use practices

(i) Fertilizer usage and application *viz* gender distribution, age categories, education levels and occupation profiles in non-protected areas

The fertilizers sources that are commonly used in non-protected areas across the study sites were nitrogen (N), phosphorus (P) and potassium (K) in form of NPK (20:39:14), Calcium Ammonium Nitrate (CAN) and urea (inorganic nutrient fertilizer sources) and animal manure (organic nutrient fertilizer source). Other minor and not included in the analysis were DAP (Diamonia Phosphate) and booster used by minority in the study areas.

Results from analysis on fertilizer usage revealed that age groups 31 - 40 and 41 and above do not significantly use animal manure, as indicated by high p-values (0.9239 and 0.2562) (Table 1). Similarly, analysis on inorganic fertilizer types and application versus age groups 31-40 and 41 and above revealed similar trends with no significantly impact as was with manure. The lack of significant influence from age groups (31-40 and 41 and above) on the use of animal manure and inorganic fertilizers suggests that factors other than age may drive fertilizer choices. The findings of this study implies that environmental and soil management strategies

should consider variables beyond demographic factors, such as sustainable practices and access to resources, to improve fertilizer use and enhance soil health, effectively. Several studies have reported that demographic factors, including age, have minimal influence on the types of fertilizers adopted by farmers (Danlami *et al.,* 2019; Gumindoga *et al.,* 2024). This suggested that education plays a more significant role in the choice between organic and inorganic fertilizers, reflecting similar patterns in fertilizer usage regardless of age (Murunga, 2022; Gumindoga *et al.,* 2024).

Gender significantly (p-value = 0.0364) influenced fertilizer use, with males tending to use more organic fertilizers than females used as reference group. These results suggest that efforts to increase fertilizer use could benefit from addressing gender-specific barriers and opportunities. These include targeted education and training programs for females' farmers, addressing access to resources, and promoting the benefits of organic fertilizers. Recognizing and leveraging these gender differences can enhance the overall effectiveness of fertilizer use strategies and contribute to more sustainable agricultural practices (Vani, 2018). Several studies by Alem *et al.* (2022), Le *at al.* (2024); Khan *et al.* (2024) have reported similar findings regarding the impact of gender on fertilizer use, particularly highlighting that men tend to use more organic fertilizers than females.

The results further showed that education level does not significantly (p = 0.7572 for primary, p = 0.4104 for secondary p = 0.7572 for tertiary) influence the use of fertilizer regardless of the sources. The high p-values for all education categories (0.7572 for primary, 0.4104 for secondary, and 0.7572 for tertiary levels) indicate that education level does not significantly influence the use of organic manure or inorganic fertilizers, suggesting that policymakers should explore other factors, such as economic incentives or cultural practices, to promote fertilizer use.

Similar studies have also highlighted the limited impact of education on fertilizer use. For instance, research conducted in Zimbabwe found that while education level was considered, did not significantly influence the adoption of both organic and inorganic fertilizers, indicating that other socio-economic factors played a more critical role in farmers' decisions (Muluneh *et al.*, 2022; Gumindoga *et al.*, 2024; Matamanda, 2024). Additionally, a study in South Asia revealed that despite educational programs, farmers often lacked awareness of proper fertilizer management, which further complicated the relationship between education and fertilizer use

(Aryal *et al.,* 2021). These findings collectively suggest that addressing barriers beyond education is essential for enhancing fertilizer adoption among farmers.

The results from the multinomial regression analysis concerning occupation at Mitundu indicated that, farmers had an estimate of 0.9209 with a standard error of 0.6851, yielding a z-value of -1.344 and a p-value of 0.025. This suggested a significant relationship with the use of organic fertilizer. Conversely, government employees have an estimate of -4.381 and a standard error of 1.423, yielding a z value of -0.605 and a p-value of 0.1789, indicating no significant impact on fertilizer use and type. These findings suggest that occupation plays a crucial role in influencing fertilizer use, particularly highlighting the importance of farmers in adopting and utilizing organic fertilizers effectively, while government employees may not engage similarly in this context. Research findings in Zimbabwe, South Asia, Nigeria and Vietnam, revealed that farmers are more likely to adopt both organic and inorganic fertilizers due to their practical experience and better understanding of fertilizer management practices compared to non-farming occupations, such as government employees (Aryal, Sapkota, *et al.*, 2021; Fami *et al.*, 2021; Olumba *et al.*, 2024). In particular, farmers involved in cooperative societies exhibited more effective fertilizer use, emphasizing the importance of access to information and resources (Mase & Prokopy, 2014). Additionally, studies have shown that training in modern farming techniques significantly enhances fertilizer application among farmers, highlighting the crucial role of occupation in shaping agricultural practices (Uzonna & Qijie, 2013; Rahman & Zhang, 2018).

For inorganic fertilizers, results showed that farmers' coefficient was -0.7164, indicating that there were no association between farmers and inorganic fertilizer type and use. This was substantiated by the t-value of -1.359 and the p-value of 0.374 that suggests that there was not a statistically significant difference. Similarly, for the Government employees, the coefficient was -0.250, which also indicates a decrease in the use of inorganic fertilizer. The t-value of -1.770 and the *p*-value of 0.281 indicated there were no statistically significant differences either. This suggested that the relationship is weak. Therefore, the trend toward reduced use of inorganic fertilizers and the lack of statistical significance suggests that further research should be done for effective soil and environmental management.

Other research with similar results includes the study by Aryal *et al.* (2021), which showed that many farmers are unaware of the recommended application rates, caused by lack of awareness can result in inefficient fertilizer usage and contributes to environmental problems. The study

reveals that demographic characteristics, such as age, gender, education, and occupation, have minimal influence on fertilizer use, particularly organic manure. Men tend to use organic fertilizers more than women, while education levels do not significantly impact fertilizer use.

Table 1: Influence of demographic factors (age, gender, and occupation) on fertilizers use

Variable	Estimate	Std. Error	z value	Pr (>|z|)
(Intercept)	0.9328	0.876	1.065	0.287
20-30 reference group				
31-40	0.507	0.53062	0.096	0.9239
41>	0.55070	0.48502	1.135	0.2562
Female reference group				
Male	-0.81484	0.3893	-2.093	0.0364 *
Business reference group				
Farmer	0.9209	0.6851	-1.344	0.025*
Government employees	-4.381	1.423	-0.605	0.1789
Non formal educational level reference group				
Primary level	0.67520	0.47887	1.410	0.7572
Secondary level	0.50136	0.60908	0.823	0.4104
Tertiary level	0.5560	1.79833	0.309	0.7572
Inorganic manure				
(Intercept)	1.0511	0.7542	1.394	0.163
20-30 reference group				
31-40	-0.1664	0.5091	-0.327	0.744
41>	-0.5983	0.4553	-1.314	0.189
Female reference group				
Male	0.65	0.130	-3.580	0.65
Business reference group				
Farmer	-0.7164	0.5272	-1.359	0.374
Government employees	-0.250	0.040	-1.770	0.281
Non formal educational reference group				
Primary level	16.2424	0.4708	-0.675	0.093
Secondary level	-0.1640	0.5913	-0.277	0.781
Tertiary level	0.2424	0.0543	0.009	0.893

(ii) Land preparation methods viz gender distribution, age categories, education levels and occupation profiles within the wards in non-protected areas

The Chi-square analysis of land preparation methods (tractor, oxen plough, and hand hoe) versus gender distribution reveals notable gender differences in the adoption of mechanized farming practices. Women had a low frequency respondent about 11 and 8 in Mitundu and Mgandu respectively with an average of 28.5, whereas men showed positive tendency with, high frequency of 34 and 32 in Mitundu and Mgandu, respectively, resulting in an average of 30.5 (Table 2). The Chi-square value for tractor use was 0.024, indicating a significant difference between genders. The association between gender and tractor use is statistically significant for men but not for women. These results indicated a gender gap in mechanized farming that suggested barriers such as limited access to resources and cultural norms. Result on oxen plough use for men showed a strong association with oxen plough use, showed by 28 and 26 respondents in Mitundu and Mgandu,with an averaging of 26, while women reported lower frequency numbers, with seven (7) in Mitundu and nine (9) in Mgandu, leading to an average of 6.5 of respondents. The Chi-square value for oxen plough use was 0.043, further emphasizing the disparity between genders, indicating that men use oxen ploughs more frequently than women do. On the use of hand hoe, women and men both showed no significant difference with 22 using hand hoes in Mitundu and 31 in Mgandu (average of 28), while in Mgandu and Mitundu the respondents were 20 in both wards (Mitundu and Mgandu), averaging 14. The Chi-square value for hand hoe use was 0.02, indicating that this method is equally common across the study area. This implies that gender does have influence on hand hoe usage, suggesting it is equally common practices across genders. These results imply that the use of tractors, which is more common among men, can negatively affect soil microorganisms due to soil compaction and disturbance.

In contrast, oxen ploughs provide a gentler alternative, promoting better soil health and enhancing microbial diversity. Additionally, hand hoeing, which was practiced equally by both genders, has minimal impact on soil organisms and is considered the most environmentally friendly method. The study suggests that increasing the use of oxen ploughs and hand hoeing could more effectively support long-term soil health compared to tractors, which tend to have more harmful environmental effects. Therefore, promoting these sustainable practices is essential for preserving soil microorganism diversity and overall environmental health. Similar studies by several researches including the one by Tilak *et al.* (2005) and Akinsemolu (2018), showed that tractor use, can lead to soil compaction and negatively affect soil microorganisms,

while traditional methods like oxen ploughing and hand hoeing are less disruptive to soil health. A study by Kebede *et al.* (2023) examines how gender affects agricultural practices, finds that men are more likely to adopt mechanized practices, while women tend to rely on traditional methods, which aligns with these findings regarding oxen plough and hand hoe usage.

Table 2: Influence of gender on farmland preparation on soil fungal microbes in Mitundu and Mgandu wards

Category	Variables	Mitundu ward	Mgandu ward	Average	Chi-square
Tractor	*Male*	34	32	30.5	0.024
	Female	11	8	28.5	
Oxen Plough	*Male*	28	26	26	0.043
	Female	7	9	6.5	
Hand hoe	*Male*	22	31	28	
	Female	20	20	14	0.02

(iii) Influence of education level and age on soil degradation forces in both protected and non-protected areas

The results from descriptive statistics reveal significant influence of education levels and age on soil degradation. According to Pearson's chi-square, education levels influenced land conversion to farmland, with primary education significantly (Chi-square value of 0.03) increasing the likelihood of such conversions. Individuals with primary education are more inclined to convert land into farmland compared to those with no formal education, as indicated by a frequency of 30 and 34 respondents in Mitundu and Mgandu respectively with an average of (32) of respondents (Table 3). This result contributed by fact that the dominant groups interviewed were respondents with primary education level and less in the non-formal education.

However, other education levels, such as secondary and tertiary education, do not significantly affect farmland conversion, as their average values of (3 and 1) in Mitundu and Mgandu, respectively. This result implies that farmland conversion positively influenced by primary education levels, but not by secondary or university education levels. This suggests that land degradation may be deteriorated by basic education, particularly if it is not sustainable or results in overexploitation. The study suggests that targeted educational initiatives can help raise awareness about sustainable land use practices, potentially mitigating land degradation. Several studies have reported the relationship between education levels and land conversion to

farmland, yielding results that align with the results of this study. A series of studies conducted showed that lower educational attainment is linked to less sustainable farming practices, increasing the risk of land degradation (Xue *et al.*, 2021; Li *et al.*, 2024).

Additionally, farmers with primary education are reported to be more prone to inefficient agricultural use, indicating that basic education may not provide adequate knowledge for sustainable practices (Olumba *et al.*, 2024). Furthermore, lower education levels hinder farmers' ability to adapt to land management practices, further impairing land degradation. These findings emphasize the critical role of education in fostering sustainable land management practices (Yang & Solangi, 2024). In contrast, secondary and tertiary education levels play a crucial role in mitigating overgrazing, as indicated by the low averages of 3.5 and 1.5 for secondary and tertiary education, respectively, while education had no significant effect on deforestation (Table 3). This suggests that individuals with secondary and tertiary education are less likely to contribute to overgrazing due to the understanding of sustainable land management and its environmental benefits. Similar research by Elahi *et al.* (2021), reported that farmers with higher educational backgrounds were more knowledgeable about grazing management techniques, the knowledge was associated with practices that minimized overgrazing, thereby promoting sustainable pasture management. The lack of a statistically significant association between education and deforestation indicated that other socio-economic factors might play a more critical (Ullah *et al.*, 2022). For instance, practices such as farmland expansion, wood fuel consumption, charcoal production, and urbanization can heavily impact forested areas regardless of the population's education level (Basnyat, 2009; Ullah *et al.*, 2022).

Table 3: Influence of education level on sources of soil degradation in protected and non-protected areas

Categories	Variables	Mitundu	Mgandu	Average	Chi-square
Forest conversion to farmland	None Formal Education	11	14	12.5	0.03
	Primary level	30	34	32	
	Secondary level	3	3	3	
	Tertiary level	2	0	1	
Overgrazing	None formal	14	5	9.5	0.73
	Primary level	25	14	19.5	
	Secondary level	5	2	3.5	
	Tertiary level	2	1	1.5	
Deforestation	None formal	9	12	10.5	0.5
	Primary level	19	37	28	
	Secondary level	3	2	2.5	
	Tertiary level	3	2	2.5	

The study revealed that age significantly influenced land conversion, overgrazing, and deforestation patterns. Individuals aged 41 and above were notably more likely to convert forest land to farmland, driven by economic or personal motives, as indicated by chi-square of 3.667, an average of 31 Mitundu and Mgandu wards and frequency of (24) and (11) (Table 4). Conversely, this older age group is less likely to contribute to overgrazing, suggesting they may possess greater knowledge of sustainable practices, supported by the low responses frequence. Younger age groups (20-30 and 31-40) show no significant associations with land conversion or overgrazing; indicating they may be less involved in agriculture or has differing priorities. Additionally, age does not significantly affect deforestation rates, implying that other factors, such as economic incentives and land management practices, are more critical to deforestation dynamics. Result by Basnyat (2009), Ogbuene *et al.* (2012), and Ali and Lyimo, (2022), showed that population growth, food demand, social economic needs are among the major contributors to deforestation and overgrazing.

Table 4: Influence of age on sources of soil degradation in protected and non-protected areas

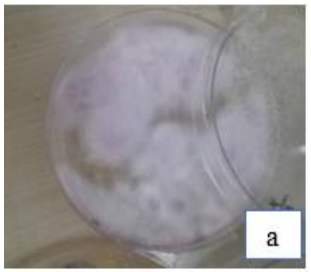
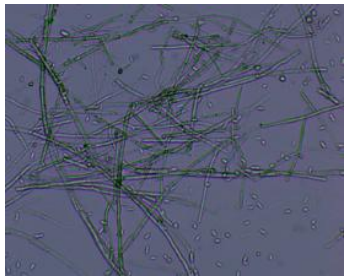
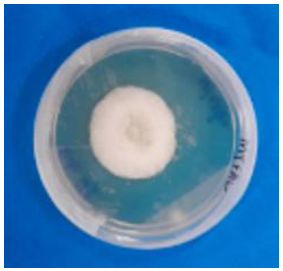
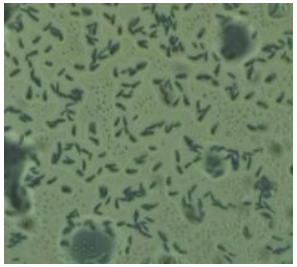
Categories	Variables	Mitundu	Mgandu	Average	Chi-square
conversion to farmland	20-30	6	10	8	0.017
	31-40	9	11	20	
	41<	31	31	31	
Overgrazing	20-30	6	5	5.5	0.645
	31-40	16	6	11	
	41<	24	11	17.5	
Deforestation	20-30	7	12	9.5	0.206
	31-40	8	13	10.5	
	41<	21	28	24.5	

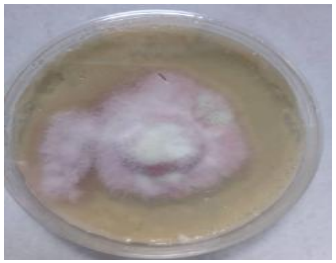
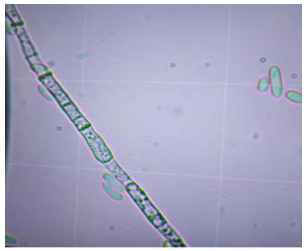
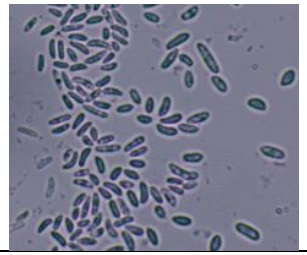
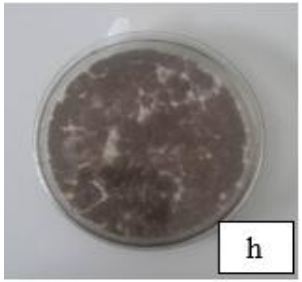
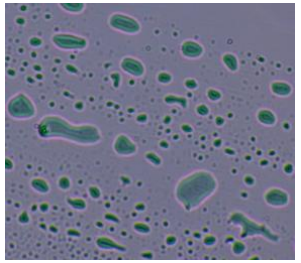
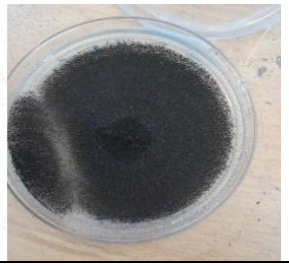
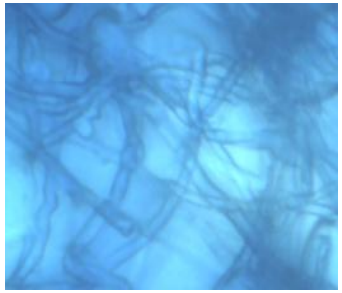
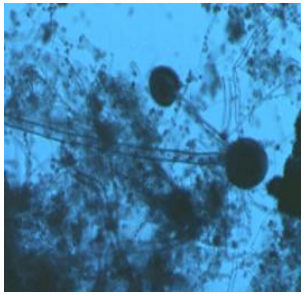
4.3 Determining the diversity of fungal communities in soils from non-protected and protected areas

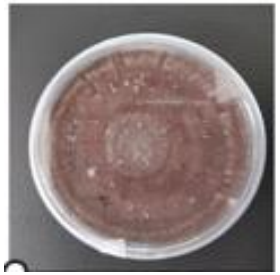
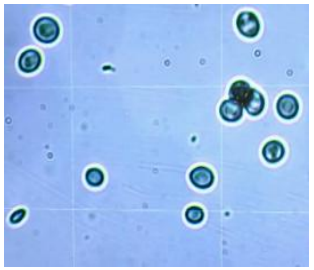
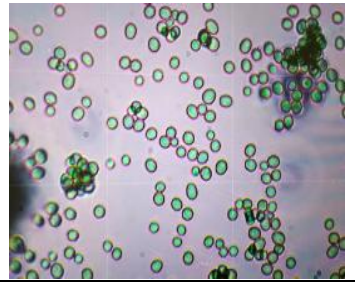
Soil fungal communities significantly influence plant health, soil structure, and nutrient cycling, making them crucial for ecosystem functioning. Understanding their diversity is essential for conservation and human intervention. Protected areas often considered biodiversity havens, while non-protected areas face more disturbances caused by human. This study examined fungal community diversity differences between protected and non-protected soils to understand the stability and resilience of ecosystems. The macro and microscopic characteristics of soil fungi were observed, as well as fungal composition, abundance and distribution.

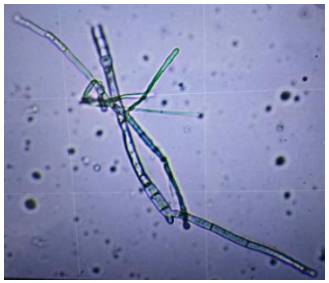
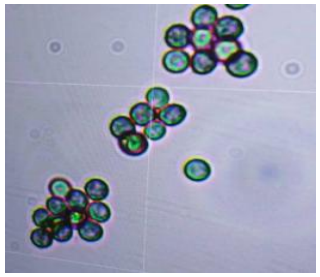
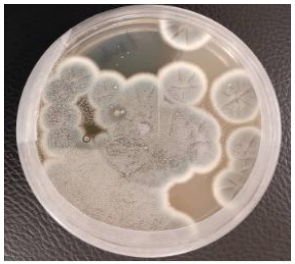
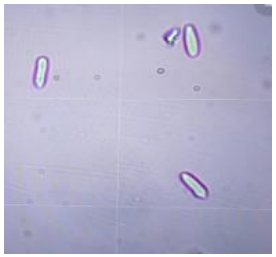
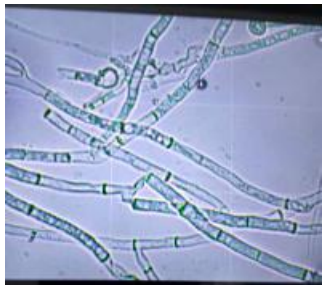
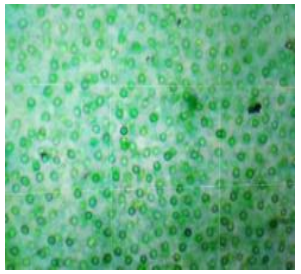
(i) Macroscopic and microscopic characteristics of isolated soil fungal species from protected and non-protected of the study areas

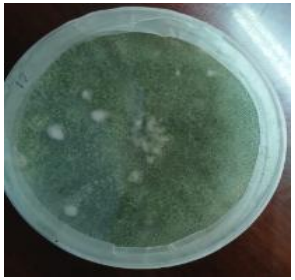
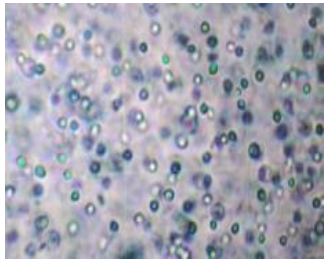
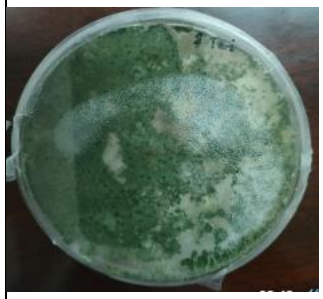
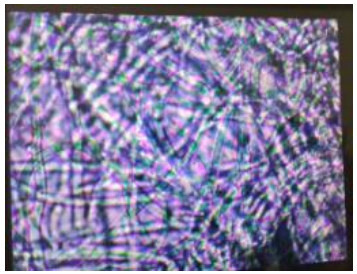
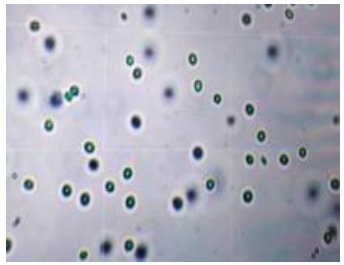
The objective on fungal diversity focused on assessing macroscopic features that included number, colour, shape and texture on the other hand microscopic features like spore shape and mycelia of the fungi were used to differentiate different fungal colonies. Identified type of colonies was compared using reference search engine and different database that include google search engine, MYCOBANK Database https://www.mycobank.org/, https://fungalgenera.org/, reviewed and published research article goggled from google scholar engine to assist in identifying the possible beneficial fungi. Fifteen (15) fungal species were studied out of 35 isolated (plate 5) due to income constrains. Fungal abundance and distribution were estimated in percentage as presented in Section 4.3 (ii).

Species	Colony description	Mycelia	Spore	Status of fungal species	Previous study
Fusarium oxysporum	White to violet-purple in color, Cottony texture 	Network and septate hyphae 	Transparent, oval/elliptical in shape 	abundance	Hussein *et al.,*2012
Fusarium soloni	white colon with cotony texture 	septate hyphae 	Crescent shape with elongated end. 	abundance	Shamsi *et al.,* 2019

Fusarium grameniu m	White to pink colon color ,wool in texxture 	septate 	Banana shaped 	abundance	(Alsohaili & Bani-hasan, 2018).
Aspergilu s tamarii	Black with white mark colony, granular texture 	Dense network and septate 	Spherical to ovoid in shape 	Abundance	Arshad *et al.,* 2023 Guerrero *et al.,* 2020
Aspergillu s niger	Dark brown to dark colony color, Velvet-like texture 	Dense network and aseptate 	Globose shape 	Abundance	Bisht *et al.,*2016

Rhizopus stolon	Grayish/ black in color , fluffy texture 	Non- septate/ aseptate 	Oval/spherical shaped 	Abundance	Arshad *et al.,* 2023
Apergillus flavus	Green yellow in color, cotony with white border , Powdery with black seed like structures 	Dense septate 	Round to oval shaped 	Abundance	Arshad *et al.* 2023

Epulorhiza spp	black in color, Velvet-like texture (compact) 	septate and well branched 	Round to ovoidal shape in cluster 	Abundance	Arshad *et al.* 2023
Penicilliu m chermesin um	gray color 	Septate branched 	ellipsoidal or cylindrical 	Function	Bani-Hasan, 2018)
Lasiodipl odia spp	white in color, Cotton dense-waxy texture 	Septate and dense mat 	Oval shaped 	Abundance	(Desvani *et al.,* 2018)

Trichoderma koningiopsis	White to light green, Velvet texture 	Branched and Septate 	Globose shape 	Function	(Hassan & Chang, 2021)
Trichoderma harzinum	White to green color, Cottony to velvet 	Branched and Septate 	ellipsoidal and globose in shape and chain like structure 	Abundance and Function	Arshad *et al.*2023
Sclerotinia sclerotiorum		Septate, heavy dense mat network 	Round shape 	Abundance	Pavlović *et al.,* 2014)

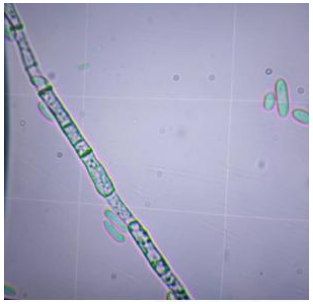
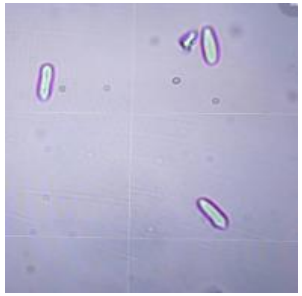
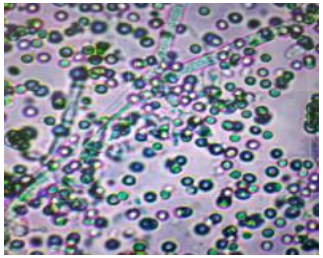
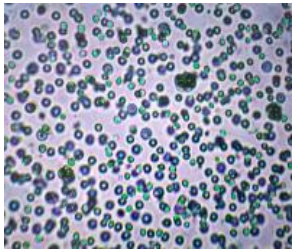
Penicilliu m chrysogen um	Greyish- gray colon color, Velvet in texture 	Septate branched 	oval shape 	abundance and function	Alsohaili & Bani-Hasan, 2018)
Trichoder ma viride	White to green colon wit cottony texture. 	Branched and septate 	Globose shape 	Function and abundance	

Plate 5: Morphological characteristics and status of soil fungal species isolated from protected and non-protected areas (Mitundu and Mgndu)

(ii) Assessment on the fungal composition and general abundance

The result from the analysis of variance indicated that all fungal species found in the study area differed significantly (Fig. 5**)** and (Table 10 in appendices). Duncan's Multiple Range Test was performed to obtain a mean separation test for fungal species composition and overall prevalence within the ecosystem. Result indicated that *Trichodema hamatum (2.4%), Aspergillus citrinum (5.6%) cladosporium oxysprorum (9%), Aspergillus oryzae (11%), Drechslera spp (11%)* were among the least abundance species in the study areas whereas Trichoderma viride (37) %, *Penicillium chrysogenum* (35%), *Trichoderma* koningiopsis (32%), and *Lasiodiplodia spp*. (31.1%). *Trichoderma harzianum* (28.62%) and *Fusarium graminearum* (28.6%) were highly abundant.

The found fungi species in study area were mostly belonging to Ascomycete with 19 species, Deuteromycetes with 15 species while Zygomycetes had one (1) species making total of 35 species composition. The Ascomycetes and Deuteromycetes were commonly abundant and found in all soil samples analyzed from the study areas. This result is similar to what (Puangsombat *et al.*, 2010), who reported that Ascomycetes and Deuteromycetes were in large number in various agricultural land and forest soils. The presence of these species in the soils implies a variety of functional roles within the soil environment, which is crucial for sustaining a healthy ecosystem and supporting numerous biological processes. The ecological roles played by such fungi as Ascomycetes and Deuteromycetes has been reported by several authors (Adedayo & Babalola, 2023; Mandal & Tiru, 2022).

Despite of lower abundance species like *Aspergillus citrinum* (5.6%) and *Trichoderma hamatum* (2.4%) are less common, they play a crucial role in maintaining ecological balance and diversity. This highlights the importance of preserving ecological stability and resilience (Shahriar *et al.,* 2022). Furthermore, it emphasizes the need for agricultural practitioners to enhance fungal biodiversity, which ultimately supports and improves ecosystem services (Bhardwaj *et al.,* 2023). Together, these findings underscore the interconnectedness of fungal diversity, ecological health, and agricultural sustainability (Shahriar *et al.,* 2022). Furthermore, the presence of these less abundant species highlighted the importance of conservation strategies that protect the entire fungal community, rather than just the dominant species (Yao *et al.,* 2023). *Trichoderma viride*'s dominance highlighted the need for continuous conservation and monitoring to protect fungal diversity, particularly in non-protected areas where agricultural practices may threaten these communities, promoting both agricultural

productivity and ecological health (Mandal & Tiru, 2022; Yao *et al.,* 2023).

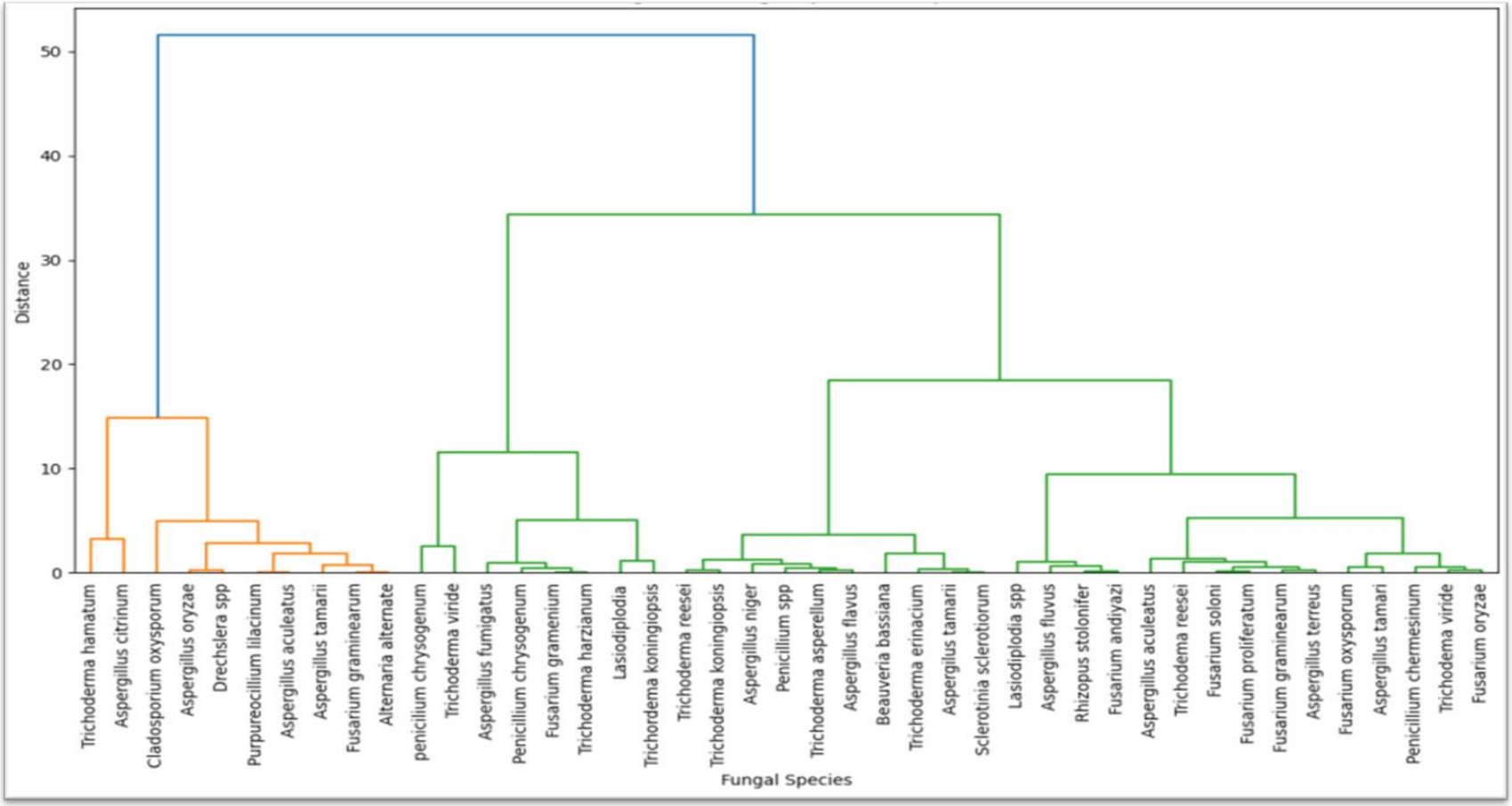


Figure 5: Dendrogram clustered diagram showing fungal species composition from protected and non-protected of Mitundu and Mgandu wards

Cluster 1 (Green): This cluster indicates species with similar or overlapping composition percentages and abundances. For example, *Aspergillus flavus* and *Fusarium graminearum* are grouped together. Cluster 2 (Orange): Indicates a low notable difference in its composition compared to the green cluster.

(iii) The influence of conservation practices on fungal abundance and distribution

The results from analysis of Chi-square on colony number, types and colony forming unit (CFU) showed that, protected areas (both Mitundu and Mgandu) consistently indicated significant higher mean fungal colony numbers (158), CFU (15766666.67), and types of colonies (17) compared to their respective non-protected areas fungal colony numbers (59), CFU (5534667), and types of colonies (7.26) (Table 5). The results suggest that fungal diversity and abundance are significantly greater in the protected areas (35 species), and non-protected areas (30 species), supporting the hypothesis that conservation areas help sustains more diverse and abundant fungal communities (Alem *et al.,* 2022). The statistical significance (p-value < 0.05) confirms that the differences between protected and non-protected areas are not due to random variation.

Table 5: Soil fungal abundance in protected and non-protected areas

Site	Metric	PA Mean	PA Std	NPA Mean	NPA Std	t-statistic	p-value	Significant ($p < 0.05$)
Mgandu	Number of Colon	158.7857143	68.31170479	58.4375	28.11635052	5.129371141	8.65E-05	TRUE
Mgandu	CFU/mL	15878571.43	6831170.479	5843750	2811635.052	5.129371141	8.65E-05	TRUE
Mgandu	Colon type	17.14285714	5.171774623	13.3125	5.016223679	2.056682282	0.049142581	TRUE
Mitundu	Number of Colon	157.6666667	63.94491677	59.8	30.06231623	5.36429802	1.03E-05	TRUE
Mitundu	CFU/mL	15766666.67	6394491.677	5534666.667	3268468.114	5.518200265	6.74E-06	TRUE
Mitundu	Colon type	15.6	6.021390442	7.266666667	3.990464826	4.467950807	0.000156576	TRUE

PA = Protected area, NPA = Non-protected area, Std = Standard Deviation, TRUE = Shows there is a significance difference.

(iv) Soil fungal distribution and abundance

Analysis of variance and mean separation test on fungal species distribution and abundance in the non-protected areas, the fungal species exhibit lower mean distribution values (Table 12), with abundances ranging from *Trichoderma hamatum* at 2.4% to *Trichoderma viride at 21.1*%. This finding aligns with the results documented by Afzal *et al.* (2021) and Avigliano *et al.* (2019), which reported a remarkably lower overall abundance of fungal species in non-protected areas compared to those in protected regions. Notably, common genera such as *Fusarium, Aspergillus*, and *Penicillium* were frequently observed in non-protected areas, suggesting that these fungi are well adapted to disturbed or less regulated environments. For example, *Fusarium graminearum* (7.8 %) and *Penicillium chrysogenum* (12.2%) highlighted the prevalence of species that thrive under conditions of ecological stress as documented by van Rhijn and Bromley (2021) in their study. While some species, like *Fusarium andiyazi* and *Trichoderma reesei*, show moderate abundance at 14.3%, the overall distribution remains less robust, underscoring the challenges faced by fungal communities in non-protected areas. These results highlighted the need for improved protection and management strategies to enhance the diversity and resilience of fungal populations, thereby supporting healthier ecosystems.

In the protected areas, a variety of *Tirichoderma* species, such as *Trichoderma harzianum* (29.65%), *Trichoderma viride* (37.5%), and *Trichoderma reesei* (20.88%) appear frequently and with high abundance. These fungi known for their roles in bio-control and might be thriving due to the favorable conditions in protected areas. Aspergillus species, such as *Aspergillus terreus* (28.97%), *Aspergillus fumigatus* (28%), and *Aspergillus niger* (27.55%), are also well represented in the protected area. These fungi are typically associated with decomposing organic matter and benefit from the diverse organic material available in protected areas (Wu *et al.,* 2021). *Penicillium* species found in protected areas were significantly higher with *Penicillium chrysogenum* having 29.87% and *Penicillium spp.* with 25.9%, whereas same species in non-protected exhibiting significantly lower abundance with *Penicillium chrysogenum* (12.2%). Fusarium species such as *Fusarium graminearum* (22.47%) and *Fusarium oxysporum* (28.17%) were notable for their higher prevalence in the protected area, although they are also common in non-protected areas with 7.8 % and 16.7 % respectively. These fungi are often plant pathogens and their distribution patterns could be influenced by plant host availability.

The results of this study showed significant difference (p-value <0.05) in fungal diversity, distribution and abundance between protected and non-protected areas (35 species and 30 species, respectively). This demonstrated the importance of conservation in maintaining ecosystem health. Similar result was reported by Park *et al.* (2019) and Gonçalves *et al.* (2023), who observed that diversity indices of the fungal assemblages detected varied across the different sampling sites but, in general, they displayed high diversity and abundance and moderate dominance. It is also reported that factors contributing to this diversity could be due to improper use of nutrient fertilizers, crop residue burning, deforestation, and overgrazing as mention by the respondents during survey (Atem, 2011).

Generally, fungal species in protected areas exhibit higher diversity, distribution, and abundance compared to those in non-protected areas. Non-protected areas host less robust fungal communities, with species like *Fusarium, Aspergillus*, and *Penicillium* more prevalent, indicating their adaptation to disturbed environments. Conversely, protected areas show a greater prevalence of species such as *Trichoderma, Aspergillus*, and *Penicillium*, thriving in stable conditions. This study emphasizes the vital role of conservation in maintaining healthy fungal communities, which are crucial for ecosystem functioning. Significant differences in fungal diversity, highlighted by a low p-value, underscore the need for improved management strategies to mitigate the impacts of human activities on fungal diversity in non-protected areas. Overall, the findings advocate for enhanced conservation measures to support fungal diversity and ecosystem health.

4.4 To identify and test potential beneficial soil fungi in non-protected and protected areas at the Mitundu and Mgandu wards

Beneficial fungi, which constitute a major portion of soil microbial communities are vital for sustaining soil health, promoting plant growth, and enhancing ecosystem resilience against environmental stressors (Devi *et al.,* 2020; Jagadesh *et al.,* 2024). Fungi such as *Penicillium spp*, contributes to plant and soil productivity by improving nutrient and water uptake, breaking down organic matter, and suppressing harmful pathogens (Mehta *et al.,* 2016)

The primary aim of this objective was to identify fungal species with potential for use in the rehabilitation of degraded soils and enhancement of plant growth under low fertility conditions. Two fungal species out of 15 fungal isolated from protected and non-protected belonging to ecologically important genera such as *Penicillium* and *Trichoderma* spp were used. From these

Penicillium spp and *Trichoderma* spp were selected for further study due to its abundance and well-documented effectiveness in improving soil quality and promoting plant development. To assess its potential, both species were tested in a pot experiment at the screen house using *Eleusine indica* and *Amaranthus spinosus* as test plants. These trials aimed to evaluate the fungi's influence on plant growth and soil improvement, providing insights into their applicability for ecological restoration and sustainable land use practices.

(i) Molecular identification of potential beneficial fungi

The research successfully utilized molecular techniques in extracting and amplifying genomic DNA from fungal microbial samples through Polymerase Chain Reaction (PCR).

(ii) Polymerase chain reaction (PCR) of isolates

In the polymerase chain reaction (PCR) conducted using a C1000 Touch Thermal Cycler, 2 µL of fungal genomic DNA was utilized for amplification with OneTaq 2X Master Mix. The PCR circumstances included an initial denaturation at 94 °C for 2 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 52.5 °C for 30 seconds, and extension at 72 °C for 30 seconds, concluding with a final extension at 72 °C for 5 minutes. DNA was extracted using universal primers for Penicillium and Trichoderma spp., specifically ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') as the forward primer and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') as the reverse primer for penicillium and (5'-AAGTAGAAGTCGTAACAAGG-3) forward and (5'-GGTTGGTTTCTTTTCCT-3') reverse primers for Trichoderma. The amplified PCR products of the Penicillium fungal species isolate were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized using a UV transilluminator (Cole Parmer), where a band corresponding to 125 bp for *Penicillium chrsogenum* and 127 bp for *Trichoderma harzianum* was observed (Fig. 5). These characterized isolates were subsequently used in a pot experiment to evaluate their effects on the growth of *E. indica* and *A. spinosus.*

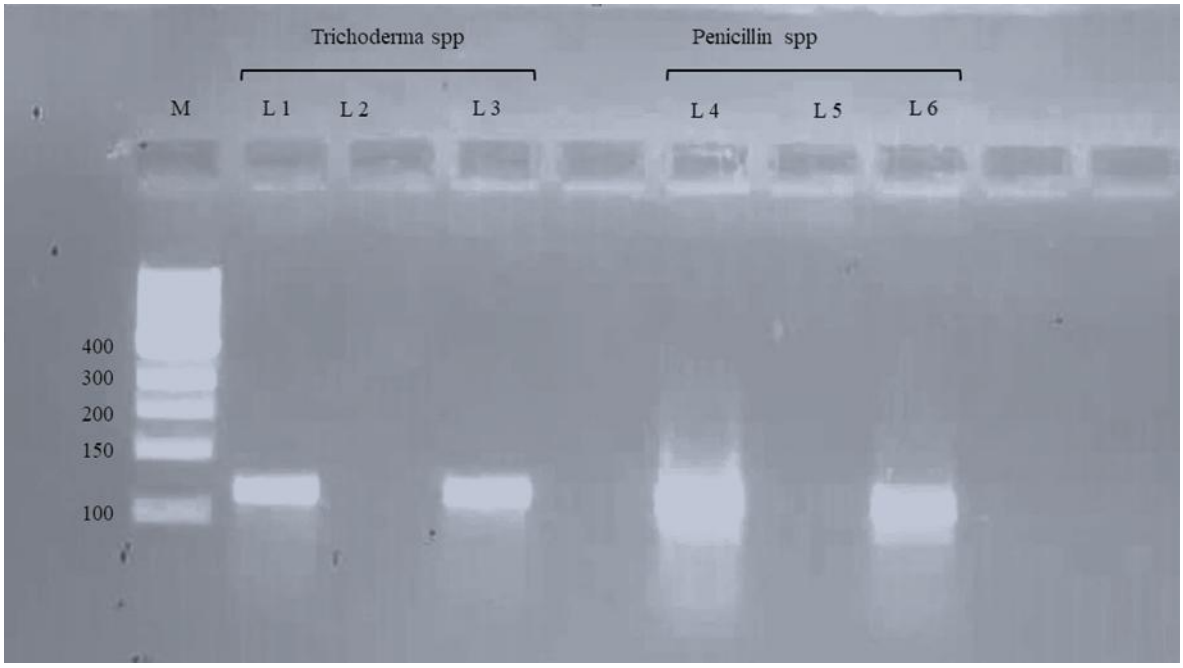


Figure 6: PCR amplification of ITS region of Penicillium chrysogenum (125bp) and Trichoderma harzianum (127bp) (isolates)

M=DNA ladder, Line (1, 2, 3)-*Trichoderma* spp, L1=Trichoderma positive control, L2=*Trichoderma* negative control, L3=*Trichoderma* amplified DNA template; Line (4, 5, 6)-Penicillium spp, L1=Penicillium positive control, L2=*Penicillium* negative control, L3=*Penicillium* amplified DNA template.

(iii) Evaluation of the efficacy of soil fungal isolates (*Trichoderma harzianum* and *Penicillium chrysogenum*) as potential plant growth promoters

The results of this study shows that inoculation of wild finger millet (*Eleusine indica*) with soil fungal isolates had a substantial impact on plant growth characteristics, *viz* plant height, leaf length, number of leaves, and tillers. Consistently, *Penicillium chrysogenum* isolates with maize bran as carrier material (PEB) significantly ($p \leq 0.05$) enhanced above ground wild finger millet plant growth parameters outperforming other experimental treatments including the positive control (Table 9 and plate 6). In comparison to the positive control (PCE), *Trichoderma harzianum* with finger millet (TEM) exhibited a significant improvement in leaf length only. Tillering was strongly influenced by fungal treatments, with significant differences found between NCE and PEM and between *Trichoderma harzianum* with maize bran (TEB) and PEM (Table 9). The strong statistical significance ($p < 0.001$) for most comparisons further reinforces the reliability of these findings. On contrary, all experimental treatments appeared to have no significant ($p \leq 0.05$) influencing root length.

The results further indicate that inoculation of wild finger millet with PEM and PEB significantly improved plant biomass on root biomass ($p \leq 0.01$) for dry shoot biomass and p ≤ 0.05 for dry root biomass) compared to other experimental treatments (Table 9 and Plate 7 A, B, D and H). The highest values for dry shoot and root biomass were recorded in plants inoculated with PEM (16.69 and 6.49g respectively), followed closely with those inoculated with PEB (13.98 and 5.75 g, respectively) (Table 9). On other hand, wild finger millet plants grown without inoculants (NCE) recorded the lowest plant biomass (8.19g) which almost similar to the observation made on plant inoculated with the positive control (PCE) (9.1g) indicating poor dry biomass accumulation. These results suggest that PEB and PEM significantly enhanced wild finger millet plant growth parameters and the overall plant biomass making them promising component for the integrated soil fertility management program for enhance plant growth and the overall plant vigor. Again, increased, root length and biomass were observed in these treatments indicating better nutrients and water uptake.

Conversely, the poor performance of NCE showed that this treatment was less effective in promoting growth, substantiating the positive effects of microbial fungi applied treatments. These results also suggest that TEB despite having a well-ramified root system did not achieve the highest biomass accumulation, indicating that root structure and system alone may not determine overall growth efficiency. Similar result is reported by Devi *et al.* (2020) that, fungi including *Penicillium* and *Trichoderma spp*. are associated with plants and plays an important role in plant growth promotion and enhanced soil fertility using different plant growth promoting mechanism such as solubilization of phosphorus, zinc, potassium; production of plant growth regulator.

It is further reported by Nosheen *et al.* (2021) that, fungi as bio-fertilizers are a promising alternative to hazardous inorganic fertilizers. This is achieved through microbial fungi interaction with the plants that enhances their immunity, growth, and development. Usharani *et al.* (2019) reported that the use of beneficial fungal microbial in agriculture lessens the need for inorganic fertilizers, which reduces ecological effects like soil degradation and nutrient losses via runoff or leaching. Study by Khan *et al.* (2020) reported that *Penicillium chrysogenum* significantly enhanced plant development by improving nutrient accessibility and approving root elongation in maize, which is similar to the findings of this current study in wild finger millet whereby PEB and PEM inoculation appeared to significantly support plant development and biomass.

Similarly, the study by Zhao *et al.* (2019); Oljira *et al.* (2020) and Ali *et al.* (2022) found that *Trichoderma harzianum* treatments improved leaf development and biomass accumulation in wheat, which aligns with the significant enhancement of leaf size and tillering observed in TEM and TEB treatments. In this study, the use of PEB and PEM as a treatment encourages environmentally friendly, sustainable farming methods in return supporting plant growth.

Again, findings by Gupta *et al.* (2022) demonstrated that inoculating maize plants with *Penicillium chrysogenum* strain 34-P led to significant increases in shoot length, dry biomass, fresh biomass, and total chlorophyll content under saline conditions. Similarly, the findings by Zhao *et al.* (2022) indicated that inoculation of wild finger millet with *Trichoderma harzianum* enhanced photosynthetic pigment concentration and root dynamics, resulting in improved plant growth and development. A study by Tatung *et al.* (2024) also found that inoculation of rise seeds with *Trichoderma harzianum* TaK12, a phosphate-solubilizing agent, significantly increased plant biomass and root length, aligning with the observed performance of, PEB, PEM and TEB inoculation in this current study.

Again Leitão, (2009) documented that Penicillium strains as soil fungus, generate extracellular enzymes such as cellulose, manganese, and pectinase, which allow them to break down hydrocarbons like phenol, halogenated chemicals, and PAHs and use agricultural waste, which makes them useful for biotechnological applications. The use of fungal microbes also enhances soil health by increasing microbial activity, inhibiting soil-borne diseases, and improving organic matter decomposition, which leads to better soil fertility and structure (Alori *et al.,* 2017; Kv *et al.,* 2019).

Environmentally, these fungi reported to contribute to sustainable agriculture by reducing the need for chemical pesticides and fertilizers, enhancing soil resilience, and promoting long-term soil productivity through improved nutrient cycling and carbon sequestration (Das *et al.,* 2025). Soil fungi further aid in rebuilding soil microbial communities, increasing nutrient availability in impoverished soils, and improving soil structure, thereby boosting agricultural productivity and ecosystem management on degraded lands. Hence, according to this study, these fungi can be used to rehabilite the degraded ecosystem in general.

Plate 6: Wild finger millet growth response to soil fungal isolation inoculation: (A) one-week after inoculation, and (B) 6 weeks after planting

Treatments included PEB (*Penicillium chrysogenum)* in maize bran carrier materials, TEM (*Trichoderma harzianum)* with millet finger carrier materials, PCE (*E. indica*) with positive control), and NCE (*E.indica*) with negative control)

Table 6: Mean Separation analysis for wild finger millet plant growth parameters and plant biomass following inoculation with soil fungi isolates Penicillium chrysogenum and Trichoderma harzianum inoculation

Treatment	Plant height	Leaf length	Number of leaves	Number of tillers	Shoot dry weight	Root dry weight	Root length
NCE	11.92 a	19.59 a	9.96 ab	1.417 a	8.19 a	1.616 a	33.59 a
PCE	13.18 a	25.42 b	10.54 ab	2.667 a	9.1 ab	3.351 ab	47.77 b
TEB	11.64 a	19.78 a	9.25 a	4.167 b	11.2 abc	2.38 a	41.34 ab
TEM	13.4 a	18.94 a	11.38 bc	4.458 bc	12.09 bc	3.904 abc	35.33 a
PEB	16.59 b	26.78 b	11.83 bc	5.833 c	13.98 cd	5.753 bc	42.22 ab
PEM	12.07 a	20.95 a	12.71 c	5.792 c	16.69 d	6.489 c	43.12 ab
CV%	25.0	34.8	30.6	58.6	25.2	60.1	23.3
LSD	1.874	4.349	1.911	1.357	3.523	2.774	11.13
P-value	<.001*	<.001*	0.006*	<.001*	<.001*	0.008*	0.132ns
df	5	5	5	5	5	5	5
SED	0.948	7.619	3.349	0.686	1.725	1.358	5.45

ns =Means with the similar letter(**s**) within a column indicates not significantly different according to DMRT (P<0.05).

***=** Means with different letter(s) within a column are significantly different at (P<0.05)

NCE=Negative control, PCE=Positive control (purchased one), TEB= *Trichoderma harzianum* in Maize bran, TEM= *Trichoderma harzianum* in finger millet PEB= *Penicillium chrysogenum* in maize bran, PEM *Penicillium chrysogenum* media, in finger millet media,

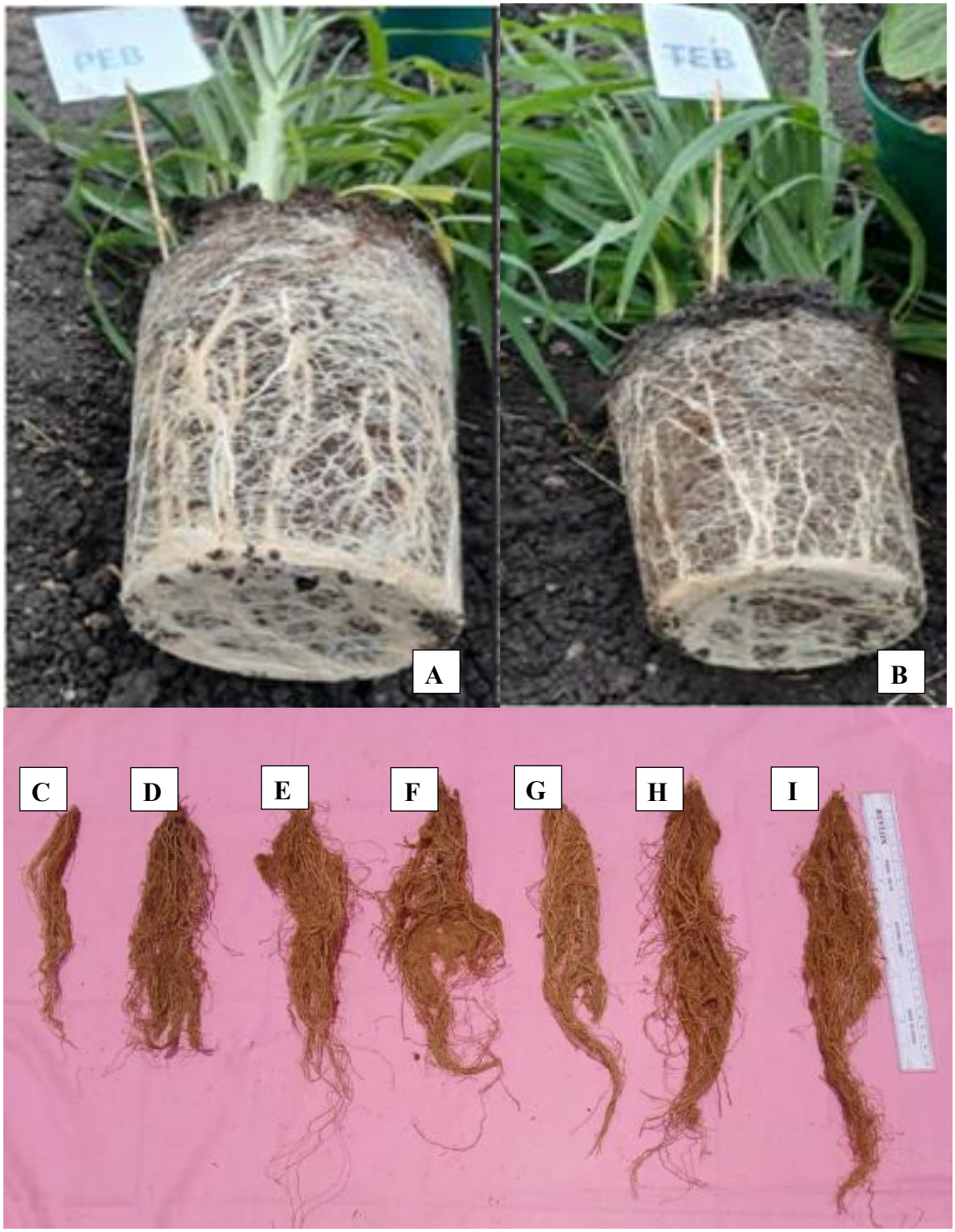


Plate 7: Wild finger millet (E. indica) roots responses following inoculation with soil fungi isolates (Trichoderma harzianum and Penicillium chrysogenum) 8 weeks after planting (A, B) PEB and TEB (C) NCE, (D) PEB, (E) TEB, (F) TEM, (G) PCE, (H) PEM and (I) PCE

TEB= Trichoderma harzianum in carrier material, TEM= Trichoderma harzianum in carrier material PEB= Penicillium chrysogenum in carrier material, PEM =Penicillium chrysogenum in carrier material, NCE=Negative control, PCE=Positive check (purchased from the nearest agro-dealer shop)

In addition, the results of this study further indicate that inoculation of *Amaranthus spinosus* with soil fungi isolates had no significant ($p \leq 0.05$) influence in promoting plant growth parameters except root length ($p \leq 0.001$) (Table 10). Amaranths plants inoculated with PAM recorded the highest value (24.62 cm) of root length compared with other experimental treatments (Table 10). The *Amaranthus* plants grown without inoculation of soil fungi recorded the smallest value (10.27 cm) for root length (Table 10 and plate 8). The observed results imply that soil fungi isolates inoculants as well as the positive control (commercial inoculatant) had no significant influence in promoting *Amaranthus* plant growth. The observed significant and positive effect of the soil fungi isolates on enhancing roots growth of the amaranths plants compared to the control treatment denotes the significance of *Penicillium* spp and *Trichoderma spp* in promoting certain aspects of plant growth.

Plate 8: (A) PAM plant showing good growth (B) PCA branches (C) PAB fresh root (D) PAM fresh root length (E) NCA, TAB, and PAM plant growth (F)PAB and PAM plants and roots PCA= Positive control of Amaranthus (6 weeks after planting)

PAB= *Penicilium* spp + Maize Bran innoculated to *Amaranthus*, PAM=*Penicillium* + millet finger inoculum treatment, TAB= *Trichoderma* spp + Maize Bran innoculated to *Amaranthus* and NCA = negative control

Table 7: Mean Separation analysis for Amaranthus spinosus plant growth parameters and plant biomass following inoculation with soil fungi isolates Penicillium chrysogenum and Trichoderma harzianum inoculation

Treatment	Plant height	Number of leaves	Plant height	Number of branches	Shoot dry weight	Root dry weight	Root length
NCA	25.44 a	11.89 a	5.833 a	5.833 a	3.378 a	0.858 a	10.27 a
PCA	31.16 a	13.22 ab	31.16 a	8.444 b	5.542 ab	1.128 a	16.77 b
PAB	23.85 a	13.61 b	23.85 a	6.5 ab	4.398 ab	1.227 a	19.82 b
TAM	27.7 a	12.67 ab	27.7 a	7.5 ab	6.065 ab	1.252 a	18.15 b
PAM	23.18 a	13.72 b	23.18 a	6.056 a	6.857 ab	1.558 a	24.62 c
TAB	24.98 a	12.78 ab	24.98 a	6.556 ab	8.367 b	1.578 a	17.31 b
CV%	57.7	17.5	40.6	40.6	53.0	46.1	20.2
LSD	9.94	1.498	1.828	1.828	3.605	0.6883	4.245
P-value	0.631	0.157	0.052*	0.052*	0.106	0.289	<.001**
df	5	5	5	5	5	5	5
SED	5.01	0.755	0.921	0.921	1.765	0.3370	2.078

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

CHAPTER FIVE

CONCLUSION AND RECOMANDATIONS

5.1 Conclusion

The study on fungal diversity and composition in the soils of protected and non-protected areas in the Itigi District, Singida Region, Tanzania, provides valuable insights into soil health and microbial ecosystems. Significant differences in fungal richness and abundance between these land uses contribute to ecological stability and resilience. Protected areas are identified as crucial reservoirs for biodiversity, housing diverse fungal communities essential for ecosystem functioning. Notable findings include high colony abundance in Mitundu and Mgandu wards, supporting beneficial fungi like *Trichoderma* spp., particularly *Trichoderma harzianum, Trichoderma viride*, and *Trichoderma reesei*, along with *Penicillium chrysogenum.*

These fungi enhance soil quality and promote the growth of wild finger millet (*E. indica*), which is vital for sustaining healthy ecosystems and promoting sustainable management practices. The study highlights the recognized benefits of *Trichoderma harzianum* and *Penicillium chrysogenum* in improving plant development and soil health, emphasizing their potential to boost agricultural output. It suggests the use of these species as biotechnological tools for ecological restoration and soil rehabilitation. Furthermore, future research should focus on classifying these fungi at the species level through sequencing to confirm their efficacy in promoting finger millet growth. Ultimately, the study underscores the importance of understanding soil fungal communities and implementing conservation measures in semi-arid regions to enhance soil health, biodiversity, sustainable agriculture, and environmental resilience.

5.2 Recommendations

Based on the study's findings, the following recommendations are proposed:

(i) To educate local populations through educational initiatives to increase knowledge of the value of healthy soil and the function of soil fungus in ecosystems.

(ii) The future studies should include younger respondents to better understand their views and needs, thereby addressing the potential gap identified in the current research.

(iii) Farmers and government employees are more likely to adopt organic fertilizers, suggesting further research for sustainable agricultural practices.

(iv) To implement programs that provides women in agriculture more resources and training, and make sure that both sexes have an equal chance to participate in land management decision-making.

(v) To invest on production of soil fungi-based bio-fertilizers *Trichoderma harzianum* and *Penicillium chrysogenum,* to enhance increase agricultural yields and soil health in degraded soils.

(vi) To initiate habitat restoration projects in degraded areas to boost native vegetation and soil microbial communities, enhance ecosystem functions, improve soil quality, and create habitats for beneficial fungi and microorganisms.

(vii) To develop comprehensive land use plans that balance agricultural production with conservation efforts, ensuring sustainable management of non-protected areas to prevent further degradation and biodiversity loss.

(viii) To establish fungal inoculation programs using *Trichoderma harzianum* and *Penicillium chrysogenum* to restore degraded soils, enhance soil health, and promote the recovery of plant growth

(ix) To conduct further studies to explore the interactions between soil fungi and various plant species in both protected and non-protected areas, focusing on their roles in nutrient cycling and soil health.

(x) To conduct further research to examine other anthropogenic activities that affecting soil fungal composition and distribution.

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APPENDICES

Appendix 1: Research questionnaires

1. **General Information**

a) Date: 11. 3. 2024

b) Name of Enumerator:……

c) District:…….

d) Ward: ……..

e) GPS Coordinates

_____

f) latitude (x.y °)

_____

g) longitude (x.y °)

_____

h) altitude (m)

_____

i) accuracy (m)

_____

1. Personal Information

a. Respondent Type

◯ Expert ◯ Community

b. **Name of respondent**

_____

c. Sex

◯ Male ◯ Female

d. Martiual Status

◯ Single ◯ Married ◯ Divorced ◯ Widow/ widower

e. Education Level

None ◯ Primary ◯ Secondary ◯ High school ◯ collage ◯ University

f. Occupation

_____

g. Name of institution

_____

h. Phone Number

Example: 0754 xxx xxx. Ask for a working number of the respondent or close relative

_____

i. Email address

_____

AGRO-ECONOMIC ACTIVITIES

1. What is your main Economic activity?

Select all that apply

◯ Livestock ◯ Keeping ◯ Crop ◯ Farming ◯ Burning

◯ Mining ◯ Firewood gathering & Charcoal ◯ Business

2. Are you involved in agriculture activities

◯ Yes ◯ No

3. How long have you lived in this village

◯ Less than 6 ◯ 6 months to 1 Years

◯ 1 to 3 Years ◯ 3 to 5 Years ◯ 6 to 9 Year ◯ More than 10 Years

4. How large is your farm?

Area in Acres:

---

5. Which crops are you mostly cultivating?

Select all that apply

◯ ◯ ◯ ◯

Sunflower Maize Millet Sorghum

Beans ◯ Tobacco ◯ Groundnut ◯ Others

Please Specify another Crop: _____

6. What type of fertilizers do you use

Select all that apply

◯ Inorganic ◯ Organic ◯ None ◯ Other

INORGANIC FERTILIZER APPLICATION

7. What kind of inorganic fertilizer did you use?

Select all that apply

◯ Urea ◯ NPK ◯ DAP ◯ CAN ◯ Others

How many Acres did you apply Urea

Area in Acres

_____

How many Acres did you apply NPK

Area in Acres

_____

How many Acres did you apply DAP

Area in Acres

_____

How many Acres did you apply CAN

Area in Acres

_____

How many Acres did you apply Other Inorganic Fertilizer

Area in Acres

_____

9. **Where do you source animal manure**

Select all that apply

◯ Cattle ◯ Swine ◯ Poultry ◯ Other

AGRICULTURAL MECHANIZATION

10. **Which Method are you using to cultivate your farm?**

Select all that apply

◯ Tractor ◯ Oxen ◯ Plough ◯ Hand Hoe

How many Acres did you cultivate by Tractor?

_____

How many Acres did you cultivate by Oxen Plough?

_____

How many Acres did you cultivate by Hand hoe?

_____

11. **Which method are you using to prepare your farm?**

Select one

◯ Residue Collecting and Burning

◯ Leaving Residues and Decomposing Them

◯ Feeding livestock residues in the Farm

12. **Has animal manure been used for fertilizer within the last year?**

◯ Yes ◯ No

SOIL DEGRADATION

13. **Is there soil degradation in your area?**

◯ Yes ◯ No ◯ I Don't Know

14. **Do you know the indicator of soil degradation?**

◯ Yes ◯ No

15. **In your opinion, what is the source/cause of soil degradation in your area**

Select all that apply

◯ Conversion to agriculture ◯ Overgrazing

◯ Deforestation ◯ Soil Erosion

◯ Forest/ grassland ◯ Over-exploitation of vegetation for domestic us

SOIL CONSERVATION PERCEPTION

16. **Is there any technology implemented to prevent, mitigate, or rehabilitate degraded soil or degraded land?**

◯ Yes ◯ No

Name that technology implemented

_____

17. **For 10 last years have you attended any project or seminar to acknowledge environmental awareness?**

◯ Yes ◯ No

18. **For 10 last years did you conduct any project or seminar to acknowledge environmental awareness?**

◯ Yes ◯ No

19. **How often did you visit the forest?**

◯ Once a week ◯ Once a month ◯ Never

20. **Do you know what types of activities are mostly undertaken in the forest?**

Select all that apply

◯ Grazing ◯ Tree pruning for wood ◯ Charcoal products

◯ Beekeeping ◯ Medicinal plant collection

21. **Have you heard about beneficial soil fungi?**

◯ Yes ◯ No

22. **What is the importance use of soil fungi in your area?**

Select all that apply

◯ ◯ ◯

Source of food Poisonous Source of soil moisture

Soil rehabilitation

23. **What is the importance use of soil fungi in your area?**

Select all that apply

Agriculture purposes source of medicine Source of soil moisture

24. **Which source of energy are you using for cooking at home?**

Select all that apply

Firewood Charcoal Solar Electricity Biogas Gas

25. **Is there any development activity/project in your community you run for the last 5 years on soil conservation?**

Yes No

26. **Please mention the activity Project**

_____

27. **Which main methods used to conserve protected forest areas?**

Select all that apply

Reforestation and afforestation Implementation of protected area regulations

Community-based Forest management Anti-poaching measures

Sustainable tourism initiatives

28. **Do fires occur in the preserved forests?**

Yes No

29. **During what season does the fire usually happen?**

Select all that apply

During the dry season (May-September)

During the wet season (November-April)

30. **For what reasons did the fire ignite?**

Select all that apply

Fires are usually ignited due to agricultural clearing

Fires are ignited for pasture management

Fires occur through poaching and honey harvesting

31. **In the past 10 years, what types of trees have been most commonly cut down in the forests?**

Hardwood species (e.g., mahogany, teak) Indigenous species (acacia

Miombo Tree species

32. **What types of trees are planted to conserve the environment and prevent soil erosion?**

- Indigenous species (acacia)
- Exotic species (eucalyptus)
- Fruit-bearing trees (mango, avocado)
- Medicinal plants (neem, moringa)

33. **What is the source of these trees? For example, are they distributed by the local government or grown independently?(Put Tick (V))**

- Distributed by the local government
- Provided by NGOs
- Grown independently by community members
- Purchased from private nurseries
- Donated by international organizations

Thank You very much for your cooperation

This is the end of our the questionnaire

Appendix 2: Tables

Table 1*:* Demographic characteristics of respondents across the study site

Variable	Wards	Mitundu%	Mgandu%	Avg l%	Chi-square	P-value
Gender	Male	64.8	69.9	67.35	0.088	0.01
	Female	35.2	30.1	32.65		
Age	<20	0	0	0		
	20-30	15.5	20.5	19.05		
	31-40	29.6	25.3	27.45		0.02
	41>	54.9	54.2	54.55		
Education Level	Non formal	*36.6*	*22.9*	29.75	1.336	
	Primary education	*50.7*	*66.3*	58.5		0.01
	Secondary education	*8.5*	*6.0*	7.25		
	Tertiary education	*4.2*	*4.8*	4.5		
Occupation	Farmer	*81.7*	*81.9*	81.8		
	Business person	*9.9*	*2.4*	6.15	5.731	0.01
	Government employee	*8.5*	*16.9*			

Table 2: Analysis of fungal species composition from protected and non-protected of Mitundu and Mgandu wards.

Suspected_fungi	Composition (%)
Trichoderma hamatum	2.4 a
Aspergillus citrinum.	5.6ab
Cladosporium oxysporum	9abc
Aspergillus oryzae	11.4abcd
Drechslera spp	11.66abcde
Aspergillus tamarii	12.33abcdef
Fusarium graminearum	12.97abcdefg
Alternaria alternate	12.99abcdefgh
Purpureocillium lilacinum	13.9abcdefghi
Aspergillus aculeatus	13.96abcdefghij
Aspergillus fluvus	15.8abcdefghijk
Rhizopus stolonifer	16.31abcdefghijkl
Fusarium andiyazi	16.44abcdefghijklm
Lasiodiplodia spp	17.04abcdefghijklmn
Aspergillus aculeatus,	18.46abcdefghijklmnop
Fusarium graminearum,	19.01abcdefghijklmnop
Aspergillus terreus	19.24abcdefghijklmnopq
Fusarium soloni	19.44abcdefghijklmnopqr
Fusarium proliferatum	19.55abcdefghijklmnopqrs
Trichodema reesei	20.09abcdefghijklmnopqrst
Penicillium chermesinum	20.72abcdefghijklmnopqrst
Trichodema viride	21.1abcdefghijklmnopqrstu
Fusarium oryzae	21.32abcdefghijklmnopqrstu
Fusarium oxysporum	21.98bcdefghiklmnopqrstuv
Aspergillus tamari	22.5bcdefghiklmnopqrstuvw
Aspergillus niger	23.17bcdefghiklmnpqrstuvw
Penicillium spp	23.6abcdefghijklmnopqrstuvw
Trichoderma asperellum	23.86bcdefghiklmnopqrstuvw
Aspergillus flavus	24.05abcdefghijklmnopqrstuvw
Trichoderma reesei	24.29abcdefghijklmnopqrstuvw
Trichoderma koningiopsis	24.5bcdefhikmnpqrstuvw
Trichoderma erinacium	25abcdefghijklmnopqrstuvw
Aspergilus tamarii	25.3abcdefghijklmnopqrstuvw
Sclerotinia sclerotiorum	25.34bcdefhikmnpqrstuvw
Beauveria bassiana	26.7abcdefghijklmnopqrstuvw
Aspergillus fumigatus	28abcdefghijklmnopqrstuvw
Fusarium gramenium	28.6abcdefghijklmnopqrstuvw
Trichoderma harzianum	28.62cdfprsuw
Penicillium chrysogenum	29 cdfpruw
Lasiodiplodia	31.1bcdefghijklmnopqrstuvw
Trichordema koningiopsis	32.2bcdefghijklmnopqrstuvw
penicilium chrysogenum	35cdefhiklmnopqrstuvw
Trichoderma viride	37.5dfiknpqrstuvw
F pr.	<.001
CV (%)	36.4
<SD	7.667
Df	47

Table 3: Analysis of mean distribution and abundance of fungal species in non-protected and protected areas

Site	species	Mean Distribution and abundance
Non-protected area	*Trichoderma hamatum*	2.4 a
Non-protected area	*Drechslera spp*	3.33 ab
Non-protected area	*Purpureocillium lilacium*	4.8 abc
Non-protected area	*Fusarium graminearum*	7.8 abcdef
Non-protected area	*Trichoderma koningiopsis*	8.38 abcdefgh
Non-protected area	*Cladosporium oxysporum*	9 abcdefghij
Non-protected area	*Aspergillus citrinum spp.*	10 abcdefghijk
Non-protected area	*Aspergillus aculeatus*	10.08 abcdefghijkl
Non-protected area	*Fusarium proliferatum*	10.66 abcdefghijklm
Non-protected area	*Aspergillus oryzae*	11.4 abcdefghijklmno
Non-protected area	*Aspergillus terreus*	11.52 abc..nop
Non-protected area	*Trichoderma harzianum*	11.91 abc..pq
Non-protected area	*Penicillium chrysogenum*	12.2 abc..qr
Non-protected area	*Fusarium andiyazi*	14.3 abc..tuvwx
Non-protected area	*Trichodema reesei*	14.3 abc...tuvwx
Protected area	*Alternaria alternate*	14.35 abc..tuvwx
Non-protected area	*Lasiodiplodia spp*	14.52 abc..xy
Protected area	*Trichoderma hamatum*	14.8 abcd...xyz
Protected area	*Cladosporium oxysporum*	15.47abcd…xyzA
Non-protected area	*Aspergillus niger*	15.5 abc..AB
Non-protected area	*Rhizopus stolonifer*	15.89 abc..CD
Non-protected area	*Aspergillus tamari*	15.96 abc..E
Non-protected area	*Fusarium oxysporum*	16.7 abc..G
Protected Area	*Fusarium andiyazi*	17.05 abc..H
Non-protected area	*Penicillium chermesinum*	17.23 abc..I
Protected Area	*Purpureocillium lilacium*	17.29 abc..K
Non-protected area	*Aspergillus fluvus*	18.14 abc..M
Non-protected area	*Fusarium soloni*	19.3 abc..MNO
Protected area	*Rhizoctonia spp*	19.3 abc…MN O
Protected Area	*Aspergillus aculeatus*	20 abc..MNO
Protected area	*Trichoderma hamatum*	20abc…MNO
Protected area	*Drechslera spp*	20abc…MNO
protected area	*Sclerotinia sclerotiorum*	20.25 bcd..NOP
Protected Area	*Trichodema reesei*	21.11 abc..NOPQRS
Protected area	*Aspergillus oryzae*	21.32 cdfghjkmn...MNOPQRS
Protected Area	*Rhizopus stolonifer*	21.93 cdfghjkmn..MNOPQRS
Protected Area	*Fusarium proliferatum*	22.28 fhjkmn..NOPQRST
Protected Area	*Fusarium graminearum,*	22.47 dfhjkm..RSTU
Protected Area	*Penicillium chermesinum*	22.55 fhjkn..UV
Protected Area	Trichoderma asperellum	23.29 jknortuvwxz..W
Protected Area	Sclerotinia sclerotiorum	23.8 cdefghijklm..WX
Protected Area	Aspergillus citrinum	23.84 cdefghijklm..WX

Site	species	Mean Distribution and abundance
Protected Area	Aspergillus flavus	24.05 fhjkm..WX
Protected Area	*Rhizopus stolonifer*	24.2 cdefghjklm..WX
Protected Area	*Trichoderma erinacium*	25 dfghjklm..WX
Protected Area	*Penicillium spp*	25.9 knortvwxzCEFGHIJKLMNO..WX
Protected Area	*Beauveria bassiana*	26.7 fhjkmnoqrstuvwxyz..WX
Protected Area	*Lasiodiplodia spp*	27.14 fhjkm..UVWX
Protected Area	*Aspergillus tamari*	27.14 rtvwxzEFIKLMNOPRSTUVWX
Protected Area	*Aspergillus niger*	27.55 wxzFIKLMOPRSTUVWX
Protected Area	*Aspergillus fumigatus*	28 hjknoqrstuvwxyz..WX
Protected Area	*Fusarium oxysporum*	28.17 xzFIKLMOPRSTUVWX
Protected Area	*Aspergillus terreus*	28.97 xzFKLMOPRSTUVWX
Protected Area	*Fusarium soloni*	29.1 knoqrst..WX
Protected Area	*Trichoderma harzianum*	29.65 xz..WX
Protected Area	*Sclerotinia sclerotiorum*	30.22 zFLMOPSTUVWX
Protected Area	*Lasiodiplodia spp*	31.1 nortvwxzCD..WX
Protected Area	*Trichordema koningiopsis*	32.2 rtvwxzCE..WX
Protected Area	*Penicilium chrysogenum*	35 FLMOPRSTUVWX
Protected Area	*Trichoderma viride*	37.5 LOSTUWX
	F pr.	<.001
	CV (%)	26.2
	<SD	5.521
	df	96

Appendix 3: Research permission letter

JAMHURI YA MUUNGANO WA TANZANIA
OFISI YA RAIS
TAWALA ZA MIKOA NA SERIKALI ZA MITAA
HALMASHAURI YA WILAYA YA ITIGI
(Barua zote zitumwe kwa Mkurugenzi Mtendaji Wilaya)

Email- ded.itigidc@singida.go.tz

Ofisi ya Mkurugenzi Mtendaji,
Halmashauri ya Wilaya ya Itigi
01 BR. YA HALMASHAURI
S.L.P. 70
43483 ITIGI SINGIDA

Unapojibu tafadhali taja

Kumb. Na. HW/ITG/L/VOL II/15

31/01/2024

Mtendaji Kata,
Kata ya Mgandu,
SLP 70.
ITIGI

YAH: KUMTAMBULISHA NDUGU REGINA JACOB

Tafadhali rejea mada tajwa hapo juu.

2. Ofisi imepokea barua yenye **Kumb. Na. NM-AIST/M030/T.22** ya tarehe **02/01/2024** kutoka chuo kikuu cha Nelson Mandela kuhusu mada tajwa hapo juu.
3. Namtambulisha ndugu Regina Jacob mwanafunzi wa Shahada ya umahiri katika chuo cha Nelson Mandela African Institute of Science and Technology (NM-AIST) anaefanya tafiti inayohusu "kukuza teknolojia ya matumizi ya fangasi katika kuboresha na kurutubisha udongo ulioharibiwa, na kukuzia mimea".
4. Anahitaji kukusanya sampuli za udongo katika misitu ya vijiji, mashamba na maeneo ya wazi, na kuuliza maswali kutumia dodoso kwa wananchi wa kata ya Kitaraka, Mgandu na Mitundu.
5. Naomba apewe ushirikiano wa kutosha.

Kny: MKURUGENZI MTENDAJI
HALMASHAURI YA WILAYA YA ITIGI
John F. Makotta
Kny. MKURUGENZI MTENDAJI (W)

Mkurugenzi aione kwenye jalada kwa taarifa.

RESEARCH OUTPUTS

(i) Publication paper

Jacob, R., Meya, A., Moyo, F., & Mbega, E. (2024). Community knowledge, land use practices, and fungal microbial volume in soil from protected and non-protected areas of Itigi District, Tanzania. *International Journal of Biosciences, 25*(4), 181–192.

(ii) Poster presentation

Diversity and composition of fungal communities in soil from pretected and non-protected areas in Itigi District, Singida Region, Tanzania