MYCORRHIZAL FUNGI ASSOCIATED WITH *Aspilia pluriseta* AND PHOSPHORUS AVAILABILITY ON SORGHUM GROWTH

MUCHOKA JAMES PETER (M EnvS)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature…… Date…….

Muchoka James Peter

Department of Agricultural Resource Management

A800/156/2015

This thesis has been submitted for examination with our approval as the University Supervisors

Signature…… Date…….

Prof. Daniel Mugendi Njiru

Department of Agricultural Resource Management

University of Embu

Signature…… Date…….

Prof. Paul Njiruh Nthakanio

Department of Agricultural Resource Management

University of Embu

Signature…… Date…….

Prof. Paul Kamau Mbugua

Department of Plant Sciences

Kenyatta University

Signature…… Date…….

Dr. Ezekiel Mugendi Njeru

Department of Biochemistry, Microbiology and Biotechnology

Kenyatta University

DEDICATION

To my wife, Hellen Njoki James and my daughters, Lillian Wanja, Betty Mukami and Maryanne Mwende for giving me an encouraging social platform that urged me on to this education pinnacle. To my granddaughter, Ellen Njoki alias “gubernatorial”, you have a benchmark to follow. Your combined prayers and words of encouragement softened this arduous task.

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LIST OF ABBREVIATIONS/ACRONYMS

$^{\circ}C$	Degrees Centigrade
Al	Aluminium
AMF	Arbuscular Mycorrhiza Fungi
ANOVA	Analysis of Variance
BLASTn	Basic Local Alignment Search Tool
Ca	Calcium
CAN	Calcium Ammonium Nitrate
CIDP	County Integrated Development Plans
DCP	Dicalcium Phosphates
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamenetetraacetic Acid
Fe	Iron
GoK	Government of Kenya
H^+	Hydrogen Ion
$H_2PO_4^-$	Dihydrogen Phosphate Ion
HPO_4^{2-}	Hydrogen Phosphate Ion
ITS	Internal Transcribed Spacer
KoH	Potassium Hydroxide
LSD	Least Significance Difference
MAP	Monoammonium Phosphates
MCP	Monocalcium Phosphates
MF	Mycorrhizal Fungi
NPK	Nitrogen, Phosphorus, Potassium
NRF	National Research Fund
OTUs	Operational Taxonomic Units
P	Phosphorus
PCR	Polymerase Chain Reaction
PHT	Phosphates Transporter
Pi	Inorganic Phosphorus
Po	Organic Phosphorus
PSB	Phosphorus Solubilizing Bacteria
PSF	Phosphorus Solubilizing Fungi

QIIME	Quantitative Insights into Microbial Ecology
RCD	Randomized Complete Design
SAS	Statistical Analysis System
SSP	Single Super Phosphates
TSP	Triple Super Phosphates
USDA	United States Department of Agriculture

ABSTRACT

Global reserves of phosphorus (P) are continually getting depleted and this poses an enormous challenge to food production. Phosphorus is one of the major limiting nutrients for plant productivity. Use of plant-mycorrhizal fungi in relation to replenishing phosphate is one of the biological techniques being considered. In this study, the association of *Aspilia pluriseta* Schweif. with mycorrhizal fungi and their role in promoting growth and enhancing P availability to gadam sorghum (*Sorghum bicolor* L.) was investigated. The main objective of this study was to determine effects of mycorrhiza in the rhizosphere of *Aspilia pluriseta* on growth and phosphorus availability to sorghum crop. The samples were taken from Tunyai and Gakurungu in Tharaka Nithi County and Kanyuambora in Embu County, Kenya. Soil samples were taken at depths 0-20cm, 21-40cm and 41-60cm. This was followed by greenhouse experiments at the University of Embu. The experiments involved use of potted plants with four treatments; *Aspilia pluriseta* vegetation covered soils; soil textural types; soil depth and mycorrhiza fungi inoculated gadam sorghum seeds. The treatment combinations were carried out in a series of two experiments on a completely randomized block design on a factorial model replicated thrice. Data obtained was subjected to ANOVA using SAS Edition 9.2 and differences between treatment means examined using Least Significant Difference (LSD) at $p \leq 0.05$. Illumina sequencing method was used on the Internal Transcribed Spacer (ITS) region on the total soil community DNA to capture the genetic fungal community within the rhizosphere. Analysis of Operational Taxonomic Units (OTUs) was done using QIIME 1.8.0 and taxonomic classification done using BLASTn on SILVA 119 database. Programming software, R was used for hierarchical clustering. The study shows that sorghum perfomed better where its seeds were inoculated with mycorrhiza-soils previously inhabited with *Aspilia pluriseta* vegetation. Spore counts varied significantly among silty clay, silt loam and sandy loam soils. The spore morphotypes was significantly higher at $p \leq 0.05$ for soils inhabited by *Aspilia pluriseta* compared to those not habited. Three hundred and seventy-three (373) OTUs were found at 3% genetic distance. Thirty-five fungal taxa were recorded in the rhizosphere of *Aspilia pluriseta*. The soil had five main phyla; Glomeromycota (90.7%), Basidiomycota (3.7%), Ascomycota (3.4%), Chytridiomycota (1.5%), and unspecified phylum fungi (0.7%). The genera *Glomus* was the most prevalent in all soil depths. The association of *Aspilia pluriseta* and mycorrhiza gave sorghum yield of 15.2 g per 1000 grains compared to yield of 13.1 g per 1000 grains in soils that did not have *Aspilia pluriseta* mycorrhiza association. This was a yield increase of 16%. Differences between gadam sorghum yields in *Aspilia pluriseta* soils and gadam sorghum seeds inoculated with mycrorihza spores was significant at $p \leq 0.05$ This study therefore, recommends use of *Aspilia pluriseta* in improving sorghum yield.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Phosphorus (P) is a macronutrient needed by crops for growth. It makes up about 0.2% of dry weight, but it is one of the most difficult nutrients for plants to acquire (Smith & Smith, 2011). In soil, P occurs mainly as insoluble phosphates and is not readily available (Schachtman *et al.*, 1998). However, with the help of microorganisms, this insoluble phosphate is solubilized to become available to plants. The majority of terrestrial plant species are capable of interacting with mycorrhiza fungi in nature and provide an effective pathway by which phosphorus is scavenged and rapidly delivered to cortical cells within the root (Smith & Read, 2008; Mike & Larry, 2011). Mycorrhiza fungi acts as a catalyst to concentrate P in a manner that makes it available to plant (Schubert & Lubraco, 2000; Calvet *et al.*, 2004; Khade & Rodrigues, 2009; Rafiullah *et al*., 2020).

Global reserves of phosphorus are fast running out (van Kauwenbergh, 2010; Neset & Cordell, 2012; Edixhoven *et al.*, 2014; Gutiérrez, 2017; Nedelciu *et al*., 2020). This poses a great challenge for global food production in the foreseeable future since plants need phosphates to grow (Gilbert, 2009; van Vuuren *et al*., 2010; Rafiullah *et al.*, 2020). Regionally, phosphorus deficiency occurs in many soils of East Africa not only due to P depletion through crop harvest and erosion but mainly due to the prevalence of high P-fixing soils in the region (Nziguheba, 2007). A natural method of solubilizing fixed phosphorus to make it available to plants would alleviate this problem. Unlike nitrogen, P replenishment, particularly in smallholder agriculture, remains a challenge as it is mainly fertilizer dependent (Sanchez, 2002; Bationo *et al*., 2004; Nziguheba, 2007; Sanginga & Woomer, 2009; Bationo & Waswa, 2011). Reduced soil fertility status is one of the main challenges facing the central highlands region (Smaling *et al*., 1997; Mugwe *et al.*, 2009; Mugo *et al*., 2020). Embu and Tharaka Nithi counties are located in this region. Reducing soil fertility, over the years, is known but not addressed (Mugwe *et al*., 2009; Mugo *et al*., 2020). This

challenge is also, documented and prioritized in the county governments' blueprint ,the Integrated County Development plans (CIDP) of 2015/2016 financial year.

Aspilia pluriseta Schweif. is a flowering plant in the daisy family hypothesized to grow in coexistence with the mycorrhiza fungi. This complex association leads to the availability of phosphorus in the soils in usable forms to plants. The herbaceous plant is common and grows naturally in the open woodlands and grasslands in western, southern, central and eastern Africa. Farmers in central-eastern Kenya reported good crop yields, particularly cereals, in farms previously grown *Aspilia pluriseta*. When uprooted, *Aspilia pluriseta* presented a huge surface area of white mycelium and a robust rooting system that was visible without any magnification (Appendix 1).

There seemed to be a relationship between the good crop yields and the mycelium on the roots of *Aspilia pluriseta*. According to Lambers *et a*l. (2008); Cheng *et al*. (2011); Lambers *et al*. (2011) and Muchoka *et al.* (2020), formation of "dense " cluster roots that produce organic anions are some of the strategies that some plants use to enhance P availability or uptake, a phenomenon that seemed to be exhibited by *Aspilia pluriseta*. Availability and uptake of P from natural and fertilizer sources remain the subject of active research (Bünemann *et al*., 2011) This research investigated the role of mycorrhizal fungi in mediating P availability within the rhizosphere of *Aspilia pluriseta* and its availability for utility by sorghum plants.

1.2 Statement of the problem

There is a growing deficieny of available Phosphate in the soils as some of it is locked and not available to plants. This compounds the deficieny problem due to overuse of soils without P replenishment. Besides, the cost of phosphate fertilizer is high and beyond the reach of many local farmers. This affects the replenishing schedule of the mineral mined from the soils. Furthermore, the manufacture of chemical phosphatic fertilizer is a high energy-intensive process and has a long-term impact on the environment in terms of eutrophication, soil fertility depletion and carbon footprint. Therefore, use of natural processes to convert insoluble to soluble P would reduce the burden of P demand among the farmers.

Co-association of mycorrhiza fungi and *Aspilia pluriseta* was hypothesized to liberate fixed organic and inorganic phosphorus to usable forms leading to improved sorghum yields. Scanty data on the kinetics of mycorrhizal fungi (MF) association with *Aspilia pluriseta*, leading to improved sorghum crop yields, was available. Mycorrhizal fungi flora inhabiting *Aspilia pluriseta* within Embu and Tharaka Nithi region has not been characterized to determine genotypes and their efficacy in increasing P availability in the soils for sorghum crop use. This research was meant to bridge this gap.

1.3 Justification of the research

Phosphorus (P) is one of the major limiting nutrients for plant productivity mainly because of its low mobility in the soil. Studies on the association of mycorrhizal fungi (MF) with *Aspilia pluriseta* and how it mediates P availability to crops is needed. Besides, there is a promising possibility of using MF to make inoculum. Increased use of MF inoculum can assist to make P more available to crops such as sorghum and thus help to offset deficiencies caused by P mining from the soil. This will reduce the amount of chemical phosphate fertilizer needed and thus reduce the cost of sorghum crop production while at the same time increasing the crop yields. In addition to more scientific knowledge and information on the mycorrhizal relationship between the two plants, the ultimate output of this research was to reduce the cost of farm P- inputs. When less of the chemical P is bought, the cost will also come down. Isolation of mycorrhiza fungi strains that are more efficient in availing P to plants will enable the commercialization of the fungal products.

Since *Aspilia pluriseta* is native, well adapted and grows in diverse ecological zones, it coud be an inexhaustible bio resource. Studies of MF in *Aspilia pluriseta* Schweif. rhizosphere including characterizing the fungi, understanding their working kinetics and dynamism of availing nutrients to plants will provide a source of P alternative to chemical commercial phosphate fertilizer. This study added to the knowledge gaps on mass generation of MF microbes, the main ingredient in making an inexpensive and sustainable biophosphate fertilizer.

1.4 **Research questions**

i. Does mycorrhizal fungi (MF) co-association in the rhizosphere of *Aspilia pluriseta* influence P availability to gadam sorghum crop?

ii. How does the intensity of MF in the rhizosphere of *Aspilia pluriseta* vary with different soil textural types in the semi-arid croplands of eastern Kenya?

iii. Is there a genetic diversity of mycorrhiza fungi found within the rhizosphere of *Aspilia pluriseta*?

iv. Do MF in the rhizosphere of *Aspilia pluriseta* influence the yield performance of gadam sorghum?

1.5 Hypotheses

i. Mycorrhiza fungi co-association in the rhizosphere of *Aspilia pluriseta* Schweif. influence phosphorus availability to gadam sorghum crop.

ii. The intensity of mycorrhizal fungi (MF) in the rhizosphere of *Aspilia pluriseta* varies across the main soil textural types in the semi-arid croplands of eastern Kenya.

iii. There is genetic diversity of mycorrhiza fungi inhabiting the rhizosphere of *Aspilia pluriseta*.

iv. Mycorrhizal fungi in the rhizosphere of *Aspilia pluriseta* influence yield performance of gadam sorghum

1.6 Objectives of this study

1.6.1 General objective

The general overall objective was to evaluate the growth effect of sorghum by the mycorrhiza fungi on the rhizosphere of *Aspilia pluriseta* Schweif.

1.6.2 Specific objectives

Specifically, the study targeted;

i. To evaluate the effects of co-association of mycorrhiza and *Aspilia pluriseta* on growth and phosphorus availability to gadam sorghum crop in Tunyai, Gakurungu and Kanyuambora regions.

ii. To determine the intensity of MF in the rhizosphere of *Aspilia pluriseta* across the main soil textural types in the semi-arid croplands of eastern Kenya.

iii. To determine genetic diversity of mycorrhiza fungi within the rhizosphere of *Aspilia pluriseta*.

iv. To determine the effect of MF co-association with *Aspilia pluriseta* on the yield of gadam sorghum crop.

1.7 Outputs of this study

The goal of this study was to look at mycorrhizal fungi diversity in the rhizosphere of wild and native *Aspilia pluriseta* shrubs in order to take advantage of root microbial symbiosis in sorghum crop development. The study enabled mycorrhizal interaction in the rhizosphere of *Aspilia pluriseta* to enhance sorghum yield from 2.2 g/1000 grains to 2.4 g/1000 grains of sorghum. Evidence of increased phosphate (available P) levels in soils inhabited by *Aspilia pluriseta* was a key out put to this study.

This study found out that there was a mycorrhizal-fungi relationship between *Aspilia pluriseta* and thirty-five fungal morphotypes in the rhizosphere of the plant that led to enhanced sorghum yields. Soil textural type was a key factor in mycorrhizal fungi proliferation with sandy loam soils exhibiting an ideal environment for commercial mycorrhiza fungal spores production.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of soil health for crop production

Soil biology has emerged over the last decade as a critical part of the knowledge base for successful and sustainable agricultural production (Shen *et al.*, 2011; Pradesh *et al.*, 2019). A key component of biology is the profound plant-mycorrhizal fungi relationship, which has enormous potential for improved management of contemporary farming systems (Khade & Rodrigues, 2009; Shen *et al.*, 2011; Chen *et al*., 2020). The vast majority of crops form an association with these specialized mycorrhizal soil fungi in order to maximize performance (Mike & Larry, 2011). Among the few but notable exceptions are members of the Brassicaceae plant family (cabbage, broccoli, cauliflower, radish, turnips, and canola), the Amaranthaceae plant family (beets, spinach, chard) and the Polygonaceae plant family (rhubarb, buckwheat). Virtually all other crop plants worldwide are host to some form of mycorrhizal association (Mike & Larry, 2011).

A healthy soil has the right nutrient balance (Hinsinger, 2001; Hinsinger *et al*., 2009). Plant roots through the rhizosphere take up soil nutrients, which is the key zone of interaction between plants and soils. Hence, root growth and rhizosphere processes have a great influence on soil nutrient transformation, mobilization, and efficient use by plants (Zhang *et al*., 2010).

Figure 2.1 shows a model of root/rhizosphere management for increasing crop productivity and nutrient use efficiency as developed by Zhang *et al.* (2010).

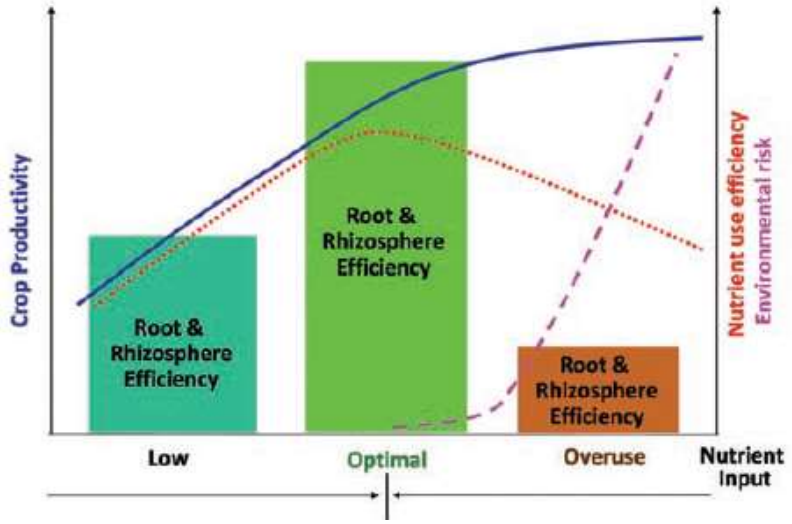

Figure 2.1. A model of root/rhizosphere management for increasing crop productivity. Source: (Zhang *et al*., 2010)

Plant roots not only regulate morphological traits to adapt to soil environmental conditions, but also significantly modify rhizosphere processes through their physiological activities, particularly the exudation of organic acids, phosphatases, and some signaling substances, proton release, and redox changes (Zhang *et al*., 2010; Marschner *et al*., 2012). The root-induced rhizosphere determine mobilization, acquisition of soil nutrients, microbial dynamics and also control nutrient use efficiency by crops, thus profoundly influence crop production and sustainability (Zhang *et al.*, 2010). Manipulating root growth and rhizosphere processes provides an effective approach to improve nutrient use efficiency and crop productivity simultaneously (Li *et al.*, 2008; Mi *et al*., 2010). Root/rhizosphere management strategies lay emphasis on maximizing the efficiency of root and rhizosphere processes in nutrient mobilization, acquisition, and use by crops rather than depending solely on excessive application of inorganic fertilizers in intensive farming systems. The efficiency of root and rhizosphere processes is highly dependent on inherent soil fertility and the status of soil nutrient supply, which is controlled by the input of external nutrients. It is well known that root growth and expansion can be greatly constrained when the available soil nutrient supply is extremely low. The efficiency of root and rhizosphere processes can be enhanced

with increasing intensity of soil nutrient supply. However, overuse of fertilizers may lead to high concentrations of nutrients in the rhizosphere, resulting in inhibition of root growth and rhizosphere processes (Li *et al*., 2008; Mi *et al*., 2010; Zhang *et al*., 2010). Synchronizing root-zone nutrient supply with crop demands spatially and temporally at an optimal level of nutrient supply in the rhizosphere is important for maximizing the efficiency of the root/rhizosphere in nutrient mobilization and acquisition (Figure 2.2).

Figure 2.2 shows a model developed by Zhang *et al*. (2010) on root/rhizosphere management in cropping systems.

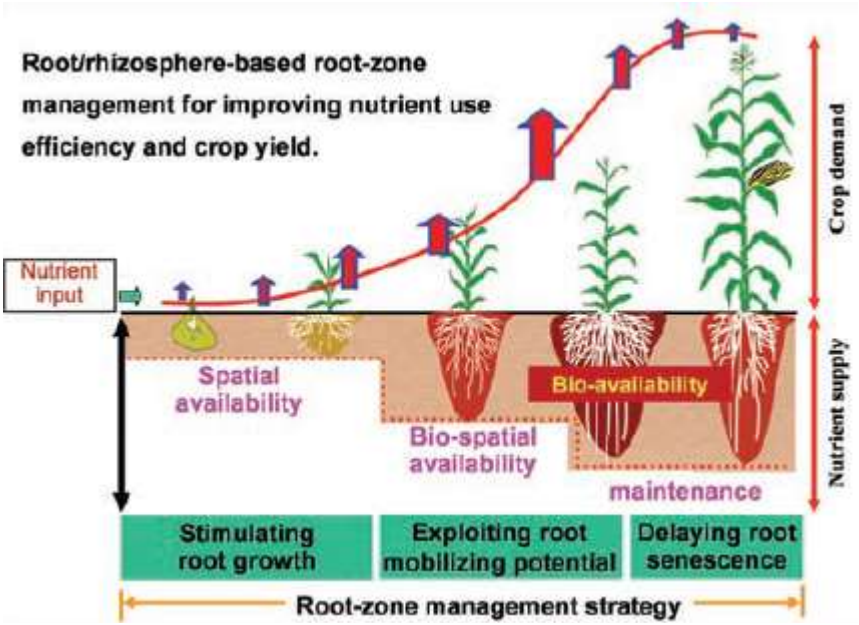

Figure 2.2 Root/rhizosphere management in cropping systems throughout the entire crop growth period. Source: (Zhang *et al*., 2010).

It is well known that the pH of the soil near the roots can differ by up to two units from that of the bulk soil. The magnitude of this differential, i.e. rhizosphere alkalinisation or acidification, is determined by plant and soil variables. Cation-anion exchange between root and soil, as well as root excretion of organic anions, have all been proposed as explanations for root-mediated pH shifts in the rhizosphere. Cation-anion exchange between the root and the soil, root excretion of organic anions, root respiration (CO2 generation), and root-induced redox reactions (O2 emission) have all been proposed as explanations. Nutritional restrictions, such as Fe and P deficiency or Al toxicity, might alter these processes, with the effect on

rhizosphere pH varying depending on the soil buffering capacity (Hinsinger et al., 2003; Neumann, 2003).

Phosphorus (P) is contained in every living plant cell and is essential for plant growth. It plays a role in energy transfer, photosynthesis, sugar and starch transformation, nutrient flow throughout the plant, and the transmission of genetic features from one generation to the next.

2.2 Soil phosphorus dynamics

Phosphorus (P) is receiving more attention as a nonrenewable resource due to increased agricultural production in this decade (Gilbert, 2009; Cordell *et al*., 2009; Ryan *et al*., 2012; Tirado & Allsopp, 2012; Alewell *et al.*, 2020). One unique characteristic of P is its low availability due to slow diffusion and high fixation in soils. What this means is that P can be a major limiting factor for plant growth. The Ministry of Agriculture in Kenya fertilizer report (Oseko & Dienya, 2015) indicates that there is need to apply manure or compost regularly to maintain and sustain the organic matter content. This in turn would alleviate aluminium toxicity thereby increasing availability of phosphorus.

Applications of chemical P fertilizers and animal manure to agricultural land have improved soil P fertility and crop production, but caused environmental damage in the past decades (Shen *et al*., 2011). Maintaining a proper P-supplying level at the root zone can maximize the efficiency of plant roots to mobilize and acquire P from the rhizosphere by an integration of root morphological and physiological adaptive strategies (Mike & Larry, 2011). A holistic understanding of P dynamics from soil to plant is thus necessary for optimizing P management and improving P-use efficiency. The aim would be to reduce consumption of chemical P fertilizer, maximize exploitation of the biological potential of root/rhizosphere processes for efficient mobilization, and acquisition of soil P by plants as well as recycling P from manure and waste. Overall P dynamics in the soil plant system is a function of the integrative effects of P transformation, availability, and utilization caused by soil, rhizosphere, and plant processes *(*Shen *et al.*, 2011).

Soil P exists in various chemical forms including inorganic P (Pi) and organic P (Po) (Figure 2.3).

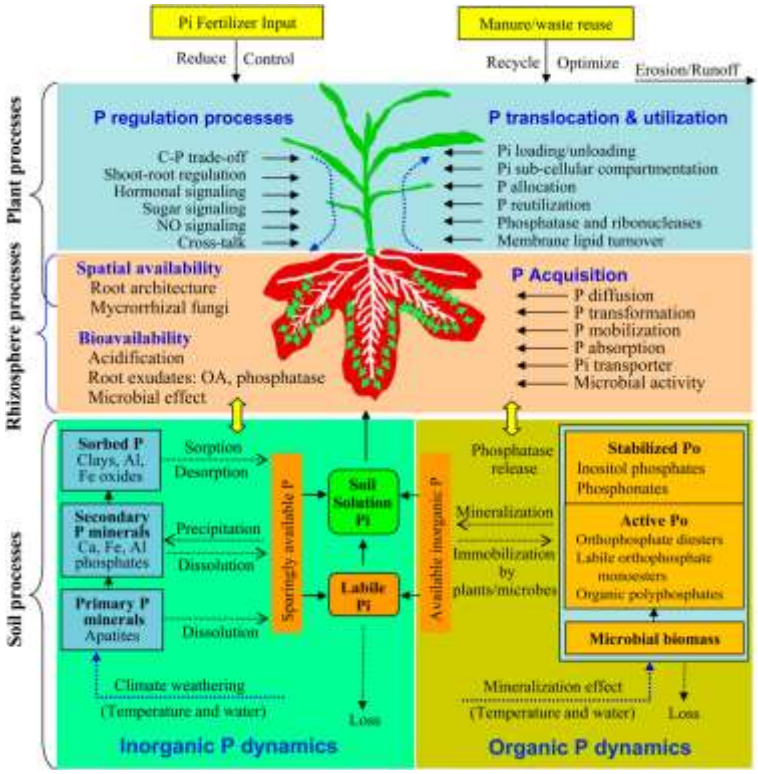

Figure 2.3. Diagrammatic expression of Phosphorus dynamics: From soil to plant Source: (Shen *et al.*, 2011)

These P forms differ in their behavior and fate in soils (Hansen *et al.*, 2004; *Turner et al.*, 2007; Ikhajiagbe *et al*., 2020). Pi usually accounts for 35% to 70% of the total P in soil (Huang *et al*., 2017). Primary P minerals including apatite, strengite, and variscite are very stable. The release of available P from these minerals by weathering is generally too slow to meet the crop demand. Direct application of phosphate rocks (apatite) has proved relatively efficient for crop growth in acidic soils (Shen *et al*., 2011; Hellal *et al*., 2019). In contrast, secondary P minerals including calcium (Ca), iron (Fe), and aluminum (Al) phosphates vary in their dissolution rates, depending on size of mineral particles and soil pH (Pierzynski *et al*., 2005; Oelkers & Valsami-Jones, 2008; Penn & Camberato, 2019). With increasing soil pH, solubility of Fe and Al phosphates increases but solubility of Ca phosphate decreases, except for pH values above 8 (Hinsinger, 2001; Penn & Camberato, 2019). The P adsorbed on various clays and Al/Fe oxides can be released by desorption reactions. All these P forms exist in complex equilibria with each

other, representing from very stable, sparingly available, to plant-available P pools such as labile P and solution P (Hansen *et al.*, 2004; Turner *et al.*, 2007; Shen *et al.*, 2011; Weeks & Hettiarachchi, 2019)

2.3 Inorganic phosphorus in soil

Applications of chemical P fertilizers are needed to improve crop growth and yield because of the low concentration and poor mobility of plant-available P in soils (Nziguheba, 2007; Bindraban *et al*., 2020) . The major forms of phosphate fertilizers in Kenya include mono ammonium phosphate (MAP), single super phosphate (SSP) and triple super phosphate (Oseko & Dienya, 2015). Application of inorganic fertilizers can significantly affect soil physicochemical properties. After application to soil, inorganic fertilizers undergo a wetting process, generate large amounts of protons, phosphate, and dicalcium phosphates (DCP), and eventually forms a P-saturated patch (Benbi & Gilkes, 1987; Yao *et al*., 2020). This Pi-saturated patch forms three different reaction zones including direct reaction, precipitation reaction, and adsorption reaction zones. The direct reaction zone is very acidic (pH = 1.0–1.6), resulting in enhanced mobilization of soil metal ions (Shen *et al*., 2011). These metal ions can also react with high concentrations of Pi in the zone thus causing further precipitation of Pi. The amorphous Fe-P and Al-P that thereby form can be partly available to plants.

In calcareous soil, new complexes of monocalcium phosphates (MCP) and dicalcium phosphates (DCP) can be formed and with time DCP is gradually transformed into more stable forms of Ca phosphates (octocalcium phosphate or apatite). Because the Pi concentration is relatively low, P adsorption by soil minerals is dominant in the outer zone (Abou *et al*., 2020). In contrast, the application of monopotassium phosphate has little influence on soil physical and chemical properties (Lindsay *et al*., 1962; Liu *et al*., 2020; Wali *et al*., 2020). Therefore, matching P fertilizer types with soil physical and chemical properties may be an efficient strategy for rational use of inorganic fertilizer P. The current advise given to farmers in the study area by the Ministry of Agriculture in Kenya is use of single super phosphate (SSP), triple superphosphate (TSP), calcium ammonium nitrate (CAN), Mavuno, compound

fertilizers (N:P:K) 23:23:0 and 20:20:0 (Oseko & Dienya, 2015). Use of soil P solubilizing microbes had not been adopted in these areas.

2.4 Manure phosphorus in the soil

Manure is applied to the soil to increase P fertility. The total P content in manure is very variable and nearly 70% of total P in manure is labile (Dou *et al*., 2000; Lu *et al*., 2020). In manure, Pi accounts for 50% to 90% (Dou *et al.*, 2000; Saleem *et al.*, 2017). Manure also contains large amounts of Po, such as phospholipids and nucleic acids (Turner & Leytem, 2004), which can be released to increase soil Pi concentrations by mineralization. Phosphorus adsorption to soil particles can be greatly reduced through applying organic substances. The humic acids contain large numbers of negative charges, carboxyl and hydroxyl groups, which strongly compete for the adsorption sites with Pi (Shen *et al*., 2011). Manure can also change soil pH and thus alter soil P availability. Organically produced manures and technologies are not only cheaper but also provide a more balanced supply of nutrients and other multiple agro ecological benefits (Donovan & Casey, 1998; Selim, 2020).

2.5 Phosphorus dynamics in the rhizosphere

The rhizosphere is the critical zone of interactions among plants, soils, and microorganisms (Richardson *et al*., 2009; Silva & Lambers, 2020). Plant roots can greatly modify the rhizosphere environment through their various physiological activities, particularly the exudation of organic compounds such as mucilage, organic acids, phosphatases, and some specific signaling substances, which are key drivers of various rhizosphere processes (Shen *et al*., 2011; Canarini *et al*., 2019). The chemical and biological processes in the rhizosphere not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient use efficiency of crops, and thus profoundly influence crop productivity (Hinsinger *et al*., 2009; Richardson *et al*., 2009; Wissuwa *et al*., 2009; Shen *et al*., 2013a)

Due to its low solubility and mobility in soil, P can be rapidly depleted in the rhizosphere by root uptake, resulting in a gradient of P concentration in a radial direction away from the root surface (Shen *et al*., 2011; Cabeza *et al*., 2017). In spite

of total soil P content usually exceeding the plant requirements (Figure 2.4), the low mobility of soil P can restrict its availability to plants.

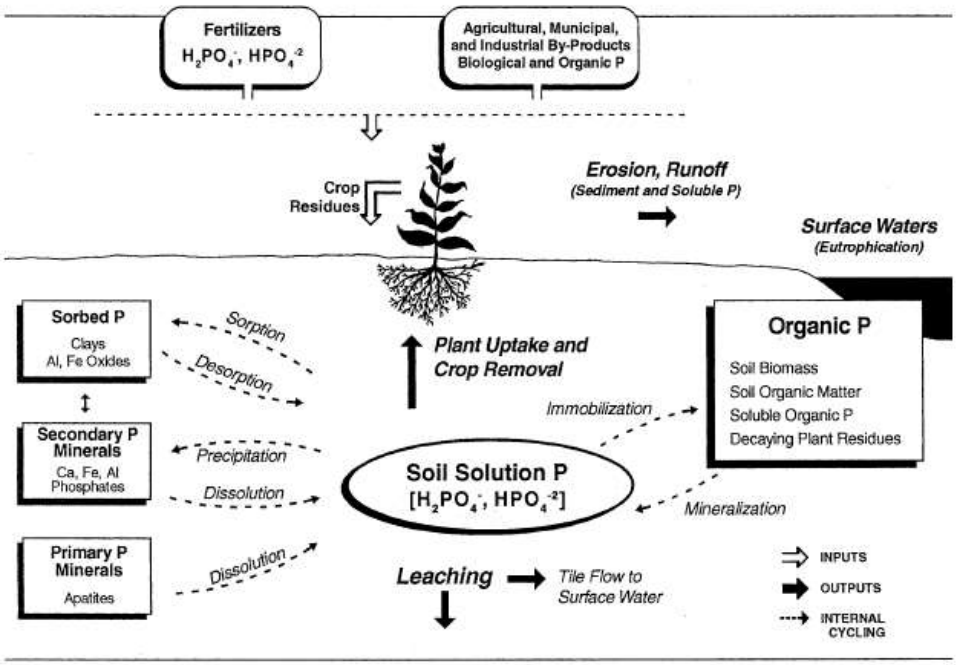

Figure 2.4. Soil Phosphorus cycle. Source: Pierzynski (2000)

Soluble P in the rhizosphere soil solution should be replaced 20 to 50 times per day by P delivery from bulk soil to the rhizosphere to meet plant demand (Marschner, 1995; Xomphoutheb *et al.*, 2020; Havlin & Heiniger, 2020). Therefore, P dynamics in the rhizosphere are mainly controlled by plant root growth and function, and also highly related to physical and chemical properties of soil (Bertin *et al*., 2003). Due to the unique properties of P in soil such as low solubility, low mobility, and high fixation by the soil matrix, the availability of P to plants is dominantly controlled by two key processes: spatial availability and acquisition of P in terms of plant root architecture as well as mycorrhizal association, and bioavailability and acquisition of P based on the rhizosphere chemical and biological processes (Shen *et al*., 2011). The latter process was the domain of this study.

2.6 Spatial availability and acquisition of soil phosphorus

2.6.1 Root architecture

Plants are able to respond to P starvation by changing their root architecture, including root morphology, topology, and distribution patterns (Hammond & White, 2008a; Kochian, 2016; Carvalho & Foulkes, 2019; Dixon *et al*., 2020). Increases in

root/shoot ratio, root branching, root elongation, root topsoil foraging, and root hairs are commonly observed in P-deficient plants, while the formation of specialized roots such as cluster roots occurs in a limited number of species (Lynch & Brown, 2008; Niu *et al*., 2013; Péret *et al*., 2014). P deficiency has been shown to reduce growth of primary roots and enhance length and density of root hairs and lateral roots in many plant species (Desnos, 2008; Niu *et al*., 2013; Péret *et al*., 2014).

P efficient genotypes of common bean *(Phaseolus vulgaris*) have more shallow roots in the topsoil where there are relatively high contents of P resources (Lynch & Brown, 2008). Some plant species, for example white lupin *(Lupinus albus*), can develop cluster roots with dense and determinative lateral roots, which are covered by large numbers of root hairs (Lambers *et al*., 2008; Schnepf *et al*., 2008; Péret *et al*., 2014). Therefore, root architecture plays an important role in maximizing P acquisition because root systems with higher surface area are able to explore a given volume of soil more effectively (Lynch, 1995; Shen *et al*., 2013b; Péret *et al*., 2014; Lugli *et al*., 2020).

Some adaptive modifications in root architecture in response to P deficiency are well documented in Arabidopsis (*Arabidopsis thaliana*) and in those species forming cluster roots (Lambers *et al*., 2006; Osmont *et al*., 2007; Desnos, 2008; Hammond & White, 2008a; Rouached *et al*., 2010). Adaptive changes of root growth and architecture under P starvation are related to altered carbohydrate distribution between roots and shoots, and these changes may be caused by plant hormones (Bertin *et al*., 2003; Nacry *et al*., 2005), sugar signaling (Hammond & White, 2008b; Vance, 2010; Camisón *et al*., 2020;) and nitric oxide in the case of cluster-root formation in white lupin (Wang *et al*., 2010). Root proliferation is stimulated when plant roots encounter nutrient-rich patches, particularly when the patches are rich in P and/or nitrogen (Drew, 1975; Hodge, 2004; Liu *et al*., 2018).

Root proliferation in P-rich topsoil layers is related to a decreased root gravitropic response under P limitation (Bonser *et al*., 1996; Brown *et al*., 2013; Rao *et al*., 2016) and ethylene may be involved in the regulation of these responses (Lynch & Brown, 2008). Root proliferation can be greatly stimulated in the P-enriched soil

patches (Hodge, 2004; McLachlan *et al*., 2020). However, the mechanisms of P-dependent changes in sorghum root proliferation in response to local P supply are not fully understood. Localized application of phosphates plus ammonium significantly enhances P uptake and crop growth through stimulating root proliferation and rhizosphere acidification in a calcareous soil (Shen *et al*., 2013b).

2.6.2 Mycorrhizal association

Mycorrhizal fungi are soil-dwelling, root-inhabiting fungi that colonize the fine absorbing roots of more than 95% of land plants (Siddiqui *et al*., 2008). Its filaments can penetrate into the smallest of soil pores and fissures to access microscopic sources of water that are unavailable to the thicker roots (Mike & Larry, 2011). Mycorrhizae are an integral part of most plants in nature (Gianinazzi *et al*., 2010; Mike & Larry, 2011; Field *et al.*, 2020; Song *et al*., 2020).

Mycorrhizal fungi increase plant nutrient uptake, especially phosphorus and several microelements (Schubert & Lubraco, 2000; Calvet *et al*., 2004; Varma *et al*., 2017). One of the many advantages of mycorrhizal fungi is that they induce plant tolerance to biotic stresses (Dehne, 1982; Barea *et al*., 1996; Diagne *et al*., 2020) and abiotic stresses (Parke *et al.*, 1983; Augé *et al.*, 2001; Schreiner *et al*., 2001; Borkowska, 2002; Shi *et al*., 2002; Swaty *et al*., 2004; Fellbaum *et al*., 2012; Diagne *et al*., 2020). Numerous research work has been carried out in regard to use of fertilizer plants to replenish soil nutrients and enhance productivity (Mugendi *et al*., 1999; Jama *et al*., 2000; Kimetu *et al*., 2004; Mureithi *et al*., 2007; Mucheru-Muna *et al.*, 2014). On the other hand, some authors reported negative influence or lack of influence on plant productivity after mycorrhization (Dosskey *et al*., 1990; Corrêa *et al.*, 2008; Conjeaud *et al*., 1996; Marschner, 1996; Eltrop & Marschner, 1996; Mahmoudi *et al*., 2020; Samba-Mbaye *et al.*, 2020). This listing is not exhaustive and does not indicate the relationship mycorrhizal fungi (MF) in *Aspilia pluriseta* would have to the growth of *Sorghum bicolor* L.

Inoculation of MF to increase yield in crops is not a new idea. Researchers have made efforts to increase the quality of legume plants through the inoculation of mycorrhizal strain alone or in combination with nitrogen fixing bacteria (Stancheva

et al., 2006; Arumugam *et al.*, 2010; Guo *et al.*, 2010). Most research in this area of promising technologies has however, concentrated on herbaceous green manure legumes (HGML) leaving out other herbaceous plants that could enhance availability of other crop macro nutrients (Cherr *et al.*, 2006; Norgrove & Hauser, 2015).

Non-leguminous plants are not usually known to have dual association of *Rhizobium* and mycorrhizal fungi (Den Camp *et al*., 2011). Interestingly, Khade & Rodrigues (2009) reported that *Rhizobium leguminosarum* bv. *viciae* was found in the non leguminous *Carica papaya* L. Further this study demonstrated a synergistic growth effect between *Rhizobium* and MF found in the rhizosphere of this plant. Similarly, this current study established a relationship between yield and productivity of sorghum and MF found on the rhizosphere of *Aspilia pluriseta*.

Considerable progress towards understanding the molecular basis of phosphorus uptake by plants has been made (Schachtman *et al*., 1998; Raghothama, 2000; Rausch & Bucher, 2002; Franco-Zorrilla *et al.*, 2004; Karandashov & Bucher, 2005; Yasmeen *et al.*, 2012; Bindraban *et al.*, 2020). Advances in plant genomics research has provided useful tools to help unravel the complexity of the regulatory pathways associated with the responses of the plant to variation in P availability mediated by mycorrhizal symbiosis (Pierre *et al*., 2014).

Mycorrhizal symbioses can increase the spatial availability of P, extending the nutrient absorptive surface by formation of mycorrhizal hyphae. In the symbioses, nutrients are transferred by mycorrhizal fungi (MF) via their extensive mycorrhizal mycelium to plants while in return the fungi receive carbon from the plant (Khade & Rodrigues, 2009; Prescott *et al.*, 2020). Mycorrhizal fungi not only influence plant growth through increased uptake of nutrients (e.g. P, Zinc, and Copper), but may also have non nutritional effects in terms of stabilization of soil aggregates and alleviation of plant stresses caused by biotic and abiotic factors (Smith & Read, 2008). The beneficial effects of MF and other microorganisms on plant performance and soil health can be very important for the sustainable management of agricultural ecosystems (Gianinazzi *et al.*, 2010; Dellag *et al*., 2020). A primary benefit of MF is the improved P uptake conferred on symbiotic crop plants. In low-P soils, mycorrhizal plants usually grow better than non mycorrhizal plants as a consequence

of enhanced direct P uptake of plant roots via the MF pathway. However, plant growth can be suppressed even though the MF pathway contributes greatly to plant P uptake (Smith & Read, 2008). The growth inhibitions might be caused by the down-regulation of the direct root P-uptake pathway (Kobae, 2019). Recent gene expression study (Feddermann *et al.*, 2010) shows that plants induce a common set of mycorrhiza-induced genes. However, variability indicates that there exists functional diversity in MF symbioses. The differential expression of symbiosis-associated genes among different MF associations is related to the fungal species, plant genotypes, and the environmental factors (Feddermann *et al*., 2010; Savary *et al*., 2020).

2.6.3 Bioavailability and acquisition of soil phosphorus

Root-induced chemical and biological changes in the rhizosphere play a vital role in enhancing the bioavailability of soil P (Hinsinger, 2001; Fageria & Stone, 2006; Jacoby *et al*., 2017). These root-induced changes mainly involve proton release to acidify the rhizosphere, carboxylate exudation to mobilize sparingly available P by chelation and ligand exchange, and secretion of phosphatases or phytases to mobilize Po by enzyme-catalyzed hydrolysis (Bertin *et al*., 2003; Shen *et al*., 2013a). Root-induced acidification can decrease rhizosphere pH by 2 to 3 units relative to the bulk soil, resulting in substantial dissolution of sparingly available soil P (Marschner, 1996; Hinsinger *et al*., 2003; Erel *et al.*, 2017). The pH change in the rhizosphere is mainly affected by cation/anion uptake ratios and nitrogen assimilation. Ammonium supply to plant roots causes rhizosphere acidification, whereas nitrate supply causes alkalization. Legumes take up excess cations over anions, resulting in proton release.

Phosphorus deficiency in white lupin stimulates proton release and citrate exudation by cluster roots in association with an inhibition of nitrate uptake (Neumann *et al*., 1999) The changes of rhizosphere pH are also related to soil-buffering capacity, microbial activities, and plant genotypes. Besides proton release, carboxylate exudation such as that of citrate, malate, and oxalate greatly enhances Pi acquisition through chelation as well as by ligand exchange. Organic acid excretion and function in increasing P mobilization is well documented (Vance *et al*., 2003; Hinsinger *et al.*, 2003; Raghothama & Karthikeyan, 2005; Touhami *et al.*, 2020).

Some molecular physiological response mechanisms that underlay the survival and resistance to both P deficiency and Al toxicity have been reported in some plants such as white lupin (Wang *et al*., 2007). This may require further investigation to determine the mechanism. Plants can secrete phosphatase to mobilize Po through enzyme-catalyzed hydrolysis (Bertin *et al*., 2003; Shen *et al*., 2013a; Dell'Aquila *et al*., 2020). The activities of phosphatases are up-regulated under P deficiency (Vance *et al.*, 2003; Hammond & White, 2008a; Dixon *et al.*, 2020). However, the efficacy of these phosphohydrolases can be greatly altered by the availability of substrate, interactions with soil microorganisms, and soil pH, depending on soil physical and chemical environments (George *et al*., 2005; Jacoby *et al.*, 2017). Root-induced bioavailability and acquisition of P in association with root exudation need to be systemically evaluated in the soil/rhizosphere-plant continuum.

Some soil and rhizosphere microorganisms except mycorrhizal fungi (for example, plant growth promoting rhizobacteria) particularly P-solubilizing bacteria (PSB) and fungi (PSF) can also enhance plant P acquisition by directly increasing solubilization of P to plants, or by indirect hormone-induced stimulation of plant growth (Richardson *et al*., 2009). P-solubilizing microorganisms (PSM; PSB plus PSF) account for approximately 1% to 50% in P solubilization potential (Chen *et al.*, 2006). The PSB or PSF may mobilize soil P by the acidification of soil, the release of enzymes (such as phosphatases and phytases), or the production of carboxylates such as gluconate, citrate, and oxalate (Shen *et al*., 2011; Nannipieri *et al.*, 2011). What is not very clear, however, is whether after removal of the host plant, the enzymatic activity of P solubilizing microorganisms continues and benefits another plant in the succession.

2.7 Phosphorus uptake and utilization by plants

Plant roots absorb P as either of $H_2PO_4^-$ or HPO_4^{2-}. Because the concentrations of these ions in soils are in the micromolar range, high affinity active transport systems are required for Pi uptake against a steep chemical potential gradient across the plasma membrane of root epidermal and cortical cells (Shen *et al*., 2011). This process is mediated by high-affinity Pi/H+ symporters that belong to the PHT1

(phosphate transporter) gene family (Shin *et al*., 2004; Ai *et al.*, 2009; Nussaume *et al*., 2011; Młodzińska & Zboińska, 2016; Cao *et al*., 2020). Disruption of PHT1 gene expression results in a significant decrease of P acquisition by roots (Shin *et a*l., 2004; Ai *et al*., 2009; Młodzińska & Zboińska, 2016; Cao *et al*., 2020). In addition, some members of this family are expressed specifically and/or up-regulated in roots colonized by mycorrhizal fungi, indicating their function in transport of Pi via a mycorrhizal-dependent pathway (Bucher, 2007; Campo & San Segundo, 2020; Wang *et al*., 2020). This research aimed at utilizing the relationship in sorghum farming using *Aspilia pluriseta* as the source of mycorrhizal inoculum.

2.8 Classification, description and distribution of *Aspilia*

Aspilia is a genus of flowering plants in the daisy family. Historically, plants in this genus were used in Mbaise and most Igbo speaking parts of Nigeria to prevent conception, suggesting potential contraceptive and anti-fertility properties (Kayode *et al.*, 2007).

Aspilia pluriseta Schweif. is a prostrate perennial herb, with branches that are 30-60 cm long. It has sessile leaves with serrated margins, obovate in shape and abruptly ending in a long and very narrow, scaberulous tip. Flower rays are yellow with achenes that are obovoid, flattened and pubescent (Plate 1.1, page 2). *Aspilia pluriseta* is a common herbaceous plant whose natural habitat is in the open woodlands and grasslands. It is native in Democratic Republic of Congo, Burundi, Rwanda, Uganda, Kenya, Tanzania, Malawi, Mozambique, Zambia, Zimbabwe and South Africa (Kayode *et al*., 2007). The common names of *Aspilia pluriseta* are; *Dwarf aspilia*-English, *Mukushamvura*-Shona, South Africa, *Mahuti*- Kikuyu, Kenya, *Mauti*- Meru, Kenya (Kayode *et al.*, 2007).

Farmers in central eastern Kenya report good crop yields, particularly cereals, in farms previously growing *Aspilia pluriseta* Schweif. When uprooted, *Aspilia pluriseta* Schweif. presents huge surface area of white mycelium that is visible without any magnification (Plate 1.2). This study brought out facts on the effect *Aspilia pluriseta* Schweif. has to the growth of gadam sorghum crop.

2.9 Research gaps

In an era of rising ecological degradation, climate change, soil erosion, and biodiversity loss, global population expansion poses a danger to food security. A lot of research work has been done on application levels of inorganic fertilizers seeking paradigm shifts to crop production revolutions. However, the perspective of utilizing naturally occurring biotrophs such as mycorrhiza fungi has not been fully known and practised. Furthermore, mycorrhiza morphotypes are not well understood neither are their ecological niches in the rhizosphere of plants determined. This research seeks to address some of these gaps.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study area was in Embu and Tharaka-Nithi counties of eastern Kenya with a semi-arid climatic set up. The study sites were three situated at co-ordinates 00°21'00" S, 37°28'30" E, 00°10'00" S, 37°50'00" E and 00°12'00" S, 37°51'00" E (Appendix 2, sampling sites). Elevation for all the studied sites was below 1000 m above sea level. Rainfall in the selected areas was below 700 mm/year with two distinct but unreliable wet seasons in the months of March to May and October to December. Dry spells are more prolonged with temperature mean of 27°C. Scanty natural tree and shrub vegetation found was mainly *Aspilia pluriseta*, *Cassia sp, Euphorbia sp, Accasia sp*, *Balanites aegyptiaca*, *Cenchrus ciliaris* and *Hyperhenia rufa* grasses that were interspersed in trees and shrubs. The selected sites had silty clay, silt loam and sandy loam soil textural types and all of them had *Aspilia pluriseta* growing in the natural environment. Farmers practise dryland agroforestry mostly rearing animals that utilize products from *Accassia* products and *Balanites* species.

In the study area, soil pH ranges from moderate acidity 5.4 to neutral 7.16 (Oseko & Dienya, 2015). In spite of farmers practicing agro-pastoralism and therefore owning animals, most of the manure is sold out to the more affluent farmers in the upper parts of the counties in this study. Soils are mainly sandy with pockets of clay and loam.

3.2 Experiments conducted and procedures

3.2.1 General experimental set up

Controlled (greenhouse) experiments were carried out at the University of Embu. Reconnaissance survey as well as soil sampling was carried out in Tunyai and Gakurungu (Tharaka Nithi county) and Kanyuambora in Embu county (Figure 3.1).

The first controlled greenhouse experiment involved use of potted plants with four treatment factors; *Aspilia pluriseta* vegetation covered soils; soil textural types; soil depth and mycorrhizal fungi (MF) inoculated gadam sorghum. The second green house experiment had similar treatment factors but *Aspilia* soil was that which grew the *Aspilia* plants for six months and cut back and the pots used to grow sorghum. The soils used to grow *Aspilia* in the greenhouse were got from patches that were not growing *Aspilia pluriseta* in the field sites but the aspect of soil depth and soil texture maintained. Sorghum was then planted in the pots with either seeds that were inoculated or un-inoculated. All other as aspects, including layout in the greenhouse was maintained like that of experiment one.

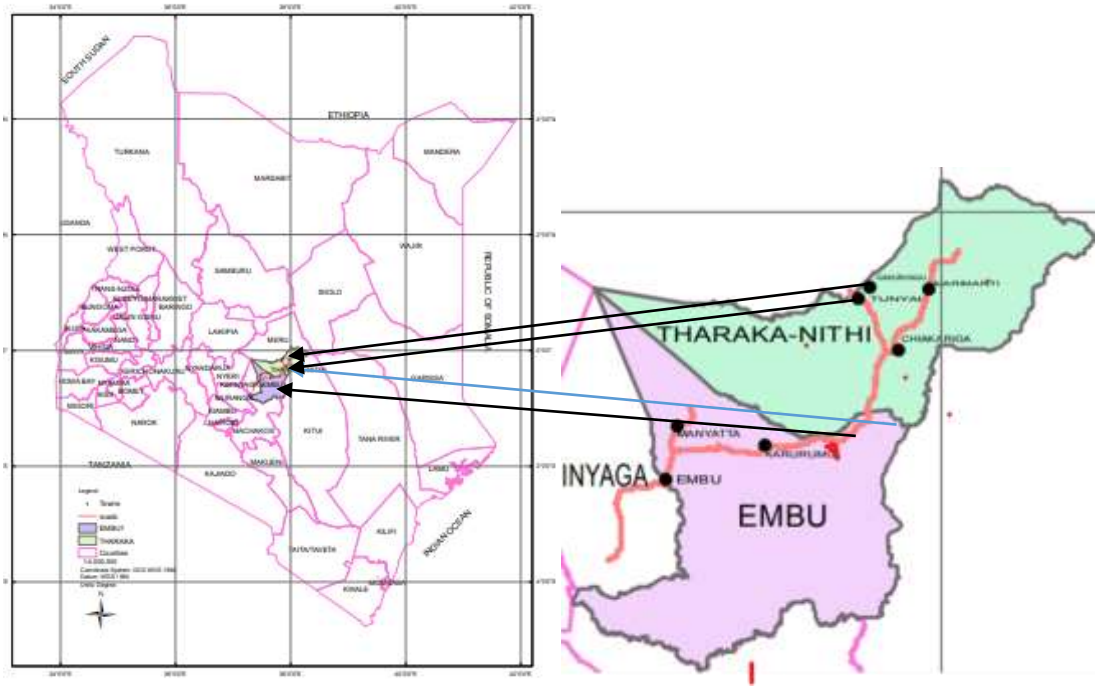

Figure 3.1. Field sampling sites at Gakurungu & Tunyai in Tharaka Nithi county and Kanyuambora in Embu county

3.2.2 Field soil sampling for laboratory analysis and greenhouse use

Soils were sampled from Gakũrũngũ, Tunyai and Kanyuambora field sites. A reconnaissance survey was initially carried out in which natural seed-bearing *Aspilia pluriseta* plots were mapped out for each of the three sites. Each of the site measured 4km^2 and was within an administrative sub unit. In each site, three sub-sites measuring 100m by 50 m were identified which formed the plots. Soil was sampled in the rhizosphere of *Aspilia pluriseta* vegetation and from patches without. Soils from these sub-sites were combined to form one composite mixture for each depth sampled (depth$_1$, 0-20 cm; depth$_2$, 21-40 cm, depth$_3$, 41-60 cm).

Soil sampling was purposive for each of the selected sites based on the soil textural types in the area: sandy loam, silty clay and silt loam soils. based on soil categorization through use of soil sieves with known particle diameter (Rawls, 1983; Gee & Or, 2002), the sites had dorminant soil characteristics as follows;

Site	Soil characteristics
Gakurungu	Silty clay: 45% silt, 45% clay,5%Sand Silt loam: 70% silt, 20%sand,10%clay Sandy loam: 60% sand, 20% clay,20%silt
Tunyai	Silty clay: 40% silt, 50% clay,10% sand Silt loam:40% silt, 50% sand,10%clay Sandy loam: 50% sand, 40% silt,10% clay
Kanyuambora	Silty clay: 50% silt, 45% clay,5%Sand Silt loam: 80% silt, 10%sand,10%clay Sandy loam: 60% sand, 20% clay,20%silt

About 1m^2 was cleared in the selected natural site where mature test plants were located. A standard soil auger (SOD-GP Dormer sampling equipment) was used to collect soil samples taken from the test plant rhizosphere at depths 0-60 cm. The patches without the test plant were either bare or covered with *Hypharhenia rufa* grass. Vertical measurements (in centimetres) along the profile were taken. Three samples for each parameter of interest was taken, each with a volume of 40cm^3 (the volume the soil auger could scoop) for the various depths (0 - 20 cm, 21- 40 cm and 41- 60 cm) in different sub-sites and were mixed together to form a single sample. Three soil samples from each sub-site (plots) and for every soil depth under

consideration were taken, giving the number of soil samples taken as 27 in every soil textural group for the plots that the *Aspilia pluriseta* grew. Similar procedure was followed for the sampling plots without this vegetation type. Overall the number of soil samples taken was 486. Samples were carefully labeled and put into separate sterilized hessian bags before taking them to the laboratory for analysis and greenhouse use. This soil was then thoroughly mixed but maintaining the site, sub site and soil depth of the collected samples. The samples were homogenously mixed and physico-chemical analysis done as outlined in procedure 3.2.4. Soils in patches without *Aspilia pluriseta* were similarly analysed. For use in the greenhouse, bigger volumes of about 20kg from each subsites was obtained in the same specific areas that sampling for laboratory analysis was undertaken.

3.2.3 Greenhouse experiments

A greenhouse experiment was set out to investigate the influence of test factors: *Aspilia pluriseta* vegetation covered soils (extracted from the field); soil textural types; soil depth and mycorrhizal fungi (MF) inoculated gadam sorghum on growth parameters of sorghum plants. The growth parameters of interest were; seedling emergence and plant count, sorghum height, the number of leaves per plant, the leaf length and sorghum yield. In the first experiment, mature plots of *Aspilia pluriseta* vegetation were identified in pre-selected sites as described in soil sampling section above. At the greenhouse, the soil was thoroughly mixed for each respective soil type, depth and presence or absence of *Aspilia* vegetation. Sterilized pots (30 cm × 40 cm) were filled with this homogenous soil to about one third full. Two seeds of mycorrhiza fungi-inoculated gadam sorghum (inoculation protocol according to Habte and Osorio (2001) were planted into pots and a similar number planted with uninoculated sorghum seeds. Two kilograms of *A. pluriseta* rhizosphere soil earlier tested and found to contain mycorrhiza fungi of the order Glomerales was mixed with 1kg gadam sorghum seeds. Each of the four treatment factors (Figure 3.2) as mentioned was replicated four times, giving the total number of pots as 144. Each pot was watered after every two days using a two-litre watering can for the first one week. Thereafter, the watering regime was reduced to once a week but ensuring the pots remained moist. Watering was done uniformly to all the pots. This was maintained for thirty-five days when watering regime was reduced to once every two

weeks. Data on plant growth attributes was taken every week and corroborated with treatment factor given in the pots. The experimental set up has 36 treatments replicated four times giving n=144 (Figure 3.2). Appendix 4 gives a comprehensive list of test factors and variables for the entire growing period of gadam sorghum crop.

Cs D2Oa	**Cs D1wpa**	**Sl D2Oa**	**Sl D2Oi**	**Sl D3Oa**	**Ls D2wpa**	**Sl D1wpa**	**Ls D3Oa**	**Cs D2wpi**	**Sl D2wpi**	**Sl D3wpa**	**Cs D3wpi**
Cs D1Oa	**Ls D3wpa**	**Cs D3Oa**	**Cs D2Oi**	**Sl D1Oa**	**Cs D3Oi**	**Cs D2wpa**	**Cs D1wpi**	**Sl D1wpi**	**Sl D3wpi**	**Cs D3wpa**	**Sl D2wpa**
Ls D1Oa	**Ls D1Oi**	**Ls D2Oa**	**Ls D2Oi**	**Sl D1Oi**	**Ls D3Oi**	**Ls D1wpa**	**Ls D1wpi**	**Sl D3Oi**	**Ls D2wpi**	**Cs D1Oi**	**Ls D3wpi**

Cs D2Oa	**Cs D1wpa**	**Sl D2Oa**	**Sl D2Oi**	**Sl D3Oa**	**Ls D2wpa**	**Sl D1wpa**	**Ls D3Oa**	**Cs D2wpi**	**Sl D2wpi**	**Sl D3wpa**	**Cs D3wpi**
Cs D1Oa	**Ls D3wpa**	**Cs D3Oa**	**Cs D2Oi**	**Sl D1Oa**	**Cs D3Oi**	**Cs D2wpa**	**Cs D1wpi**	**Sl D1wpi**	**Sl D3wpi**	**Cs D3wpa**	**Sl D2wpa**
Ls D1Oa	**Ls D1Oi**	**Ls D2Oa**	**Ls D2Oi**	**Sl D1Oi**	**Ls D3Oi**	**Ls D1wpa**	**Ls D1wpi**	**Sl D3Oi**	**Ls D2wpi**	**Cs D1Oi**	**Ls D3wpi**

Cs D2Oa	**Cs D1wpa**	**Sl D2Oa**	**Sl D2Oi**	**Sl D3Oa**	**Ls D2wpa**	**Sl D1wpa**	**Ls D3Oa**	**Cs D2wpi**	**Sl D2wpi**	**Sl D3wpa**	**Cs D3wpi**
Cs D1Oa	**Ls D3wpa**	**Cs D3Oa**	**Cs D2Oi**	**Sl D1Oa**	**Cs D3Oi**	**Cs D2wpa**	**Cs D1wpi**	**Sl D1wpi**	**Sl D3wpi**	**Cs D3wpa**	**Sl D2wpa**
Ls D1Oa	**Ls D1Oi**	**Ls D2Oa**	**Ls D2Oi**	**Sl D1Oi**	**Ls D3Oi**	**Ls D1wpa**	**Ls D1wpi**	**Sl D3Oi**	**Ls D2wpi**	**Cs D1Oi**	**Ls D3wpi**

Cs D2Oa	**Cs D1wpa**	**Sl D2Oa**	**Sl D2Oi**	**Sl D3Oa**	**Ls D2wpa**	**Sl D1wpa**	**Ls D3Oa**	**Cs D2wpi**	**Sl D2wpi**	**Sl D3wpa**	**Cs D3wpi**
Cs D1Oa	**Ls D3wpa**	**Cs D3Oa**	**Cs D2Oi**	**Sl D1Oa**	**Cs D3Oi**	**Cs D2wpa**	**Cs D1wpi**	**Sl D1wpi**	**Sl D3wpi**	**Cs D3wpa**	**Sl D2wpa**
Ls D1Oa	**Ls D1Oi**	**Ls D2Oa**	**Ls D2Oi**	**Sl D1Oi**	**Ls D3Oi**	**Ls D1wpa**	**Ls D1wpi**	**Sl D3Oi**	**Ls D2wpi**	**Cs D1Oi**	**Ls D3wpi**

Figure 3.2. Experimental lay out in the greenhouse at the University of Embu

Key: Soil textural class (Sl-sandy loam; Cs-silty clay; Ls-silt loam); Vegetation (O-soils without *Aspilia pluriseta*; wp-soils with *Aspilia pluriseta*); Inoculation (a-gadam seeds not inoculated; i-gadam seeds inoculated); Soil depth (D1-soil depth level 1, 0-20 cm; D2-soil depth level 2, 21-40cm; D3-soil depth level 3, 41-60 cm)

Experiment two involved growing of *Aspilia pluriseta* in the greenhouse for a period of six months after which the vegetation was cut back and gadam sorghum planted in the pots. After six months, soil in the pots was analyzed for pH, MF concentration, soil moisture content, soil phosphate levels and major elements' content, nitrogen and phosphorus (N & P). This was compared to the original soil used in the pots before growing *Aspilia pluriseta*. In this second experiment, soil from the field without *Aspilia pluriseta* was used to grow the plants but maintaining the other study factors as used in experiment one. The design of the experiment was similar to experiment one.

Pots, measuring 30cm by 40cm of strong polythene material was used for each experiment. Each of the treatrment factors was replicated four times. Crop growth parameters measured were seedling emergence; plant stand count; leaf elongation (mm); growth in height (mm); number of leaves and total grain yield (g/1000 grains).

3.2.4 Measurements of physico-chemical information on soil rhizosphere depths in the study sites

Physico-chemical analysis of the rhizosphere soil was done for factors that would influence fungal populations and distribution. Soil pH for each rhizosphere depth was taken with a portable pH meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10). *In situ* soil temperature was taken using an electrical chemical analyzer (Jenway – 3405). 10 g of the composite soil from every rhizosphere depth studied was analyzed in the laboratory for mycorrhiza fungi determination using Varma, (1998) protocol. The same soil sample was analysed for soil phosphorus in ppm using (Olsen *et al.*, 1954b) protocol. Soil moisture and soil nitrogen content were determined using Johnson, (1962) and Bremner, (1960) protocols respectively. samples were also analysed for soil phosphates using Mildred, (1942) protocol and organic matter content using the method by (Schulte & Hoskins, 2009). See Appendix 3 giving the arrangement/layout of the test factors and variables for physico-chemical soil attributes tested.

3.2.5 Root colonization by arbuscular mycorrhiza fungi on *Aspilia pluriseta*

Roots of *Aspilia pluriseta* were cleared in 10% potassium hydroxide (KOH) and autoclaved for 15 minutes (Trouvelot *et al.,* 1986). After cooling, the roots were washed in tap water and then bleached in ammonia, hydrogen peroxide and water solution for 2 hours. After bleaching the roots, they were rinsed in water before adding 1% hydrochloric acid. The acidified roots were then stained using 0.05% acidified glycerol stained with trypan blue and autoclaved for 3 minutes. The roots were then rinsed in water and stored temporarily in acidified glycerol without the stain. A minimum of 30x1cm roots were chosen randomly and 30 fragments placed parallel to each other on the slide. Polyvinlylactoglycerol (PVLG) was used as a mounting reagent and the slide was covered using a 24x50 mm coverslip. The roots were squashed gently to reveal the arbuscular mycorrhizal fungi (AMF) infection. The roots were assessed under the compound microscope using a linear eyepiece moving along each root fragment. The roots were rated according to the ranges of classes by (Trouvelot *et al.,* 1986). The classes gave a rapid estimation of the abundance of arbuscules, vesicles and the level of mycorrhizal colonization of each root fragment (Trouvelot *et al.,* 1986). The information obtained was exported to the Mycocalc software program for analysis.

3.2.6 Mycorrhiza fungi sampling and characterization

3.2.6.1 Sample collection

Soils were sampled from Gakũrũngũ, Tunyai and Kanyuambora field sites using a standard soil auger (SOD-GP Dormer sampling equipment). A reconnaissance survey was initially carried out in which natural seed-bearing *Aspilia pluriseta* plots were mapped out. Besides the seed bearing vegetation of interest, the selected areas of the survey for each of the three sites had to have three soil textural types (sandy loam, silt loam and silty clay). In each site, a quadrant measuring one metre by one metre was thrown at random in each sub-site (a sub-site consisted of an area within the site with one soil textural type). In case the quadrant contained more than one *Aspilia pluriseta* plant, the one closest to the centre of the quadrant was chosen for collection of rhizosphere soil fungal spores. The quadrant was thrown five times in each sub-site and soil was sampled at $depth_1$, 0-20 cm; $depth_2$, 21-40 cm, $depth_3$, 41-60 cm using a soil auger with a scooping capacity of $40cm^3$ of soil. The sampled soil

was put together for each rhizosphere depth from each sub-site and homogenously mixed. One hundred grams (100 g) of the mixture was put into khaki paper bags for soil and root DNA analysis in the laboratory.

3.2.6.2 Soil and rootlets total DNA extraction

Ten grams (10 g) of composite soil from the rhizosphere of *Aspilia pluriseta* comprising of the plant's rootlets was weighed using an electronic balance. The weighed soil was put into 100 ml beaker and about 50 ml tap water was added. The mixture was placed and mixed on an electronic stirrer overnight. After 12 hours the mixture was washed several times by passing it through a 710 µ sieve placed on top of a 45 µ sieve (Varma, 1998). The 710 µ sieve collected the roots and course debris while the 45 µ prevented the spores from passing through. The roots and course debris from the 710 µ sieve were put into a mortar and air-dried in a hood while the process of sieving continued by collecting sieved water and soil mixture in a 1-litre cylinder (Varma, 1998).

The washing and decanting process was done several times until near-clear water was obtained. This was followed by filling the centrifuge tubes with the sieved content. Centrifugation was done for 5 minutes at 1500 revolutions per minute (rpm) and the filtrate was poured off while the supernatant remained at the bottom of the tube. 48 % sucrose solution was added to the supernatant at equal volumes (50ml) and centrifuged for 1 minute at 1500 rates per minute (Varma, 1998). The filtrate was collected on the 45 µ sieve while the supernatant was disposed off. The filtrate was then washed with slowly flowing tap water to wash off the sucrose. The washed content was then collected in a 50ml plastic cylinder and the contents poured into a filter paper. Using a fine pair of forceps, the contents were picked and transferred to eppendorf tubes.

The dried plant roots in the mortar were crushed into a fine powder using a pestle and the contents added to fungal spore cells in the eppendorf (Lee *et al.*, 1988) . The content in the eppendorf was re-suspended in 100 µl of solution A {100mM Tris-HCL (pH 8.0), 100Mm EDTA (pH 8.0); added to 5 µl of lysozyme (from a 20mg/ml solution) and incubated at 37°C for 30 minutes in a water bath}. 400 µl of lysis buffer (solution B) comprising 400 mM Tris-HCL (pH 8.0), 60 mM EDTA (pH 8.0), 150

mM NaCl, 1% sodium dodycyl sulfate and the tube was left at room temperature for 10 minutes. 10 µl of Proteinase K (20mg/ml) was mixed gently and incubated at 65°C for 1 hour in a water bath. An equal volume of chloroform/ isoamyl alcohol was added and centrifuged at 13200 rpm for 5 minutes at 4°C. The supernatant was transferred to new tubes. In the new tubes, 150 µl of sodium acetate (pH 5.2) and an equal volume of isopropanol alcohol was added accordingly. The tubes were briefly mixed through inversion. The mixture was then incubated at –20°C overnight. The tubes were then spun at 13200 rpm for 30 minutes and the supernatant was discarded.

The resultant DNA pellets were washed in 300 µl of 70% ethanol. The pellets were then spun at 10000 rpm for 1 minute and the supernatant discarded. The resultant DNA pellet was air dried in the hood and dissolved in 50 ml of 13 Tris-EDTA. Genomic DNA (5–15 ng) in 10 µl of ddH2O was used for RAPD amplification using 1.5% agarose gels and images obtained confirming presence of DNA (Lee *et al.*, 1988). Nine samples (3 samples each from $depth_1$, 0-20 cm; $depth_2$, 21-40 cm and $depth_3$, 41-60 cm) were dried using LABCONCO machine. About 30 µl of the confirmed DNA was shipped to mrdnalabs (USA) for next generation sequencing with the primers as diversity assay bTEFAP® average inhouse ITSwanda. Illumina was used as the sequencing technology method.

3.2.6.3 Amplicon library preparation and sequencing

The primers used for amplification of the Internal Transcribed Spacer (ITS) on Polymerase Chain Reaction (PCR) was ITS_1 (TCCGTAGGTGAACCTGCGG) and ITS_4 (TCCTCCGCTTATTGATATGC). The primers barcode was according to (White *et al.*, 1990). Using HotStarTaq Kit (Qiagen, USA) Plus Master with an initial heating of 94°C for 3 min, followed by 28 cycles of denaturation at 94°C for 30 seconds, amplification proceeded in a 30 cycle PCR Mix. Annealing was done at 53°C for 40 seconds and extended at 72°C for 1 min. Final elongation step was done at 72°C for 5 min.

visualization of polymerase chain reaction (PCR) products was done on 2% agarose gel and its success determined by the relative intensity of the bands. DNA concentrations determined the pooling together of multiple samples in equal proportions. Calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) purified pooled samples. DNA library was prepared using the pooled and

purified PCR product in accordance to (Yu & Zhang, 2012) illumina sequencing protocol. Next generation sequencing was done at MRDNA laboratory in the United States of America (USA) using a MiSeq platform and following the manufacturer's instructions.

3.2.6.4 Sequencing, taxonomic classification and submission of the data

Barcodes and primers were depleted from the sequences obtained in the Illumina sequencing platform using a proprietary pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed by the manufacturer. Using protocol developed by (Reeder & Knight, 2010), low quality sequences were identified by denoising and were filtered out of the dataset. Sequences less than 200 base pairs after quality check through phred20- based quality trimming, ambiguous base calls and those with homopolymer runs exceeding 6bp were removed.

Analysis of sequences was done by a script optimizer for high-throughput data to weed out potential and definite as described by Gontcharova *et a*l. (2010). De novo operational taxonomic units (OUT) clustering was done using standard UCLUST method with the default settings as implemented in QIIME pipeline Version 1.8.0 at 97% similarity level (Caporaso *et al.*, 2010). Taxonomy was assigned to each OTU using BLASTn against SILVA SSU Reference 119 database at default e-value threshold of 0.001 in QIIME (Quast *et al.*, 2013). Resulting raw sequences were submitted to the National Centre for Biotechnology Information (NCBI) sequence read archive with study accession number SRP320693.

3.2.7 Mycorrhiza fungi inoculum application to sorghum seed

Soil confirmed to contain arbuscular mycorrhiza fungi (procedure 3.2.2, above) was obtained from the study sites. This soil, from mature *Aspilia pluriseta* plots was homogenized on a polythene sheet, ensuring big soil clods were broken down using a hand trowel. On a separate polythene sheet, one kilogram of gadam sorghum seed (enough seed to plant in the pots) was placed. The seed and the mycorrhiza containing soil were mixed in the ration 1:0.5 (one kilogram of gadam sorghum seed to half kilogram of the soil). Using a pipette, five drops of water were added to the soil-seed mixture before thoroughly mixing the ingredients. The resultant mixture was ready to plant in the pots.

3.3 Data analysis

3.3.1 Greenhouse data

Data from the greenhouse was analysed using the general linear model $yijk = \mu + \tau i + \beta j + (\tau\beta)ij + \gamma k + (\tau\gamma)ik + (\beta\gamma)jk + (\tau\beta\gamma)ijk + \varepsilon ijk$.

Where, μ is a constant; τi, βj and γk and εijk were the factors considered in the model (The block effects, soil textural type, soil depth, *Aspilia* vegetation cover and inoculation respectively). (τγ)ik, (βγ)jk and (τβγ)ijk are interaction effect among the factors. SAS edition 9.2 was used to compute the variables. Differences in the treatment means was separated using least significant difference (LSD) at $p \leq 0.05$. Physico-chemical data was similarly analysed.

3.3.2 Molecular data

Illumina sequencing platform was used on total *Aspilia pluriseta* rhizosphere genome soil. A script optimizer analysed sequences for high-throughput data. De novo operational taxonomic units (OTUs) clustering was done using standard UCLUST method with the default settings as implemented in QIIME pipeline Version 1.8.0 at 97% similarity level. Taxonomy was assigned to each OTU using BLASTn against SILVA SSU Reference 119 database at default e-value threshold of 0.001 in QIIME. Data was analysed using R programming software.

CHAPTER FOUR

RESULTS

4.1 Overview of the results

The study investigated mycorrhiza fungi in the rhizosphere of *Aspilia pluriseta* and their influence on growth and availability of phosphorus to gadam sorghum plants. The results showed that spore counts varied significantly among soil textural types, sample locations and soil depth with sandy loam soil textural type having the highest spore counts. The results also showed that spore count decreased significantly with the depth of soil along the rhizosphere with the intensity of mycorrhiza spore morphotypes significantly higher for soils whose vegetation was covered with *Aspilia pluriseta* than those without. Results from taxonomic analysis revealed that there was fungal richness along the plant's rhizosphere with the genera *Glomus* being the most prevalent in all tested soil depths. Further, the results showed that all growth parameters were enhanced in both mycorrhiza fungi inoculated gadam sorghum seeds and in pots whose soils were taken from the rhizosphere of *Aspilia pluriseta* plants and that growth attributes had a positive correlation to yield at 95% confidence level. The results also showed that soil phosphate level was enhanced where seed inoculation with mycorrhiza was done and in soils previously grown *Aspilia pluriseta* vegetation.

4.2 Effects of co-association of mycorrhiza and *Aspilia pluriseta* on growth and phosphorus availability to gadam sorghum crop

4.2.1 Seedling emergence and plant count growth parameter

The response of gadam sorghum plant to test factors for the first 35 days on the crop's emergence and stand count was as presented in Table 4.1.

Table 4.1 Response of seedling emergence and plant count on the test factors for the first 35 days of sorghum growth in the greenhouse at the University of Embu

Factor	SC 7	SC 14	SC 21	SC 28	SC 35
Silty Clay	83.3b	83.3b	83.3b	84.4b	82.3b
Silt Loam	96.9a	96.9a	94.8a	94.8a	94.8a
Sandy Loam	90.2ab	90.2ab	90.2ab	90.2ab	90.2a
LSD	7.2	7.2	7.6	7.6	7.8
Block 1	83.3b	83.3b	83.3b	84.7b	81.9b
Block 2	91.7ab	91.7ab	91.7ab	91.7ab	91.7a
Block 3	98.6a	98.6a	95.8a	95.8a	95.8a
Block 4	86.9b	86.9b	86.9b	86.9b	86.9ab
LSD	8.3	8.3	8.7	8.8	9
NoAp	83.8b	83.8b	83.8b	84.4b	83.1b
Ap	96.5a	96.5a	95.1a	95.1a	95.1a
LSD	5.9	5.9	6.2	6.2	6.3
0-20 cm	95.8a	95.8a	95.8a	95.8a	95.8a
21-40 cm	90.6ab	90.6ab	90.6a	90.6a	90.6a
41-60 cm	84.0b	84.0b	81.9b	82.9b	80.8b
LSD	7.2	7.2	7.6	7.6	7.8
SOi	83.8b	83.8b	83.8b	84.4b	83.1b
SNi	96.5a	96.5a	95.1a	95.1a	95.1a
LSD	5.9	5.9	6.2	6.2	6.3
Mean	90.1	90.1	89.4	89.8	89.1
p-value	<.0001	<.0001	<.0001	0.0002	<.0001
R^2	0.3	0.3	0.2	0.2	0.2
Co. Variation	19.6	19.9	20.9	21.0	21.6
Root MSE	17.9	17.9	18.7	18.9	19.2

Values followed by the same letter within the column are not significantly different from each other at $p \leq 0.05$ (LSD test).

KEY: SC7-Plant count day (7, 14, 21, 28 & 35 are days after planting); LSD-Least significance difference; NoAp-Soils without *Aspilia pluriseta;* Ap-*Aspilia*

pluriseta soils; SOi-gadam sorghum seeds not inoculated; SNi-Sorghum seeds inoculated.

Results indicated that within the first 35 days of gadam sorghum plants' growth in the controlled greenhouse environment, sorghum plants in the silt loam textural soil type exhibited the highest seed emergence and stand count. It can be observed that soil textural type influenced gadam sorghum seeds emergence. Greater seedling emergence was experienced in silt loams at 97% germination within the first seven days. Results show that seeds planted in silty clay soils had the lowest seedling emergence in the first week and subsequently lower plant population over time. The crop stand (plant population) from pots in silty clay soil differed significantly at $p \leq 0.05$ with those from silt loam (Table 4.1). Further analysis on the interaction between test factors that could affect emergence and stand count parameter revealed that there was interaction between soil textural type, presence or absence of *Aspilia* and soil depth but this interaction was not significant at $p \leq 0.05$ (Apendix 5). All other factors had no interaction. There was more seedling emergence and plant count in the top soil, 0-20 cm compared to others (Table 4.1). From the results, seed emergence was highest in soils that had *Aspilia pluriseta* compared to those without and this difference was significant at $p \leq 0.05$. The percentage sorghum seed that emerged and the subsequent plant count for the inoculated seed was significantly higher than the seed that came from un-inoculated pots.

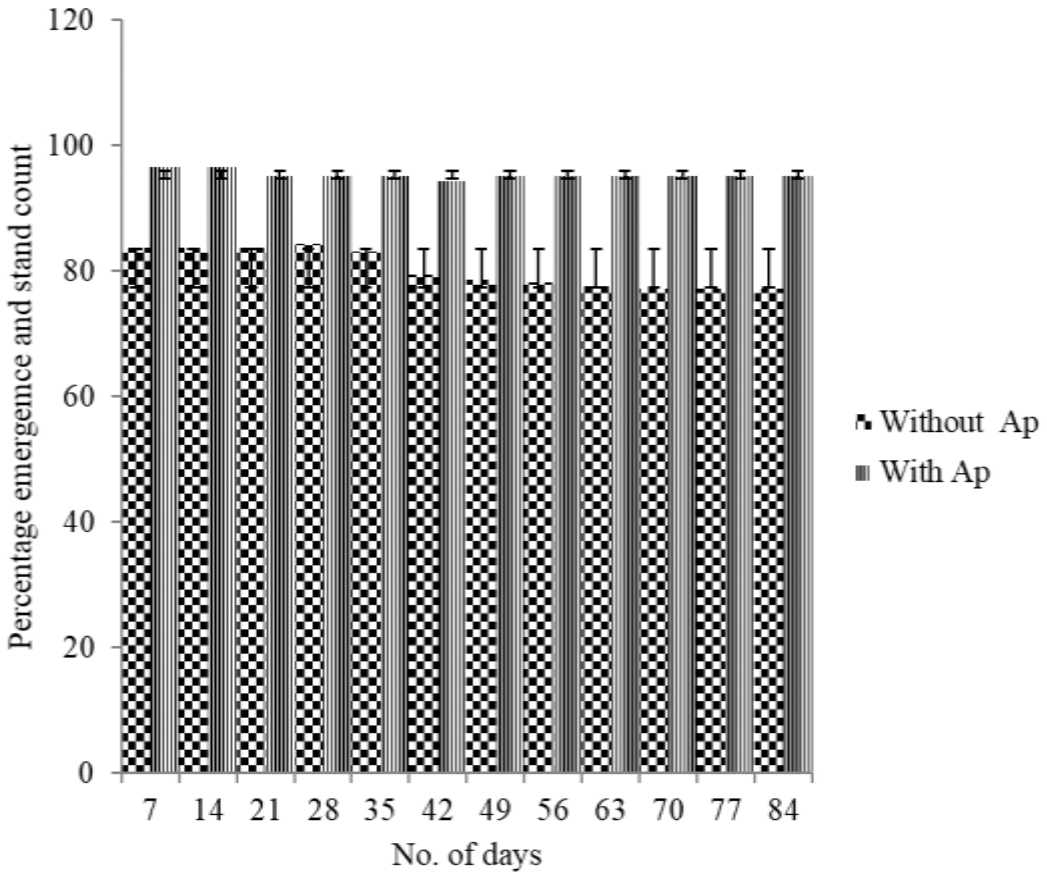

Figure 4.1. Percentage sorghum seedling emergence and stand count over time in the greenhouse at the University of Embu

Results of sorghum seedling emergence and stand count in the greenhouse indicate that gadam seedling emergence and early growth were highest in soils that had *Aspilia pluriseta* vegetation (Figure 4.1) with 96.5% seedling emergence and early growth in the first week differing significantly at $p \leq 0.05$ with seeds planted in soils that were not previously growing *Aspilia pluriseta*. Figure 4.1 also demonstrates that the crop's establishment was almost maintained in soils with *Aspilia pluriseta* vegetation compared to those without this type of vegetation.

Results presented in Figure 4.2 show that gadam sorghum seeds that were inoculated with soils inhabited by *Aspilia pluriseta* had better seedling emergence and improved seedling survival over time.

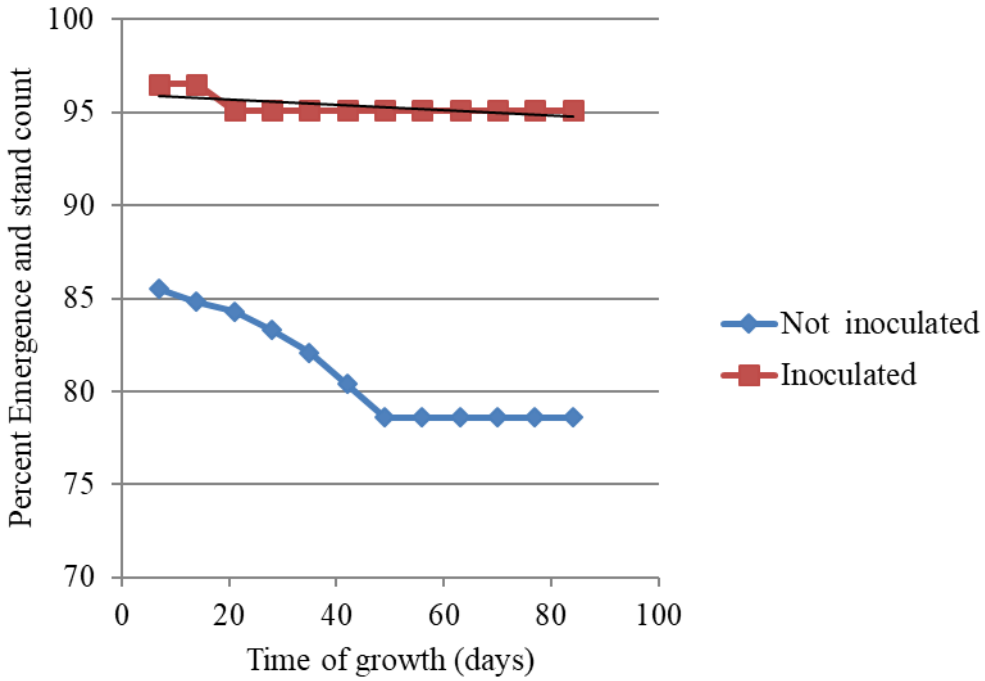

Figure 4.2. Gadam sorghum emergence and stand count for inoculated and un-inoculated seedlings grown at the greenhouse in the University of Embu

Figure 4.2 shows that the inoculated gadam sorghum seeds had higher percent seedling emergence and stand count (establishment) compared to the un-inoculated. Aggregate gadam crop establishment was higher in inoculated sorghum seeds than in seeds that were not inoculated.

4.2.2 Sorghum plant height growth parameter

Results on table 4.2 show the response of plant height on the test factors of the experimental set up at the greenhouse.

Table 4.2 Response of sorghum plant height on the test factors for the first 35 days of sorghum growth in the greenhouse at the University of Embu

Factor	H7	H14	H21	H28	H35
Silty Clay	6.2a	19.3a	48.3a	94.7a	113.6a
Silt Loam	6.3a	19.1a	46.9a	93.4a	119.1a
Sandy Loam	5.2b	16.0b	39.1b	72.3b	100.5b
LSD	0.6	1.8	3.6	6.5	5.5
Block 1	6.2ab	19.6a	48.8a	96.9a	114.6a
Block 2	6.7a	19.5a	48.4ab	90.8ab	115.0a
Block 3	5.7b	17.7a	44.3b	86.0b	115.5a
Block 4	5.0c	15.7b	37.6c	73.4c	99.1b
LSD	0.7	2	4.2	7.5	6.3
NoAp	5.3b	17.1b	43.6a	84.2a	112.5a
Ap	6.5a	19.1a	45.9a	89.3a	109.6a
LSD	0.5	1.4	3	5.3	4.5
0-20 cm	7.1a	20.8a	50.5a	93.7a	115.9a
21-40 cm	5.8b	18.2b	44.0b	84.2b	109.0b
41-60 cm	4.8c	15.4c	39.7c	82.4b	108.3b
LSD	0.6	1.8	3.6	6.5	5.5
SOi	5.3b	17.1b	43.6a	84.2a	112.5a
SNi	6.5a	19.1a	45.9a	89.3a	109.6a
LSD	0.5	1.4	3	5.3	4.5
Mean	5.9	18.1	44.8	86.8	111.1
p-value	<.0001	<.0001	<.0001	<.0001	<.0001
R^2	0.5	0.3	0.4	0.4	0.3
Co. Variation	24.4	24.2	20.0	18.6	12.2
Root MSE	1.4	4.4	9.0	16.1	13.4

Values followed by the same letter within the column are not significantly different from each other at $p \leq 0.05$ (LSD test).

KEY: H7-Plant height in centimetres (7, 14, 21, 28 & 35 are days after planting); LSD-Least significance difference; NoAp-Soils without *Aspilia pluriseta;* Ap-*Aspilia pluriseta* soils; SOi-gadam sorghum seeds not inoculated; SNi-Sorghum seeds inoculated.

From the results, it was noted that the plant height in silty clay and silt loam was higher compared to that of sandy loam in the first 35 days of crop's growth. This difference was significant at $p \leq 0.05$.

Sorghum plants grown in soils formerly grown *Aspilia pluriseta* attained higher growth heights per unit time compared to plants that were grown in soils devoid of this vegetation cover (Table 4.2). Differences in plant height means between the two treatments was significant at $p \leq 0.05$ through out the period of growth (Table 4.2 and Appendix 4).

Sorghum plants attained higher plant height in pots with top soil (0-20 cm) compared to the crop planted with either the sub soil or the bottom soils and this difference was significant at $p \leq 0.05$. Means of plant height (cm) for sorghum plants in soils that had inoculated seed were higher and significantly different at $p \leq 0.05$ from those that were not inoculated (Table 4.2). The model used showed interaction between soil textural type, presence or absence of *Aspilia* and soil depth but this interaction was not significant at $p \leq 0.05$. All other factors had no interaction effects (Apendix 5).

Figure 4.3 is a simulated polynomial model showing that gadam sorghum plants attained higher heights at maturity in pots whose soil was from *Aspilia pluriseta* vegetated areas.

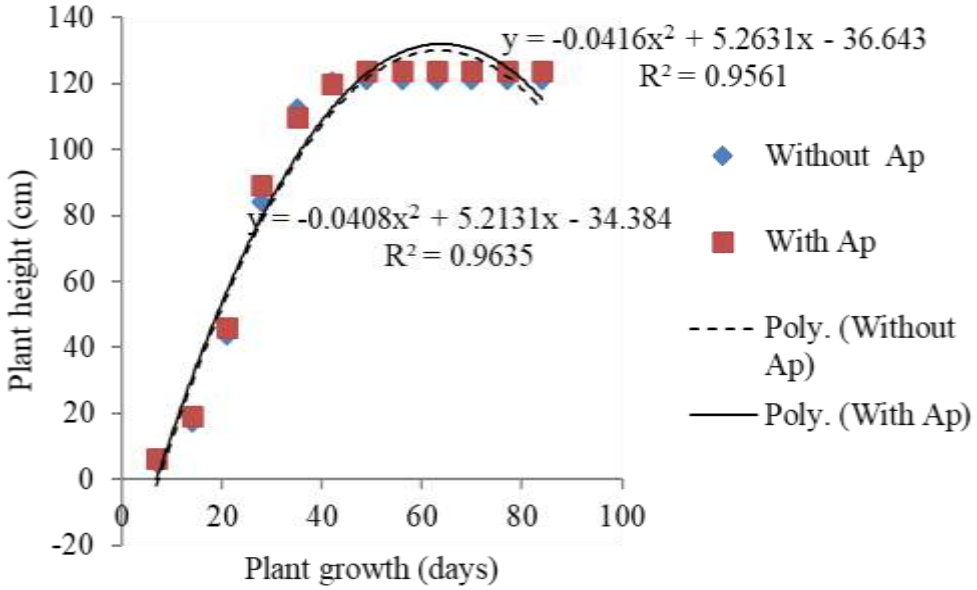

Figure 4.3. Growth in height of gadam sorghum in the greenhouse at the University of Embu estimated through a polynomial function

From the results presented it can be observed that the average height of sorghum plant was 124 cm at physiological maturity (Figure 4.3). Although both growth curves were normal, the curve with soils where *Aspilia pluriseta* previously grew peaked higher.

There was observable difference in the height of sorghum seedlings between those that were established from inoculated seeds compared to those that were not. Sorghum seedlins that were grown from seeds that were inoculated were taller and had given an inflorescent. This applied also to pots from *Aspilia* sites.

Plate 4.1. Height of potted gadam sorghum plants under different treatments in the greenhouse at the University of Embu

4.2.3 Sorghum plant number of leaves growth parameter

Results given in Table 4.3 indicate the number of leaves per plant of sorghum in response to the test factors of the experimental set up at the greenhouse.

Table 4.3 Gadam sorghum number of leaves variation per plant on the test factors for the first 35 days of sorghum growth in the greenhouse at the University of Embu

Factor	NL7	NL14	NL21	NL28	NL35
Silty Clay	1.9b	2.7a	2.9a	3.1a	3.6a
Silt Loam	1.9ab	2.8a	3.0a	3.0a	3.7a
Sandy Loam	2.0a	2.8a	2.8b	3.1a	3.5a
LSD	0.2	0.2	0.1	0.1	0.2
Block 1	1.8b	2.8ab	3.0a	3.1a	3.6bc
Block 2	2.0a	2.7b	2.9a	3.0b	3.7ab
Block 3	2.0a	2.9a	3.0a	3.1a	3.8a
Block 4	1.9ab	2.7b	2.8b	3.0b	3.4c
LSD	0.2	0.2	0.1	0.1	0.2
NoAp	1.9b	2.7a	2.9a	3.0a	3.6a
Ap	2.0a	2.8a	2.9a	3.1a	3.6a
LSD	0.1	0.1	0.1	0.1	0.1
0-20 cm	2.1a	2.9a	3.0a	3.2a	3.8a
21-40 cm	1.8b	2.9a	3.0a	3.0b	3.5b
41-60 cm	1.8b	2.4b	2.8b	3.0b	3.4b
LSD	0.2	0.2	0.1	0.1	0.2
SOi	1.9b	2.7a	2.9a	3.0b	3.6a
SNi	2.0a	2.8a	2.9a	3.1a	3.6a
LSD	0.1	0.1	0.1	0.1	0.1
Mean	1.9	2.7	2.9	3	3.6
p-value	<.0001	<.0001	<.0001	<.0001	<.0001
R^2	0.2	0.3	0.3	0.3	0.2
Co. Variation	19.6	13.6	8.5	7.2	12.2
Root MSE	0.4	0.4	0.2	0.2	0.4

Values followed by the same letter within the column are not significantly different from each other at $p \leq 0.05$ (LSD test).

KEY: NL7-Number of leaves (7, 14, 21, 28 & 35 are days after planting); LSD-Least significance difference; NoAp-Soils without *Aspilia pluriseta;* Ap-*Aspilia pluriseta* soils; SOi-gadam sorghum seeds not inoculated; SNi-Sorghum seeds inoculated.

Table 4.3 potrays that there was no significant difference in the means of the number of leaves attained after 35 days of planting except in the first week of crop's growth for the soil textural types. The table also shows that the number of leaves did not differ significantly at $p \leq 0.05$ for soils with *Aspilia pluriseta* and those without except for the first week of growth. However, the number of leaves in pots that were inoculated differed from those not inoculated in the first and fourth week of crop's growth at $p \leq 0.05$.

4.2.4 Sorghum plant leaf length growth parameter

The average leaf length of gadam sorghum crop in response to test factors is given in Table 4.4. The difference in leaf length growth of sorghum in silt clay pots compared to other soil textural types was significant at $p \leq 0.05$ in the first three weeks (Table 4.4).

Table 4.4 Response of gadam sorghum average leaf length on the test factors for the first 35 days of sorghum growth in the greenhouse at the University of Embu

Factor	LL7	LL14	LL21	LL28	LL35
Silty Clay	5.4a	15.8a	37.1a	74.4a	93.7a
Silt Loam	4.5b	12.8b	30.7b	69.2a	93.7a
Sandy Loam	3.8c	10.9c	25.5c	48.2b	74.0b
LSD	0.5	1.6	3.7	7.6	5.5
Block 1	5.3a	15.6a	37.5a	76.0a	91.2ab
Block 2	4.9ab	14.9a	33.1b	69.3a	96.9a
Block 3	4.3b	11.7b	28.6b	59.9b	87.0b
Block 4	3.6c	10.5b	25.2c	50.6c	73.5c
LSD	0.6	1.9	4.3	8.8	6.3
NoAp	4.3b	12.8a	31.0a	61.8a	88.9a
Ap	4.8a	13.6a	31.2a	66.1a	85.4a
LSD	0.4	1.3	3	6.2	4.5
0-20 cm	5.4a	15.6a	35.8a	70.9a	89.3a
21-40 cm	4.1b	12.0b	27.8b	57.8b	84.0a
41-60 cm	4.1b	11.9b	29.7b	63.2b	88.1a
LSD	0.5	1.6	3.7	7.6	5.5
SOi	4.3b	12.8a	31.0a	61.8a	88.9a
SNi	4.8a	13.6a	31.2a	66.1a	88.4a
LSD	0.4	1.3	3	6.2	4.5
Mean	4.5	13.2	31.1	64	87.1
p-value	<.0001	<.0001	<.0001	<.0001	<.0001
R^2	0.3	0.3	0.3	0.4	0.4
Co. Variation	29.1	30.8	29.7	29.5	15.5
Root MSE	1.3	4.1	9.2	18.9	13.5

Values followed by the same letter within the column are not significantly different from each other at $p \leq 0.05$ (LSD test).

KEY: LL7-Leaf length, in centimetres (7, 14, 21, 28 & 35 are days after planting); LSD-Least significance difference; NoAp-Soils without *Aspilia pluriseta;* Ap-*Aspilia pluriseta* soils; SOi-gadam sorghum seeds not inoculated; SNi-Sorghum seeds inoculated.

Sorghum leaf length growth was significantly different at p≤0.05 for top soils (0-20 cm) compared to either the sub soil (21-40 cm) or the bottom soil (41-60 cm). In pots with *Aspilia* soils the mean was significantly different with pots that did not have *Aspilia* in the first one week of growth. A similar scenario was witnessed in pots whose seed ws inoculated compare to pots whose seed was not.

4.2.5 Changes in pH, phosphorus and phosphate levels before and after gadam sorghum harvest

Change in the level of soil pH, soil phosphorus and phosphates as a result of soil textural treatment factor is presented in Table 4.5.

Table 4.5 Changes in soil pH, phosphates and phosphorus before and after harvesting gadam sorghum in the greenhouse at the University of Embu

Factor	pH1	pH2	Phos1	Phos2	Phr1	Phr2
Silty Clay	6.3a	6.2a	96.2a	96.8a	25.7a	24.2a
Silt Loam	6b	5.9b	76.9a	78.9a	33.5a	32.8a
Sandy Loam	6.3a	6.1a	80.8a	80.3a	35.3a	34.5a
LSD	0.2	0.2	39.2	39.6	15.8	15.9
NoAp	6.3a	6.2a	65.6b	63.3b	31.8a	31a
Ap	6b	5.9b	103.6a	107.7a	31.2a	30a
LSD	0.2	0.2	32	32.3	12.9	13
0-20 cm	6.4a	6.3a	108.6a	108.4a	40.3a	39a
21-40 cm	6.1b	5.9b	65.3b	67.3b	28.5a	27.8a
41-60 cm	6.1b	6b	80ab	80.8ba	25.7a	24.7a
LSD	0.2	0.2	39.2	39.6	15.8	15.9
Mean	6.2	6.1	84.6	85.5	31.5	30.5
p-value	0.0007	0.0029	0.0675	0.0511	0.327	0.3252
R^2	0.8	0.7	0.5	0.6	0.4	0.4
Co. Variation	2.6	2.6	36.8	36.8	40	41.5
Root MSE	0.2	0.2	31.2	31.5	12.6	12.7

Values followed by the same letter within the column and along the rows are not significantly different from each other at p≤0.05 (LSD test).

Key: pH1- Initial soil pH; pH2-pH one week after harvesting; Phos1-Initial soil phosphates in ppm; Phos2-Soil phosphates in ppm one week after harvesting; Phr1-Initial soil phosphorus in ppm; Phr2-Soil phosphorus in ppm one week after harvesting; Ap-*Aspilia pluriseta*; NoAP-Without *Aspilia pluriseta*

Results obtained and presented in Table 4.5 indicate that silty clay and sandy loam soils did not show any significant difference in pH, but were significantly different compared to the silt loam at $p \leq 0.05$. After growing sorghum, the pH and phosphorus reduced in all the soils while phosphate levels increased (Table 4.5).

Results presented in Table 4.5 show that phosphate levels in soils grown with *Aspilia pluriseta* vegetation were higher compared to soils without this vegetation. Soil pH was correspondingly lower in those areas where soil was sampled in *Aspilia pluriseta* plots. The calculated phosphate linear regression eaquation was $P_2O_4 = 78.555 + 18.42B + 20.914S - 27.967L - 15.07D$ where,

B= the block effects,

S= Effects of soil textural type

L=Effects of location, whether soils were from *Aspilia* vegetation cover or not

D=Effects of soil depth (0-20 cm; 21-40 cm & 41-60 cm)

The observed results in Table 4.5 also depict that the pH, phosphates and phosphorus were generally higher in soil depth 1 (0-20 cm) compared to the lower soil depths both before and after planting sorghum. However, there were significant differences in pH and phosphates at $p \leq 0.05$ for soils in depth 1 compared to depth two and depth three. The pH level was higher in soils of depth one even in the second experiment where *Aspilia pluriseta* was grown in the pots and later cut back (Table 4.6).

Table 4.6:Physico-chemical parameters in the soil after growin sorghum in the pots earlier planted *Aspilia pluriseta* in the greenhouse at the University of Embu.

Factor	pH	P_2O_4	SM	N	P	SOM
Gakurungu	5.7ba	70ba	17.3a	0.04b	22.2a	8.0a
Tunyai	6.3a	91ba	17b	0.05b	30.8a	17b
Kanyuambora	5.8b	109a	17.5a	0.11a	33.9a	17.5a
LSD	0.6	27	0.3	0.02	18.9	1
Silt clay	6.2a	87.1a	18.9a	0.06a	22.9a	10.9a
Silt loam	5.8ba	99.3a	18.1b	0.08a	33.9a	10.0a
Sandy loam	5.7b	54.9a	14.9c	0.07a	30.0a	7.3b
LSD	0.4	45.1	0.3	0.03	14.9	2.2
With *Aspilia*	5.8a	99.0a	17.6a	0.07a	31.4a	11.0a
Without *Aspilia*	5.9a	64.8b	17.0b	0.07a	26.5a	7.7b
LSD	0.5	28.1	0.2	0.02	12.2	1.8
Depth 1 (0-20 cm)	6.1a	77.4a	17.3a	0.09a	37.3a	9.7a
Depth 2 (21-40 cm)	5.7a	73.9a	17.4a	0.07ba	26.2a	8.2a
Depth 3 (41-60 cm)	5.8a	94.5a	17.2a	0.05b	23.4a	10.1a
LSD	0.4	28.3	0.3	0.03	14.9	2.2

Values followed by the same letter within the column are not significantly different from each other at $p \leq 0.05$ (LSD test).

Key: pH-Level of acidity; MF-Mycorrhiza fungi; P_2O_4-Phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus; SOM-Soil organic matter

Table 4.6 potrays that even though the values are not significant, the pH values in *Aspilia pluriseta* soils was lower than in soils without. Soils originally sourced from Kanyuambora had more phosphates, soil moisture, Nitrogen content and soil organic. Silty clays had higher pH, soil moisture and soil organic matter content compared to the other soils.

Mycorrhizal infectivity potential of *Aspilia pluriseta* is shown in Table 4.7. The table shows readings observed through a compound microscope for 30 pieces of *Aspilia pluriseta* roots, each 1 cm showing the level of arbuscules, vesicles and total colonization as guided by Trouvelot *et al.* (1986). Results show that only one slide had zero total mycorrhiza colonization while three slides had five out of a possible mycorrhiza colonization scale of five. Twenty slides had more than three scores on a total scoring matrix of five. From these results the calculated mycorrhizal root colonization percentage (F %) in *Aspilia pluriseta* shrub using Mycocalc application software was 96.7% while the intensity of the mycorrhizal colonization in the root

system (M) was 42.2%. The intensity of arbuscular mycorrhizal fungi (AMF) colonization in the root fragments (m) was 43.6%, the arbuscule abundance in mycorrhizal parts of root fragments (a) was 56.1% and arbuscules abundance in the root system (A) was 23.7%.

Table 4.7 *Aspilia pluriseta* mycorrhiza fungi root colonization for samples taken from Gakurungu, Tunyai and Kanyuambora

Slide number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
M (Total colonization)	3	2	4	3	2	3	4	2	4	5	2	5	4	2	3	3	4	4	4	4	4	5	4	3	3	2	2	0	2	2
A (Arbuscules)	1	1	3	2	2	2	3	2	3	2	0	3	1	1	3	3	2	3	2	2	1	0	2	2	1	2	1	0	1	1
V (Vesicles)	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Recorded values of total colonization (M), arbuscules (A) and Vesicles (V) were exported to Mycocalc application software

Mycorrhizal species from molecular analysis indicating their relative populations in the rhizosphere of *Aspilia pluriseta* in the selected locations of study are as shown in Table 4.8.

Table 4. 8 Mycorrhiza fungal species and populations in the rhizosphere of *Aspilia pluriseta*, from soils in Gakurungu, Tunyai and Kanyuambora

Species identified	Species count in *Ap* rhizosphere soils			Species count in adjacent non *Ap* soils		
	d_1	d_2	d_3	d_1	d_2	d_3
Septoglomus funneliformis constrictum	1312	479	119	423	147	56
Aspergillus oryzae	4	0	0	16	8	3
Limacella glischra	0	6	0	0	0	0
Malassezia restricta	0	0	9	0	0	0
Archaeospora trappei	0	0	8	0	0	0
Glomus sp.	61282	34879	20750	343	259	160
Glomus indicum	430	374	11	12	9	1
Rhizophagus glomus proliferum	876	20	13	5	0	0
Penicillium herquei	0	0	25	4	6	33
Paraglomus laccatum	3411	65	49	121	20	9
Diversispora celata	1101	33	25	19	5	2
Septoglomus glomus viscosum	14408	3477	870	146	37	12
Geranomyces variabilis	0	0	36	0	0	0
Diversispora glomus eburneum	19	2	1	0	0	0
Aspergillus cervinus	20	1	0	2	1	0
Rhizoctonia solani	15	1	0	0	0	0
Crepidotus applanatus	57	2	1	7	7	0
Ambispora sp.	11	0	0	0	0	0
Pachyella clypeata	39	1	0	0	0	0
Conocybe apala	5	5	0	0	0	0
Hortaea werneckii	29	1	0	0	0	0
Ambispora gerdemannii	376	9	2	3	1	0
Cladosporium cladosporioides	0	0	4	0	0	0
Minutisphaera fimbriatispora	25	15	0	1	0	0
Rhizophlyctis rosea	442	7	4	10	0	0
Hydropus marginellus	3	1	0	3	1	0
Funneliformis mosseae	394	340	248	5	1	1
Rhizophagus intraradices	6139	2090	141	33	12	4
Ramularia mycosphaerella punctiformis	0	0	3	0	0	0
Paraglomus occultum	119	3	3	9	1	0
Diversispora aurantia	4	1	0	0	0	0
Archaeorhizomyces sp.	2	1	0	0	0	0
Rhizophagus glomus iranicum	50	48	0	3	0	0
Mortierella hyaline	4	3	0	0	0	0
Sclerocystis glomus sinuosum	1207	1027	167	7	3	2

Next-generation sequencing results from soil and rootlets samples, species count; d_1 (0-20 cm); d_2 (21-40 cm); d_3 (41-60 cm); *Ap-Aspilia pluriseta*

From the results of molecular work, 35 arbuscular mycorrhizal fungal species were recorded from the rhizosphere where *Aspilia pluriseta* vegetation grew. Species

composition and diversity varied along the rhizosphere with more abundance in depth 1 (0-20 cm). *Archaeospora trappei* mycorrhizal fungi dominated the lower rhizosphere zone (depth 3, 41-60 cm). *Glomus sp* was the most abundant in the rhizosphere of *Aspilia pluriseta* shrub.

4.3 Intensity of MF in the rhizosphere of *Aspilia pluriseta* across the main soil textural types

Table 4.9 shows the number of mycorrhizal spores in the sampling sites for different soil textural types. The results of the three tested soil textural types (silty clay, silt loam and sandy loam) showed that proliferation of mycorrhizal fungal spores was different among the soil types (Table 4.9). Sandy loam had the highest spore counts in 10g of soil. However, the spore counts in sandy loam soil did not vary significantly with those of silt loam soil at $p \leq 0.05$ but differed significantly with spore counts in silty clay soil.

Table 4. 9 Number of arbuscular mycorrhizal fungi spores in different sampling sites for soil textural types at Gakurungu, Tunyai and Kanyuambora

Sites	Sandy loam	Silt loam	Silty clay	LSD
Gakũrũngũ	394.0 [a]	299.0[a]	224.5[b]	153.2
Tunyai	377.3[a]	343.3[a]	265.0[b]	75.3
Kanyuambora	441.3[a]	348.3[a]	292.7[b]	103.9

Values followed by the same superscript letter within the row are not significantly different from each other at $p \leq 0.05$ (LSD test).

Figure 4.4 shows the number of mycorrhizal fungal spores in the tested sites in locations with *Aspilia* compared to those sites where *Aspilia* vegetation was not previously growing. The results show that mycorrhizal spores were more in soils with *Aspilia* compared to those without *Aspilia pluriseta* vegetation previously growing and that the difference was significant at $p \leq 0.05$ (Figure 4.4).

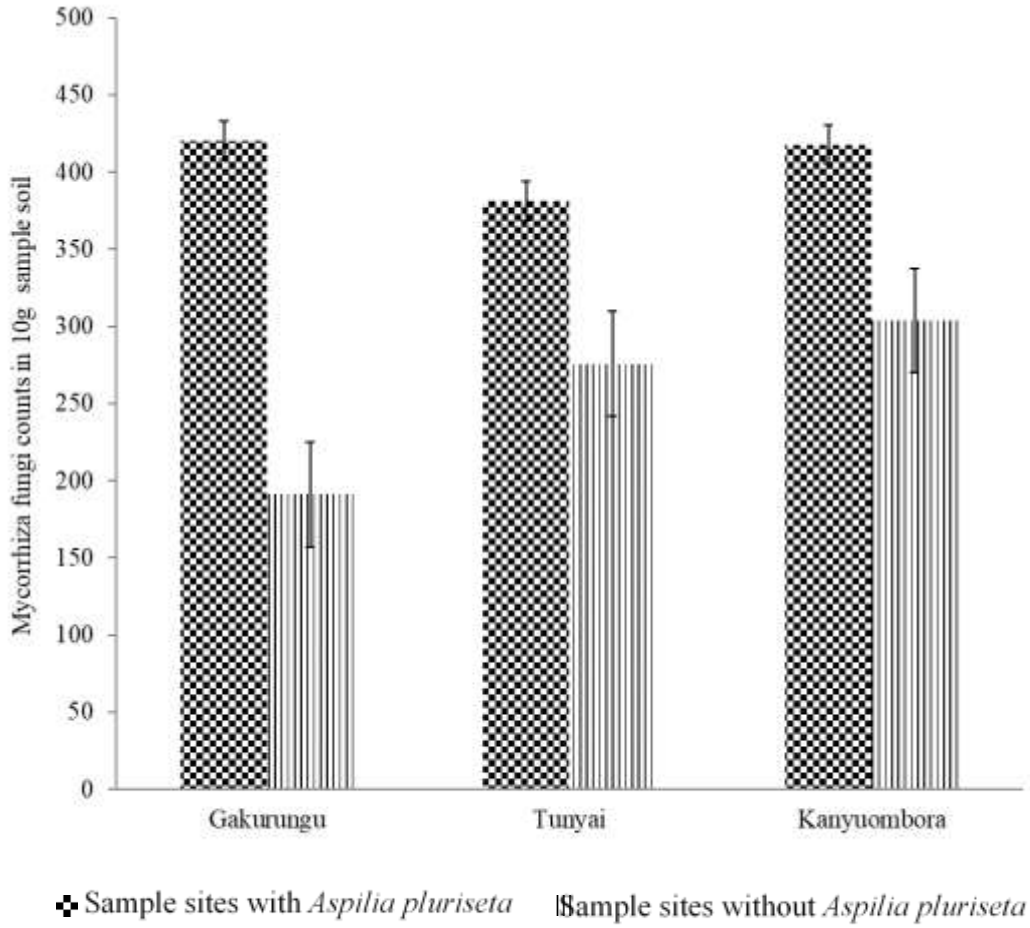

Figure 4.4 Arbuscular mycorrhiza fungi spore counts in Gakurungu, Tunyai and Kanyuambora soils with *Aspilia pluriseta* compared to those without. Letters represent LSD error bars

The number of mycorrhizal fungal spores in different rhizosphere depths of *Aspilia pluriseta* at Gakurungu, Tunyai and Kanyuambora is shown in Table 4.10.

Table 4.10 Number of mycorrhiza spores in different soil depths in Gakurungu, Tunyai and Kanyuambora

Site	Depth 1 (0-20 cm)	Depth 2 (21-40 cm)	Depth 3 (41-60 cm)	LSD
Gakũrũngũ	509.50[a]	253.33[b]	154.67[b]	153.2
Tunyai	508.00[a]	296.67[b]	181.00[c]	75.3
Kanyuombora	524.00[a]	338.67[b]	219.67[c]	103.9

Values followed by the same superscript letter within the row are not significantly different from each other at $p \leq 0.05$ (LSD test).

From the results, it was observed that the number of spores varied along the plant's rhizosphere with soil depth 1 (0-20 cm) having more spores per 10g of soil compared to depth 2 and 3 (21-40 cm and 41-60 cm respectively). Spore counts in soil depths at Tunyai and Kanyuombora varied significantly at $p \leq 0.05$ but spore count variation at Gakurungu site did not for soil depth 2 and 3 (Table 4.10).

Table 4.11 show the interaction between the various treatments for soil samples that were experimented. Results indicated that the treatment combination that gave the highest number of spore counts was sandy loam soil at depth 1 (0-20 cm) with *Aspilia* vegetation previously growing on the soil at 784±6.1 spore counts (Table 4.11)

Table 4.11 Interaction among treatments on soil samples taken at Gakurungu, Tunyai and Kanyuambora (n=3)

Soil textural type	Soil depth (cm)	Arbuscular mycorrhizal fungi spore counts	
		With *Aspilia pluriseta*	Without *Aspilia pluriseta*
Sandy loam	0-20 cm	784±6.1	592±3.6
Sandy loam	21-40 cm	448±4.4	336±4.4
Sandy loam	41-60 cm	304±2	184±3.6
Silt loam	0-20 cm	480±3.5	416±7.2
Silt loam	21-40 cm	368±4.4	384±3.6
Silt loam	41-60 cm	256±5.6	186±3.6
Silty clay	0-20 cm	592±4.4	280±3.6
Silty clay	21-40 cm	320±3.6	176±2.6
Silty clay	41-60 cm	208±2.6	180±4.4

4.4 Genetic diversity of fungi within the rhizosphere of *Aspilia pluriseta*

4.4.1 Physico-chemical information on soil rhizosphere depths in the study sites

The physico-chemical characteristics of rhizosphere soils for the three soil depths are as shown in Tables 4.12a, b and c.

Table 4.12a *Aspilia* rhizosphere soil depth$_1$ (0-20 cm) physico-chemical parameters in Gakurungu, Tunyai and Kanyuambora

Site	Soil textural type	Physico-chemical attributes							
		pH	MF Counts /10 g soil sample	$P_2O_{4\ (ppm)}$	SM %	N%	P (ppm)	OM%	Soil Temp (°C)
Gakurungu	Sandy loam	6.4	960	48.1	6.0	0.2	21	2	25.8
	Silt loam	5.9	720	57.3	6.9	0.0	25	3.7	25.5
	Silty clay	6	413	120.2	10.8	0.2	52.5	2.3	25.4
Tunyai	Sandy loam	6.2	688	41.2	8.6	0.2	18	3.0	25.7
	Silt loam	6.3	640	177.5	12.4	0.2	77.5	3.9	25.4
	Silty clay	6.7	448	65.1	13.2	0.1	20.5	5	25.3
Kanyuambora	Sandy loam	6.1	784	89.3	6.1	0.2	39	1.3	25.5
	Silt loam	5.7	480	81.3	8.1	0.2	35.5	2.2	25.3
	Silty clay	5.9	592	76.7	7.2	0.2	33.5	1.3	25.4
Average		6.1	636	84.1	8.8	0.2	35.8	2.7	25.5

Key: MF-Mycorrhizal fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus; OM-Soil organic matter

Table 4.12b *Aspilia* rhizosphere soil depth$_2$ (21-40 cm) physico-chemical parameters in Gakurungu, Tunyai and Kanyuambora

Site	Soil textural type	Physico-chemical attributes							
		pH	MF Counts /10 g soil sample	$P_2O_{4\ (ppm)}$	SM %	N%	P (ppm)	OM%	Soil Temp (°C)
Gakurungu	Sandy loam	6.7	376	25.2	6.2	0.2	11	1.8	25.6
	Silt loam	5.7	440	65.3	3.3	0.1	28.5	3.5	25.4
	Silty clay	5	224	25.2	9.9	0.1	11	2.1	25.3
Tunyai	Sandy loam	5.7	336	80.2	10.4	0.1	35	3.2	25.5
	Silt loam	5.8	368	64.1	12.9	0.1	28	3.7	25.3
	Silty clay	7.4	296	80.2	12.5	0.1	35	5.4	25.1
Kanyuambora	Sandy loam	6.1	448	61.8	9.4	0.2	27	2.1	25.4
	Silt loam	4.7	368	208.4	7.7	0.0	91	2.8	25.3
	Silty clay	5.8	320	47.0	9.8	0.1	20.5	1.4	25.3
Average		5.9	353	73	9.1	0.1	31.9	2.8	25.4

Key: MF-Mycorrhizal fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus; OM-Soil organic matter

Table 4.12c *Aspilia* rhizosphere soil depth$_3$ (41-60 cm) physico-chemical parameters in Gakurungu, Tunyai and Kanyuambora

Site	Soil textural type	Physico-chemical attributes							
		pH	MF Counts /10 g soil sample	$P_2O_{4\ (ppm)}$	SM %	N%	P (ppm)	OM%	Soil Temp ($^{\circ}$C)
Gakurungu	Sandy loam	6.6	208	53.8	6.5	0.2	23.5	1.9	25.5
	Silt loam	6	284	50.4	5.2	0.1	22	2.6	25.3
	Silty clay	5	160	246.8	7.2	0.1	10.2	1.7	25.1
Tunyai	Sandy loam	5.6	296	73.3	7.0	0.1	32	2.9	25.4
	Silt loam	5.9	200	95.04	15.8	0.0	41.5	4.8	25.2
	Silty clay	8	160	44.7	12.6	0.1	19.5	5.3	25.0
Kanyuambora	Sandy loam	6.6	304	90.5	7.2	0.0	39.5	1.3	25.3
	Silt loam	5.4	256	19.5	7.5	0.1	8.5	3.1	25.2
	Silty clay	4.8	208	81.3	8.1	0.1	35.5	1.9	25.1
Average		6	231	83.9	8.6	0.1	25.8	2.8	25.2

Key: MF-Mycorrhizal fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus; OM-Soil organic matter

From the results, it was observed that soil pH for the three studied depths was slightly acidic with a range from 5.9-6.1 (Table 4.12a, 4.12b & 4.12c). The middle depth (21-40 cm, Table 4.12b) appeared more acidic with a pH of 5.9 compared to 6.1 and 6.0 in the first and third soil depth respectively. There was more organic matter content in depth two at 9.1% compared to 8.8% and 8.6% for depth one and depth three respectively. Soil temperatures declined with increasing rhizosphere depth from 25.5°C in depth one to 25.4°C and 25.2°C in depth two and three respectively. Mycorrhizal fungi (MF) spore counts along the rhizosphere of *Aspilia pluriseta* plant had an inverse relationship to soil depth with the top soil, depth one having an average 636 spores (Table 4.12a) per 10g of the sample soil tested compared to 353 (Table 4.12b) in depth two and 231 spores (Table 4.12c) in depth three.

4.4.2 Fungal communities in the rhizosphere of *Aspilia pluriseta*

Figure 4.5 show the distribution of unique and shared operational taxonomic units (OTUs) of the samples taken at Gakurungu, Tunyai and Kanyuambora.

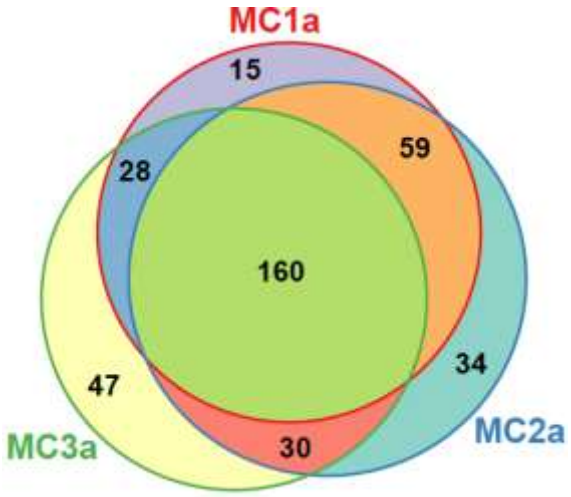

Figure 4.5 Distribution of unique and shared OTUs from soil samples taken at Gakurungu, Tunyai and Kanyuambora.

From the results of BLASTn using SILVA SSU Reference 119 database, 373 fungal operational taxonomic units (OTUs) were obtained, and all matched with accessions in SILVA database. Out of the 373 OTUs, 323 were of fungal origin and spread in 5

phyla; Glomeromycota, Basidiomycota, Chytridiomycota, Ascomycota and an unknown fungal phylum.

The observed results indicated that $MC2_a$ consisting of rhizosphere soil depth 21-40 cm had a higher overall number of OTUs (283) compared to MC1a (0-20 cm) and $MC3_a$ (41-60 cm) with 262 and 265 overall OTUs respectively. One hundred and sixty OTUs were shared among all sample types (Figure 4.6).

Figure 4.6 show the relative abundance of the predominant fungal genera in soil samples collected from the rhizosphere of *Aspilia pluriseta* in the study sites.

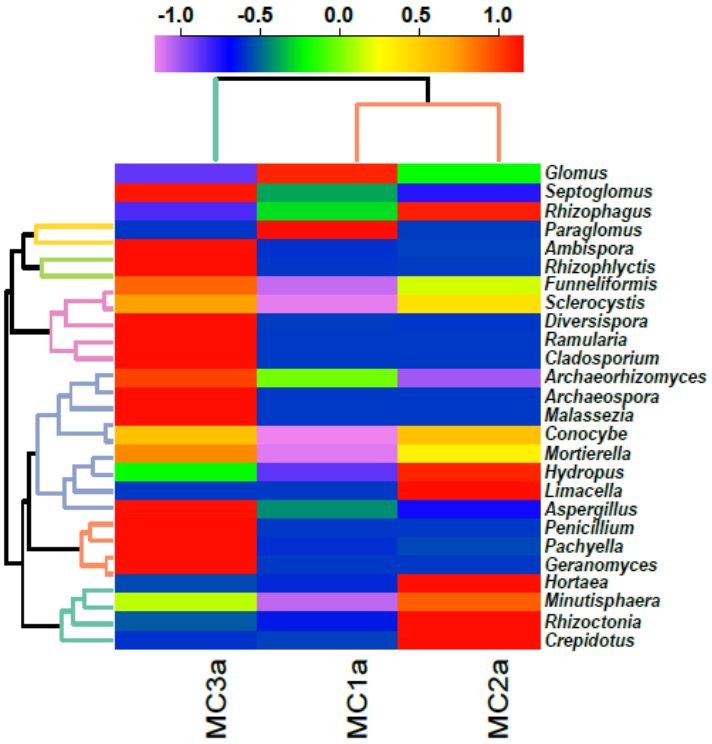

Figure 4.6 Heat map showing the levels of fungal genera in the three *Aspilia pluriseta* rhizosphere depths in Gakurungu, Tunyai and Kanyuambora

From the results, it was observed that fungal phylum Glomeromycota was more abundant in rhizosphere depth one (0-20 cm) with 232 OTUs compared to depth two (21-40 cm) and depth three (41-60 cm) which had 229 and 213 OTUs respectively. This phylum was represented by most genera as shown in Figure 4.6. The results also

depict the dominant species in the rhizosphere as *Glomus sp* and *Paraglomus laccatum.*

Figure 4.7 shows the relationship existing between the sample size sequenced and the operational taxonomic units in the tested soil samples.

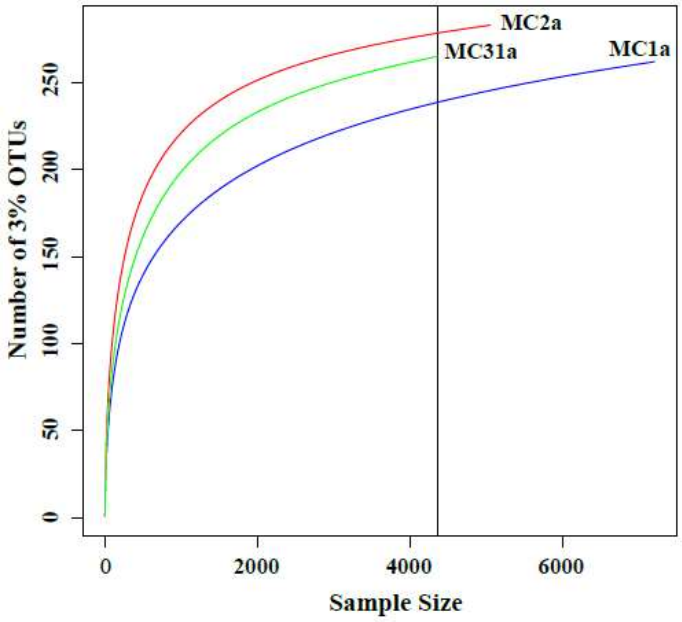

Figure 4.7 Relationships between the sample size sequenced and operational taxonomic units in the tested soil samples of Gakurungu, Tunyai and Kanyuambora

Results obtained from hierarchical clustering between samples collected from the rhizosphere of *Aspilia pluriseta* indicated that samples from the second and third studied soil levels (21-60 cm) to be closer than from the sample in the first soil level, 0-20 cm (Figure 4.7). From the results obtained, it was observable that it was easier to obtain OTUs from smaller sample sizes in soil depth 2 (MC2a) than in MC1a (soil depth 1) as the latter required larger soil samples to obtain equivalent number of OTUs (Figure 4.7).

4.4.3 Fungal richness and diversity indices

Table 4.13 gives a computation of diversity indices from OTU-based taxonomic units.

Table 4.13 Indices of diversity for OTU-based fungal taxonomic units from the rhizosphere of *Aspilia pluriseta* at Gakurungu, Tunyai and Kanyuambora

Sample	Filtered sequences	Number. of OTUs	Richness (S)	Evenness (J)	Shannon (H)	Inverse Simpson (I/D)
MC1a	72,093	283	42	0.0457	2.5	4.8
MC2a	50,539	262	58	0.0978	3.3	12.8
MC3a	43,596	265	62	0.0711	2.9	7.3
Totals	166,228	323	162			

Results obtained showed that the index by Richness (S) estimated the rhizosphere depth MC3a (41-60 cm) to be the richest site, constituting 62 taxa while MC2a (21-40 cm) and MC1a (0-20 cm) had 58 and 42 taxa respectively. Evenness (J') values ranged soil samples from the three soil depths from 0.0457 – 0.0978 (close to 0.1). Soil depth 21-40 cm had the best evenness of species (Table 4.13) and still maintained a higher Simpson (1/D) value at 12.8 compared to soil depth 41-60 cm and 0-20 cm that had 7.3 and 4.8 Simpson values respectively. The Shannon's index (H) value was higher in MC2a (21-40 cm) than in other soil depths, though the degree of variation between the sampled depths was low (H = 2.5–3.3).

Figure 4.8 show hierarchical clustering of DNA samples collected from the rhizosphere depth of *Aspilia pluriseta.* The results show connectivity of distance matrix with threshold dissimilarity of 1 indicating that data of the three samples were connected, hence there were no significant differences in the soil community structure in the samples at 95% level of confidence (p value≤0.05).

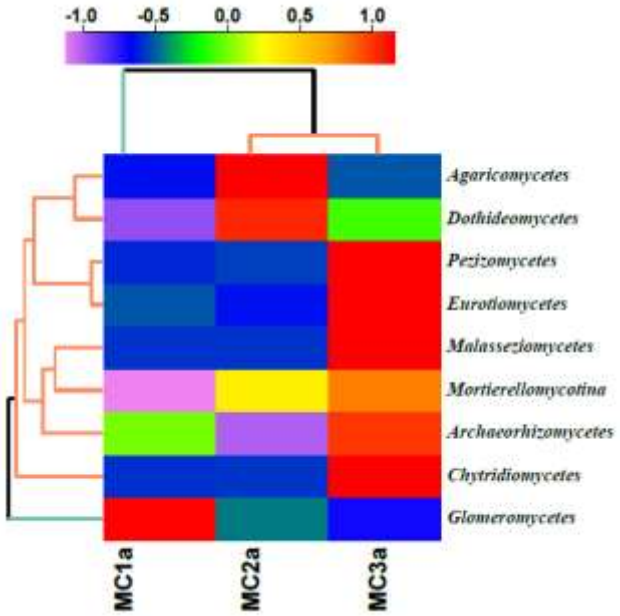

Figure 4.8 DNA hierarchical clustering from samples collected in the rhizosphere depth of Gakurungu, Tunyai and Kanyuambora soils

Table 4.14 shows the response of growth indices for gadam sorghum after six months of growing *Aspilia pluriseta* in pots of different soil textural types. No significant difference among the treatements was observed. The table also indicate that sandy loam textural soil type gave the highest sorghum yields at 15.8 g/1000 grains compared to those in other soil textural types (Table 4.14).

Table 4.14 Evaluation of growth indices of gadam sorghum planted six months after growing *Aspilia pluriseta* in pots of different soil textural types from Gakurungu, Tunyai and Kanyuambora

	Soil textural types			
Duration (days)	Silty Clay	Silt Loam	Sandy Loam	LSD
	Stand count (average No. of plants per pot)			
7	2a	2a	2a	0
14	1.8b	1.8ba	1.9a	0.1
21	1.6b	1.8ba	1.9a	0.2
28	1.6b	1.8ba	1.9a	0.2
35	1.5b	1.8a	1.9a	0.2
	Plant eight (cm)			
7	3.5b	4.1ba	4.4a	0.7
14	11.7b	13.7ba	14.8a	2.3
21	22.2b	26.1ba	28.7a	4.5
28	42.2b	49.7ba	54.6a	8.6
35	43.7b	51.5ba	56.6a	9
	No. of leaves			
7	1.8b	2a	1.9a	0.1
14	2.4b	2.6ba	2.7a	0.2
21	2.5a	2.6a	2.7a	0.3
28	3.4b	3.7ba	4.1a	0.4
35	3.6b	4.0ba	4.4a	0.5
	Leaf length (cm)			
7	2.6b	3.1ba	3.3a	0.6
14	7.0b	8.4ba	8.9a	1.6
21	16.9b	20.2ba	21.9a	4
28	32.1b	38.3ba	41.6a	7.5
35	33.0b	39.4ba	42.8a	7.8
Total yield (g/1000 grains)	12.2b	14.4ba	15.8a	1.5

Values followed by similar letters along the row are not significantly different at $p \leq 0.05$ (LSD test)

Observed results (Table 4.14) show that the initial stand count (plant population) of gadam sorghum in the first one week was not significant at $p \leq 0.05$ in all the textural soil types. However, in subsequent growth period, indices of all growth parameters showed differences at $p \leq 0.05$ between silty clay textural soil type on one hand and silt loam and sandy loam soils on the other. Sandy loam soils exhibited the highest growth values for all the tested growth parameters after the first week of growth. The results also indicate that sandy loam textural soil type gave the highest sorghum

yields at 15.8 g/1000 grains compared to those in other soil textural types (Table 4.14).

The response of growth indices for gadam sorghum after six months of growing *Aspilia pluriseta* compared to pots that had not been growing any vegetation is shown in Table 4.15. There was a signifincance difference with *Aspilia* and without *Aspilia* treatment.

Table 4.15 Comparison of growth indices of gadam sorghum planted 6 months in pots with *Aspilia pluriseta* and those without in the greenhouse at the University of Embu

Duration (days)	Location		t-test
	With *Aspilia*	Without *Aspilia*	
	Stand count		
7	2.0a	2.0a	0
14	1.9a	1.8a	0.1
21	1.8a	1.8a	0.1
28	1.8a	1.8a	0.1
35	1.8a	1.9a	0.2
	Plant height (cm)		
7	4.3a	3.7b	0.6
14	14.4a	12.5b	1.9
21	27.2a	23.8b	3.7
28	52.4a	45.2b	7
35	54.3a	46.8b	7.3
	No. of leaves		
7	2.0a	1.9b	0.1
14	2.6a	2.5a	0.1
21	2.7a	2.6a	0.2
28	3.8a	3.6a	0.3
35	4.2a	3.8b	0.4
	Leaf length (cm)		
7	3.3a	2.7b	0.5
14	8.8a	7.4b	1.3
21	21.3a	18.0b	3.2
28	1.8a	1.7a	0.2
35	41.5a	35.3a	6.3
Total yield (g/1000 grains)	15.2a	13.1b	2

Values followed by similar letters along the row are not significantly different at $p \leq 0.05$ (LSD test)

From the results, it was observed that gadam sorghum stand count means were not significantly different at $p \leq 0.05$ between sorghum crop grown in *Aspilia* pots and those that were not for the initial five weeks of growth. However, from the results, plant height differed significantly at the same confidence level in pots with *Aspilia pluriseta* soils compared to those that did not have this vegetation. The difference in the length of the leaf was significant between the two treatments in the first three weeks of crop' growth (Table 4.15).

Table 4.16 shows the response of growth indices for gadam sorghum after six months of growing *Aspilia pluriseta* in pots with soil from different rhizosphere depths.

Table 4.16 Comparison of growth indices of gadam sorghum on the effects of rhizosphere soil depths of *Aspilia pluriseta* after being planted 6 months in pots at the greenhouse, University of Embu

Duration (days)	Rhizosphere soil depth			
	0-20 cm	21-40 cm	41-60 cm	LSD
	Stand count			
7	2.0a	2.0a	2.0a	0.0
14	2.0a	1.9a	1.7b	0.1
21	2.0a	1.9a	1.5b	0.2
28	2.0a	1.9a	1.5b	0.2
35	2.0a	1.8b	1.4c	0.2
	Plant height (cm)			
7	6.4a	3.5b	2.1c	0.7
14	21.4a	11.8b	7.0c	2.3
21	41.3a	23.1b	12.7c	4.5
28	78.5a	43.8b	24.1c	0.6
35	81.3a	45.4b	25.0c	9.0
	No. of leaves			
7	2.1a	1.8b	1.8b	0.1
14	3.0a	2.6ba	2.1c	0.2
21	3.5a	2.6b	1.8c	0.3
28	4.8a	3.6b	2.7c	0.4
35	5.4a	3.9b	2.8c	0.5
	Leaf length (cm)			
7	4.9a	2.7b	1.4c	0.6
14	13.1a	7.3b	3.9c	1.6
21	32.3a	17.9b	8.8c	4.0
28	61.4a	33.7b	16.7c	7.5
35	63.3a	34.8b	17.2c	7.8
Total yield (g/1000 grains)	22.8a	12.7b	7.0c	2.5

Values followed by similar letters along the row are not significantly different at $p \leq 0.05$ (LSD test)

From the results, it was observed that gadam sorghum yield (g/1000 grains) was inversely proportional to soil depth with the yield of soil depth 0-20 cm being significantly higher at $p \leq 0.05$ than the yield from depth 20-40 cm and 41-60 cm (Table 4.16). The results also indicate that all the growth indices differed

significantly at p≤0.05 in most of the weeks with the 0-20 cm depth displaying the highest values followed by 20-40 cm and lastly by 41-60 cm depth.

Correlation of various test factors to the yield of gadam sorghum crop is shown in Table 4.17.

Table 4.17 Correlation coefficient values on test factors to the yield of gadam sorghum at the greenhouse, University of Embu

Test factor	p-value	Correlation value
Site[1]	0.0064	0.23
Inoculation[2]	0.0064	0.23
Block	0.1614	0.12
Soil	0.0173	0.12
Depth	<.0001	-0.35
Stand count	<.0001	0.39
Plant height	<.0001	0.46
No. of Leaves	<.0001	0.42
Length of Leaves	<.0001	0.33

[1]Whether or not *Aspilia pluriseta* had grown in the soils [2]whether or not gadam sorghum seeds were inoculated with Arbuscular mycorrhiza fungi (AMF). N=144, Prob > |r| under H0: Rho=0

Observed results show that except for the soil depth that had an inverse relationship to yield, all the other growth parameters measured had a positive correlation to yield (Table 4.17). From the results, blocking was not significant to the yield of gadam sorghum at p≤0.05. The plant height and the number of leaves gadam crop plant acquired during growth contributed strongly to the yield of the plant at indices +0.46 and +0.42 respectively (Table 4.17). Results also show that the correlation between the number of leaves per plant and the length of leaves to total yield was highly significant at p≤0.05.

CHAPTER FIVE

DISCUSSIONS

5.1 Introduction

This chapter discusses the results given in chapter four.

5.2 Co-association of mycorrhiza and *Aspilia pluriseta* on growth and phosphorus availability to gadam sorghum crop

5.2.1 Soil textural types on gadam sorghum seedling emergence and stand count

Seed germination and eventual crop stand is a process that influence crop yield and quality (Sohindji *et al.*, 2020). Gadam sorghum germination and subsequent plant survival was significantly affected by the soil textural type in which the plant was grown. Past research noted the importance of soil textural type to seed germination and good seedling establishment (Valdés-Rodríguez *et al.*, 2013) for bigger seeds like the *Jatropha curcas*. However, little is documented on germination and seedling establishment for small grains in relation to soil textural types. Results obtained in this study differ from the principal findings by Danso *et al*. (2011) and Díaz-Chuquizuta *et al.* (2017) that germination and survival of plant seeds was best done using sandy soil. In this study, silt loam soils had the best seed emergence percentage at 96.9% compared to 90.2% and 83.3% for sandy loam and silty clay respectively (Table 4.1). This difference was significant at $p \leq 0.05$.

Whereas past research on germination reported on bigger seed types, this research gives credence to silt loam soil as being more superior for smaller grain germination. The aim of this current study was to determine the effect of three soil textural types on seed germination rates and seedling survival in *Aspilia pluriseta* growing areas. The lowest sorghum seedling emergence percentage was obtained in silty clay soil (Table 4.1). On the overall, the more coarse soil particles provided better aeration to the seeds, which may have favored higher germination rates in the silt loam and sandy loam as was reported by Valdés-Rodríguez *et al*. (2013).

In this research, results indicated that water retention capacity of the different soil textural types affected stand count with time. The smaller the particles were, the more the water holding capacity and with time, seeds that germinated dried up due to low oxygen levels. Sladonja *et al*. (2014) obtained similar results on pyrethrum seedling emergence tested for different soil types while Idu *et al.* (2003) observed that germination and emergence of *Helianthus annuus* L. were low in clay treatments compared to other soil treatments with bigger particle size. Anderson *et al*. (2004) varied rainfall, sowing time, soil type, and cultivar influence on plant population for wheat in Western Australia and obtained similar results.

5.2.2 *Aspilia pluriseta* effect on gadam sorghum seeds germination and early seedlings growth

Early growth of gadam sorghum plant was influenced by the use of soils that had *Aspilia pluriseta* initially growing in them. A number of mechanisms are at play and explain how species coexist in a given community structure and hence exhibit negative intraspecific species interactions giving rise to competition (Chesson, 2000; Hubbell, 2006 and Bever *et al*., 2010). To coexist, species trade-off or enhance antagonist trade-offs and avoidance. Varga, (2015) noted that mycorrhiza fungi negatively influenced seed germination but at the same time improved plant growth. This partly explained the behavior of sorghum seedlings stand count stability in pots that had soil from *Aspilia pluriseta* growing sites compared to seedlings in soils that had not been growing *Aspilia pluriseta*. Arbuscular mycorrhizal fungi (AMF), in the recent past, has received widespread attention in restructuring plant communities (Hart *et al.*, 2003; Urcelay & Díaz, 2003; Klironomos *et al*., 2011).

Previous studies show AMF to influence interspecific plant competition (Danieli-Silva *et al*., 2010; Wagg *et al.*, 2011a; Mariotte *et al.*, 2013) and one way that has been mentioned by many scholars is the ability of AMF to control interspecific competition due to net benefit that AMF provide to many different plant species (O'Connor *et al*., 2002; Urcelay & Díaz, 2003). The variation in mycorrhizal response lead to the common concept of 'mutualism–parasitism continuum', occurring in obligate and facultative mycorrhizal plants (Johnson *et al*., 1997). Hoeksema *et al*. (2010) corroborated this continuum through a meta-analysis in

which he found that AMF inoculation of plant species differed for each plant functional group. Hetrick *et al.* (1990) explained this phenomenom by arguing that there exists differences in plant traits among different plant functional groups therefore exhibiting different mycorrhizal responses.

Plant characteristics can affect the manner in which plant species rely on AMF for nutrient acquisition from the soil (Brundrett, 2002). When C4 grasses are compared to C3 plants, the latter often have more fibrous (i.e. thinner), highly branched root systems with a lower dependency on AMF for nutrient uptake (Hetrick *et al.*, 1990; Wilson & Hartnett, 1998) and hence make C3 plant species poorer in response to AMF inoculation. However, the thicker roots found in C4 grasses enable the plants develop stronger interdependence on AMF thereby exploring soil and acquiring more nutrients. In the event of AMF colonization suppression, C4 grasses compete less effectively with C3 grasses (Hartnett *et al.*, 1993; Hetrick *et al*., 1994). In addition, with the high phosphorus (P) requirements for nitrogen (N) fixation in N-fixing plants, these plants naturally have a high AMF dependency for absorbing P and hence arbuscular mycorrhizal fungi may be expected to increase the competitive ability of N-fixing plants (Wagg *et al.*, 2011a).

Interspecific competition between plant species may be affected by the plants' mycorrhizal responses and this has been suggested as a reason that may influence plant diversity and community structure (Vogelsang *et al.,* 2006). In his conceptual model, Urcelay & Díaz (2003) suggested that the AMF effects on plant diversity could be given by the relative mycorrhizal response of dominant vs. subordinate plants. When the mycorrhizal response of dominants is high, he argued that AMF at this point could enhance competitive ability of dominants, thereby causing a decrease in plant diversity (Hartnett & Wilson, 1999). Vogelsang *et al*. (2006) explained changes in plant diversity by manipulating AMF inoculation and P conditions. In addition, van Der Heijden *et al.* (1998) argued that if the subordinates are highly mycotrophic, AMF inoculation may increase plant diversity by enhancing competitive ability of subordinates. Many other factors studied could influence AMF effects on plant interspecific competition and community structure, among them being abiotic soil conditions (P availability) and AMF species identity and diversity

(Collins *et al*., 2009; Wagg *et al.*, 2011b). AMF has been found to promote plant biodiversity and productivity but this effect declined with increasing soil P availability (Birhane *et al*., 2014). Furthermore, according to Maherali & Klironomos (2007), differences between species of AMF lead to distinct colonization strategies and functional roles in nutrient uptake and pathogen protection. Indeed, past studies have shown that AMF species diversity and identity can influence AMF effects on plant community structure and interspecific competition (Vogelsang *et al*., 2006; Wagg *et al.*, 2011b). However, to fully understand the magnitude and direction of AMF effects on plant community structure and interspecific competition, systematic tests examining every factor is required and this was outside the scope of this current study.

5.2.3 Effect of gadam sorghum seeds inoculation with mycorrhizal fungi on seedling emergence, stand count and plant height

Inoculation with mycorrhizal fungi improved seedling emergence, stand count and plant height in this experiment. Results obtained closely mirrored the observation made by Caravaca *et al.* (2002) that rhizosphere aggregate stability of afforested semiarid plant species was significantly improved upon mycorrhiza fungi inoculation. Not only does mycorrhizal association with plants improve drought tolerance (Fitter, 1988) but also seedling survival (Janos, 1980).

Gadam sorghum is a short stature crop (Chimoita *et al.*, 2019) and this is confirmed in this experiment showing the plant as having an average height of 124 centimetres at physiological maturity. Having a small height is one of the drought escaping mechanism of plants in dry land areas as assimilate partitioning is done early during the plant's life (Basu *et al.*, 2016). It is in scholarly domain that nutrient acquisition of crops is mainly propelled by soil and root microorganisms (Reid & Greene, 2013) that initiate a key role in nutrient circulation and absorption and in fighting against soil pathogens (Ismail & Hijri, 2012; Ismail *et al.*, 2013). Agricultural ecosystems depend on beneficial soil micro-organisms (Janzen *et al*., 2011; Reid & Greene, 2013; Rodriguez & Sanders, 2015) and have been used in agriculture more frequently over the past few decades. Nevertheless, the cultivation of the target microorganism is not a simple task, given the large number of microbes, their functional diversity, and the complexity of microbial assemblages. This experiment

confirms that AMF in the rhizosphere of *Aspilia pluriseta* establish close symbiosis with sorghum plants just like past research by Redecker *et al.* (2000) established. Adaptability of host plants is improved tremendously when arbuscular mycorrhizal fungi (AMF) colonizes plant roots and in addition offers supplemental phosphorus (Juniper & Abbott, 1993; Watson *et al.*, 2001), nitrogen (N), and zinc (Smith & Read, 2008) to plants. According to Smith & Read (2008), nutrient uptake was increased by more than 100-fold after root systems were extended, increasing the root surface as a result of symbiosis with AMF. This way, the plants under the influence of AMF were able to attain more biomass and consequently, enhanced yield.

A large category of agricultural crops such as cotton, soybean, onion, pulses, rice, tomato, potato, corn and wheat can form symbiotic relationships with AMF (Gao *et al*., 2020). This current study confirms that *sorghum bicolor*, L. forms similar symbiotic relationship with mycorrhiza fungi. The influence level of AMF on crop yield and economics of farming is still uncertain, in some crops (Rodriguez & Sanders, 2015). However, AMF have been determined to increase total crop yield in potatoes by 9.5% (Hijri, 2016). In addition, AMF may function throughout the entire growth period (McGee *et al.,* 1999) and affect whole-plant physiological responses (Berta *et al.*, 2014; Bona *et al.*, 2015; Bona *et al.*, 2017). This study, therefore, affirmed the efficacy of mycorrhiza fungi in forming a symbiotic relationship to sorghum leading to improved plant biomass and yield.

5.2.4 Changes in pH, phosphorus and phosphate levels before and after gadam sorghum harvest

There is a significant reduction of soil pH at $p \leq 0.05$ for soils with *Aspilia pluriseta* compared to those without that is clearly discernable (Table 4.5). In this case, the strategy that rhizosphere arbuscular mycorrhiza fungi in *Aspilia pluriseta* is exploiting in solubilizing P in the soil is by lowering the soil pH through microbial production of organic acids (Khan *et al.*, 2006; Kumar *et al.*, 2018; Kalayu, 2019). phosphate can precipitate to form calcium phosphates, including rock phosphate (fluorapatite and francolite) in alkaline soils and these precipitations are insoluble soil solutions (Kalayu, 2019). Solubility of these precipitations increase with

decrease in soil pH. Mycorrhiza and other soil microorganisms are instrumental in increasing P availability after lowering the soil pH through production of organic acids (Satyaprakash *et al.*, 2017).

Past studies have posted strong positive correlation between solubilization index and organic acids produced (Alam *et al.*, 2002). Phosphorus solubilizing micro-organisms create acidity through evolution of CO_2 (Yousefi *et al.*, 2011) as observed in solubilization of calcium phosphates (Buddhi & Yoon, 2012). P solubilization was brought about by production of organic acid as well as decrease of the pH through the action of microorganisms (Selvi *et al.*, 2017). The divalent and trivalent forms of inorganic P, $HPO4^{-2}$ and $HPO4^{-3}$ increases in the soil as the soil pH increases. Different organisms contribute differently to the type and amount of organic acid produced. Solubilization efficiency is dependent on the strength and nature of acids. Tri- and dicarboxylic acids as compared to monobasic and aromatic acids more effectively do phosphate solubilization. Aliphatic acids are also more effective compared to phenolic, citric, and fumaric acids (Buddhi & Yoon, 2012; Kalayu, 2019). Phosphates solubilization is effectively done by organic acids such as gluconic, glutaric tartaric, propionic succinic, oxalic, malonic, butyric citric, glyoxalic lactic, 2-ketogluconic, adipic, glyconic, fumaric, acetic and malic acid (Yousefi *et al.*, 2011; Ahmed & Shahab, 2012; Satyaprakash *et al*., 2017; Selvi *et al.*, 2017; Kumar et al., 2018). Gluconic acid and 2-ketogluconic acids appear to be the most frequent agent of mineral phosphate solubilization (Rodríguez & Fraga, 1999; Buddhi & Yoon, 2012; Satyaprakash *et al.*, 2017).

Inorganic and Organic acids produced by phosphate solubilizing micro-organisms, of which mycorrhiza is part, chelate cations by dissolving the insoluble soil phosphates thus compete with phosphate for adsorption sites in the soil (Pradhan & Sukla, 2006; Khan *et al*., 2009). Chelation of the cations bound to phosphate by the carboxyl and hydroxyl groups of the acids converts it into soluble forms. However, this study model could not prove that this second method of phosphate solubilization happened. Sorghum plants used as the follower crop utilized mycorrhiza spores to their advantage and this explains why phosphate levels tended to increase after harvest as phosphorus levels reduced. The study by Kavanová *et al*. (2006) revealed

that phosphorus was critical for cell division and elongation on the grass plants at the early stages of growth. This phenomenon could have contributed towards greater plant height for seeds that were inoculated with mycorrhizal fungi therefore enhancing rapid utilization of phosphorus at the root zone. This current experiment further corroborates research by Bhuiyani *et al*. (2008) that Phosphorus inoculation was found to be positive and significant on mungbean plant height. Besides, Bam *et al*. (2006) showed that germination and vigor in rice improved significantly when seeds were soaked in potassium and phosphorus salts.

Soil that had *Aspilia pluriseta* previously growing, had mycorrhiza that acted as a source of inoculation and therefore continued with phosphates synthesis with gadam sorghum as the host plant. These results therefore agree with Tiamtanong *et al*. (2015) that mycorrhiza fungi inoculation enhances the development of soil phosphates by increasing phosphates enzyme activity. The phosphorus in the soil was converted to phosphates through the action of mycorrhiza. According to Courty *et al.* (2010) and Burke *et al*. (2014), mycorrhizal fungi can increase P availability by secreting phosphatases into soil to degrade organic P and also excrete organic acids that mobilize P from mineral complexes. The connection between mycorrhizal phosphatase production and availability of P has not been cultured yet (Olsson *et al.*, 2002; Nygren & Rosling, 2009), but non-mycorrhizal fungi are known to increase phosphatase activity under P starvation (Kaffman *et al.*, 1994; Aarle *et al.*, 2001). Since availability of P is linked to soil pH, change in soil P may be inherently linked to changes in soil acidity in response to mycorrhizal fungi.

Past research show soil pH as a primary factor affecting diversity, biomass and fungal and bacterial community structure in the soil in a variety of ecosystems (Bååth & Anderson, 2003; Fierer & Jackson, 2006; Lauber *et al.*, 2009; DeForest & Scott, 2010; Rousk *et al.*, 2010). Change in soil pH has been shown to influence mycorrhizal fungal species richness (Lauber *et al.*, 2009), colonization of the root (Cairney & Meharg, 1999; Coughlan *et al.*, 2000; Aarle *et al.*, 2002), biomass (Aarle *et al.*, 2002) and community structure (Lehto, 1994; Wallander *et al.*, 1997; Erland & Taylor, 2002; Kjøller & Clemmensen, 2009; Rineau & Garbaye, 2009; Dumbrell *et al.*, 2010). It can be argued that soil acidification alters mycorrhizal fungal

community composition both directly and indirectly, particularly through its effect on P availability. According to Lauber *et al*. (2009), soils with low pH may harbor different mycorrhizal fungal species as compared to high-pH soils mainly because those species capable of maintaining growth and cellular function in acidic environments would survive over those adapted to more basic conditions.

5.3 Intensity of MF in the rhizosphere of *Aspilia pluriseta* across the main soil textural types

5.3.1 Soil textural type on the intensity of mycorrhizal fungi along the rhizosphere of *Aspilia pluriseta*

In all the soil textural types, mycorrhiza spores were recorded, albeit in differing numbers, confirming ubiquitousness of mycorrhiza fungi microflora (Smith & Read, 2008). Soil textural types played a significant role in determining the number of mycorrhiza spores in the rhizosphere. Other scholars like Davison *et al*. (2012), Hazard *et al.* (2013), Jansa *et al*. (2014) and Oehl *et al.* (2017), corroborate this finding on the influence of soil textural type on mycorrhizal populations. The difference in spore counts in sandy loam and silt loam soils were not statistically significant but intensity levels in silty clay soils were significantly lower at $p \leq 0.05$ compared with the other type of soils (Table 4.9). Past research asserts that this variance between soil textural types influencing arbuscular mycorrhiza fungi (AMF) communities is still a subject of study. Results of this study show sandy loam as ideal for mycorrhizal fungi proliferation owing to its bigger particle size (compared to other textural soils tested) thus better aeration. The physico-chemical factors as tested in this study are conducive and give the right micro climate for fungal proliferation. However, further studies need to be conducted to ascertain whether pure sandy soils would give better fungal population results or it was the loamy part in the sand that enhanced the observed enhanced population.

Soils inhabited by *Aspilia pluriseta* in all the three sites showed a higher number of morphotypes compared to those without this plant species (Figure 4.4). Plant, soil and climatic factors determine the development of mycorrhiza fungi and influence

establishment of the mycorrhizal symbiosis and its efficiency (Carrenho *et al*., 2007; Hindumathi & Reddy, 2011; Calvet *et al*., 2013). The ability to produce mycorrhizal propagules (spores, hyphae and colonized roots) is enhanced through the inoculation of plants with AMF. This is an important part of the process of soil microbiota recuperation (Calvet *et al.*, 2004; Gartner *et al.*, 2012). Although different factors were considered in determining AMF spore production, different plant species were tested in different locations, giving different results from this current experiment.

5.3.2 *Aspilia pluriseta* rhizosphere depth on the intensity of mycorrhizal fungi

This research demonstrated that mycorrhiza spore count in the rhizosphere of *Aspilia pluriseta* decreased with increase in soil depth (Table 4.10). The intensity of mycorrhiza spores varied significantly with soil depth from a high of 524 spores at Kanyuombora to 154.7 at Gakurungu at depths 0-20 cm and 41-60 cm respectively (Table 4.10). This inverse relationship on mycorrhiza fungi population and soil depth was exhibited in all the sites tested (Table 4.10). On comparison of the three sites, AMF population was highest in Kanyuambora and the lowest in Gakurungu (Figure 4.4). It is not rather obvious for mycorrhiza population to decrease with an increase in soil depth as suggested by Mejstrik (1972), Rillig & Field (2003) and Asghari *et al.* (2005) because studies by Gucwa-Przepióra *et al.* (2007) obtained different results. Gucwa-Przepióra *et al*. (2007) found out that there was an increase in arbuscular mycorrhiza colonization with increasing soil depth because of chemophytostabilization amendments.

An increase in arbuscular mycorrhiza (AM) colonization with increasing soil depth was observed in soils with spontaneously growing *Deschampsia cespitosa*. Similar work by Mejstrik (1972) but using different plant species indicated that the frequency and intensity of vesicular-arbuscular mycorrhiza in *Molinietum coeruleae* was soil depth dependent and that the higher the depth, the less the intensity of AMF. Rillig & Field (2003) in their research report indicated that arbuscular mycorrhiza responds to plants exposed to elevated atmospheric carbon dioxide and that this gas is a function of soil depth. In addition, Asghari *et al.* (2005) while on the study of growth response of *Atriplex nummularia* to inoculation with arbuscular mycorrhizal

fungi at different salinity levels showed higher infectivity rate with decreased salinity levels which in itself, was negatively correlated to soil depth. Curiosity of results from these scholars engineered this study on depth aspects for *Aspilia* plant. Indeed this study agrees with similar studies by Shukla *et al*. (2013), on soil depth as an overriding factor for AMF. In other studies, although not related to *Aspilia pluriseta*, mycorrhiza fungi populations have been shown to decrease with an increase in soil depth (Jakobsen & Erik, 1983; Oehl *et al.*, 2005; Cuenca & Lovera, 2010). Some researchers suggested that it might be due to the less organic content (Oehl *et al.*, 2005) and low availability of oxygen in deeper soil zones (Varma *et al.*, 2012), because fungi are sensitive to low oxygen pressure which prevails at lower depths (Ray & Brady, 1996). Studies by Rodríguez-Caballero *et al.* (2017) concluded that soil pH and levels of two micronutrients (Mn and Zn) both variants being factors of soil depth in the same continuum, play significant roles in triggering arbuscular mycorrhizal fungi (AMF) populations. According to Anderson *et al.* (2008) and Bever *et al.* (2010), AMF are generally scarce where the plant roots are sparse. Besides this phenomena, Brundrett, (2017) concludes that strong mycorrhizal plants will always have more rhizosphere spore counts compared to non-mycorrhizing plants. Based on these earlier findings, *Aspilia pluriseta* shrub satisfies the criteria of a strong mycorrhizal plant.

Molecular work identified 35 different mycorrhizal fungal species in the rhizosphere where *Aspilia pluriseta* vegetation grew compared to adjacent soils without this plant species (Table 4.7). Species composition and diversity varied along the rhizosphere with more abundance in depth 1 (0-20 cm). Mycorrhizal fungi species in the rhizosphere of *Aspilia pluriseta* shrub was important to this experiment as both their abundance and diversity would enhance the shrub's potent in soil phosphate uptake by follower crops. Studies by Radhika & Rodrigues (2010) and Torrecillas et al. (2012) concluded that relative diversity of rhizosphere mycorrhiza fungi depended on host plant species. Although these case studies mentioned did not evaluate *Aspilia pluriseta* shrub, it is evidently clear that this shrub is host to various mycorrhizal fungi of importance to ecological reconstruction.

5.4 Genetic diversity of fungi within the rhizosphere of *Aspilia pluriseta*

5.4.1 Physico-chemical information on soil rhizosphere depths in the study sdtehr6esites

Plant productivity is mainly limited by shortage of minerals in the soil whose continuous regeneration and transformation is a factor of soil microbial community (Rousk *et al*., 2009). Soil pH strongly influences fungal biomass composition (Fierer & Jackson, 2006). In this experiment, moderately acidic and sandy loam textured soils tended to favour proliferation of rhizosphere fungal growth (Table 4.12a, b & c). The level of soil organic matter was higher in the second rhizosphere layer (21-40 cm) as well as the soil moisture and this correspondingly increased the number of operational taxonomic units (OTUs).

As exhibited in the physico-chemical tables,(Tables 4.12a, b & c) an intricate balance between the physico-chemical factors contributed to fungal microbe population agreeing with the principal findings of Bhattarai (2015) that soil has diverse elements that contribute to its productivity and the proper balance between those elements is what actually matters. Despite the role played by organic matter in soil ecosystems (Lejon *et al.*, 2007), scanty information is available on the effect of soil organic matter, particularly on arbuscular mycorrhizal fungi. But it is an established fact that growth of arbuscular mycorrhizal fungi can be increased (John *et al.*, 1983; Joner & Jakobsen, 1995; Gryndler *et al.*, 2002; Albertsen *et al.*, 2006; Gryndler *et al*., 2006) and decreased (Avio & Giovannetti, 1988; Ravnskov *et al.*, 1999; Ravnskov *et al*., 2006; Gryndler *et al*., 2009) by soil organic amendments.

wfPlant materials release a lot of cellulose in the form of organic matter that enters soil from decaying. Pure cellulose can increase root colonization and external mycelial growth of arbuscular mycorrhizal fungi but only after it has been sufficiently decomposed (Gryndler *et al.*, 2002). The argument by Avio & Giovannetti, (1988), Ravnskov *et al.* (1999) and Gryndler *et al.* (2002) indicate that when cellulose is used fresh or composted for shorter periods, it can inhibit mycorrhizal symbiosis. However, in general, studies on the influence of organic matter on fungi have provided inconsistent results indicating variable effects of

different organic substrates on mycorrhizal symbiosis. Like in all mentioned studies, the current study treated decomposition of the organic amendments as being unique with specific intention of finding out the genetic variability of fungal spores along the rhizosphere of *Aspilia pluriseta* and whether or not differences in organic levels of the soil colloids in the rhizosphere caused significant differences in fungal species. As the results show, organic matter was highest in MC2a (depth zone 21-40 cm, Table 4.12a, b & c) and the same rhizosphere zone had more operational taxonomic units (Figure 4.5) confirming findings by Gryndler *et al*. (2002).

Soil microbial community is strongly influenced by soil pH as it affects abiotic factors, such as carbon availability (Kemmitt *et al.,* 2006), availability of nutrients (Kemmitt *et al.*, 2005; Kemmitt *et al.,* 2006; Aciego & Brookes, 2008), and the solubility of metals (Firestone *et al.*, 1983; Wood, 1995). Additionally, biotic factors may affect soil pH thus influencing biomass composition of fungi and bacteria (Fierer & Jackson, 2006), in both agricultural (Kohler *et al.*, 2005) and forest soils (Bååth & Anderson, 2003). A salient issue in the study of soil pH is its multiple effect on many factors. Manipulating the pH of a soil through experiments can result to changes in several other factors that are hard to separate (Bhattarai, 2015). Conversely, comparing pHs of different natural soils introduces confounding factors, frequently unidentifiable, derived from differences in soil textural type and management regimen that also vary between soils. The aim of this experiment was to assess the influence the soil pH had on fungal populations along the rhizosphere of *Aspilia pluriseta*. Moderate acidity gave better fungal population growth and diversity compared to low and high soil acidity levels.

Temperature and moisture regimes in this study also affected populations and diversity of fungal growth along the rhizosphere. While fungal growth and symbiotic functioning is affected by these factors (Grey, 1991; Augé, 2004; Heinemeyer & Fitter, 2004; Mayra *et al*., 2005), fungal survival is also influenced by similar factors. Carbon supply to the fungus can be interrupted on occasions when plants suffer setbacks on growth due to low temperature and (or) drought. This indirectly affects fungus survival as well. However, temperature and moisture may also directly affect fungus survival. For instance, low and freezing temperatures may increase fungal

mortality (Addy *et al.*, 1997; Klironomos *et al.*, 2001) and it is estimated that one-third of all metabolically active hyphae may die during the wet cold weather (Kabir *et al.*, 1997). Studies have demonstrated that viability of hyphae may remain high for long periods under dry conditions (Tommerup & Abbott, 1981; Brundrett *et al.*, 1996; Pattinson & McGee, 1997), but decline drastically when the soil is wetted periodically (Braunberger *et al.*, 1996; Pattinson & McGee, 1997). From these studies, it is obvious that temperature and moisture influence fungal population and diversity and therefore has a direct effect on fungal natural selection that could lead to adaptations to local climatic conditions and fungal survival. Differences in tolerance between fungal species may generate seasonal patterns of fungal community compositions (Klironomos *et al.*, 2001), but nothing is virtually known about potential differences within fungal species from disparate environments (Fitter *et al.*, 2004). Thus, by carrying out this study in the driest month of September, was a way of abridging it to previous studies, on soil temperature and moisture when fungal populations were expected to be at their best. However, data from the localized rhizosphere soil differences gave compelling evidence that indeed changes in soil moisture and temperature affected fungal populations and diversity.

5.4.2 Fungal communities in the rhizosphere of *Aspilia pluriseta*

Illumina's sequencing high sensitivity enabled detection of species that were rare, thus providing detailed fungal diversity information on the rhizosphere of *Aspilia pluriseta* plant (Knief, 2014). The phylum, *Glomeromycota* was most frequently identified in the plant's rhizosphere compared to *Ascomycota* and *Basidiomycota. Chytridiomycota* were least in the scale among fungi communities in the plant's rhizosphere and were represented on a smaller proportion of the rhizosphere fungal communities. The presence of unidentified fungal phylum indicate that new and potentially useful fungal communities do exist.

Results from most rhizosphere mycological research findings indicate heavy presence of *Ascomycota* and *Basidiomycota* phyla (Zimudzi *et al*., 2018; Jie *et al*., 2019; Floc *et al.*, 2020) from cultivated crops. However, from this research study, there is a clear departure on the hierarchical fungal composition of the wild semi-arid shrub (*Aspilia pluriseta*) that could prove beneficial to follower-cultivated crops. Past studies indicate that rhizosphere microorganisms mainly comprise of actinomycetes,

bacteria and fungi (Jie *et al*., 2019). This composition of microbial community in the rhizosphere is influenced by plant species and soil types (Berg & Smalla, 2009). In soybean for example, studies by Sugiyama *et a*l. (2014) indicated that microbial communities composition in the rhizosphere differs from other plants, due to its strong ability to relate symbiotically with many other microorganisms. This past research is however limited to soybean and few other crops or shrubs. The search to understand similar relationship to other crops and shrubs continues. Furthermore, soil ecological environment is reflected by the structure of the soil microbial community (Costa *et al.*, 2006). Many studies link maintenance of soil quality to abundance, structure and diversity of rhizosphere microbial communities (Garbeva *et al*., 2004; Kong *et al*., 2011) buttressing the results in this study that diverse fungal community in the rhizosphere of *Aspilia pluriseta* sets a good ecological environment for subsequent gadam sorghum growth.

5.4.3 Fungal richness and diversity indices

High spore density and diversity of mycorrhiza fungi are suggested to be advantageous for improving fungal diversity root colonization and subsequent crop growth (Hu *et al*., 2015). This experiment consistently showed high rhizosphere spore counts and root colonization (Table 4.7). Soil arbuscular mycorrhiza fungi are sensitive to changes in land-use patterns and management regimes (Mang'erere *et al.*, 2018; Martinez & Johnson, 2010; Oehl *et al.*, 2010) but this was not clearly demonstrated in this experiment as soil samples were collected *in situ* along the rhizosphere of *Aspilia pluriseta*.

Thirty-five taxa of arbuscular mycorrhizal fungi (AMF) were extracted and identified directly from the soil samples representing five genera in the three studied rhizosphere habitats. Considerably, this number is a large, given that only about 190 AMF species have so far been described worldwide (Knief, 2014), and that the samples were taken from three soil depths along a transverse section of *Aspilia pluriseta* plant rhizosphere. The number of species detected from MC1a (depth 1; 0-20 cm), MC2a (depth 2; 21-40 cm) and MC3a (depth 3; 41-60 cm) was relatively high when compared to that usually reported from corresponding habitats (Neville *et al.*, 2002**;** Cuenca & Lovera, 2010; Armansyah *et al.*, 2018**;** Song *et al.*, 2019**).** The

high species numbers could be attributed to the following reasons: (1) the high mycotrophic dependency of the sampled *Aspilia pluriseta* plant species. Previous studies by Muchoka *et al.* (2020), found out that colonization of AMF in the roots of these sampled plant species in the three soil depths surveyed had all rhizosphere depths intensively colonized by arbuscular mycorrhiza fungi. (2) Environmental conditions that were both hot and arid. It is an established fact that high temperatures and high light intensities increases the probability of AMF sporulation (Cardoso *et al.*, 2003; Koide & Mosse, 2004). Besides, susceptibility to spore predation and parasitism is less in the arid environmental conditions compared to locations with higher rainfall amounts (Lovelock *et al*., 2003). (3) Sampling was done in the hottest month of September when the sun is overhead the equator in the tropics. Past scientific reports show that spore populations are usually greatest in autumn where there are marked warm and cold seasons (David & Patricia, 1999) and that, substantially, more spores are expected in the dry season (Álvarez-sánchez, 1999). It has been asserted that spores collected during this period not only have greater spore density and population (Álvarez-sánchez, 1999; Lovelock *et al.*, 2003) but are also in better condition for identification (David & Patricia, 1999). Considering the seasonal nature of AMF, the spores' diversity in these sampled depths would no doubt increase with longer-term sampling.

More species richness was found in MC3a (41-60 cm) followed by MC2a (21-40 cm) as these were the zones of less agricultural disturbance over time. The same zones, however, had less spore density (Table 4.10) giving a sharp contrast to the findings of Boddington & Dodd, (2000) and Oehl *et al*. (2003). In the current study, however, the sampled sites were fallow lands that had a break to cultivation for a period of three years and this explains the difference in the results.

5.5 Mycorrhiza fungi co-association with *Aspilia pluriseta* on the yield of gadam sorghum crop

Crop plants are more efficiently able to obtain phosphate from the soil by the help of arbuscular mycorrhiza fungi (AMF). All globally important food crops naturally form this symbiosis with the fungi and this is good news in the fight against food insecurity (Smith & Read, 2008). In this experiment, soil that had *Aspilia pluriseta* previously growing had mycorrhiza that acted as a source of inoculation and

therefore continued with phosphates synthesis with gadam sorghum as the host plant leading to improved sorghum yield (Muchoka *et al.*, 2020). The success of AMF co-association from plant to plant in agricultural soils can be determined by many factors such as competition with native fungi species, habitat niche availability and compatibility (Verbruggen *et al*., 2013). Compatibility is particularly important for AMF inoculation, where some isolates could be host "specialists," and others are said to be "generalists" (Öpik & Moora, 2012). In this study, co-association of mycorrhiza fungi from the rhizosphere of *Aspilia pluriseta* and gadam sorghum tested showed a generalist nature, since it enhanced plant growth as evidenced by the changes in growth parameters measured (Plate 4.1 & Table 4.2). Accordingly, AMF that are considered plant host generalists have a high establishment rate in several crops (Öpik & Moora, 2012).

Results presented in this report show that both sorghum and *Aspilia pluriseta* were effectively colonized, indicating a low specificity by the host *Aspilia* plants. Although the potential of AMF to contribute to improved crop yields has been known for decades and in spite of an extremely strong research focus on this symbiosis, there are remarkably few published studies demonstrating that large-scale inoculation of globally important crops, in an agricultural situation, resulting to significant increases in food production (Ceballos *et al.*, 2013). This experiment reasonably showed success to improved yields from a host surrogate plant (*Aspilia pluriseta*) to a benefitting plant (gadam sorghum).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Overview of the chapter

This chapter concludes the results and discussions in chapters four and five respectively and and gives recommendations arising from this research study.

6.2 Conclusions

This study examined mycorrhizal fungal diversity in the rhizosphere of wild and native *Aspilia pluriseta* shrub with an aim to exploit root microbe symbiosis for sorghum crop production. The study found out that sorghum growth was enhanced through mycorrhizal association in the rhizosphere of *Aspilia pluriseta*. Evidence was adduced from this study on increased phosphate (available P) levels in soils inhabited by *Aspilia pluriseta* and/ or where sorghum seed was inoculated with mycorrhiza from *Aspilia* soils.

More specifically,

i. Seedling emergence and stand count were enhanced at $p \leq 0.05$ in both mycorrhiza fungi inoculated gadam sorghum seeds and in pots whose soils were taken from the rhizosphere of *Aspilia pluriseta* plants. Soil phosphate level was enhanced where seed inoculation with mycorrhiza was done and in soils previously grown *Aspilia pluriseta* vegetation. *Aspilia pluriseta* bush fallows can be used for phosphate bio-remediation and cover crop in arid and semi-arid Gakurungu, Tunyai and Kanyuambora environments.

ii. The intensity of spore morphotypes was significantly higher at $p \leq 0.05$ for soils whose vegetation was covered with *Aspilia pluriseta* than those without. *Aspilia pluriseta* vegetation used together with sandy loam soil could culture commercial mycorrhiza fungi production for use in agrisystems.

iii. Three hundred and seventy three operational taxonomic units (OTUs) were realized at 3% genetic distance while 35 fungal taxa were realized in the rhizosphere of *Aspilia pluriseta* with five main phyla; Glomeromycota (90.7%), Basidiomycota (3.7%), Ascomycota (3.4%), Chytridiomycota

(1.5%), and unspecified phylum fungi (0.7%). The genera *Glomus* was the most prevalent in all soil depths with 85.6 % of the OTUs in depth 0-20 cm, 69.0 % in depth 21-40 cm and 48.5 % in depth 41-60 cm. Obligate arbuscular mycorrhiza fungi was found in the soil sample and if commercially cultured could enhance phosphates uptake in crops.

iv. Mycorrhiza fungi effectively colonized both sorghum and *Aspilia pluriseta*. *Aspilia pluriseta* served as a host surrogate plant and gadam sorghum as a benefitting plant. The co-association led to improved sorghum crop yield from 13.1 g/1000 sorghum grains in soils that had not grown *Aspilia pluriseta* (and/or) not inoculated to 15.2 g/1000 grains in soils previously grown *Aspilia pluriseta* (and/or inoculated). This is a yield increase of about 16% in one. Growth attributes had a positive correlation to total yield at 95% confidence. Mycorrhiza fungi in the rhizosphere of *Aspilia pluriseta* has the potential to contribute to improved sorghum crop yields reducing hunger and vulnerability in the arid and semi-arid Gakurungu, Tunyai and Kanyuambora locations in eastern Kenya.

6.3 Recommendations

6.3.1 Recommendations for further research from this work

i. Organisms assess and respond to habitat heterogeneity by allocating time or effort in response to variability in hazards, opportunities and competitors. There is need to conduct research experiment on possible competition of *Aspilia pluriseta* and field crops (inter competition between species and intra competition between *Aspilia pluriseta* species alone) and gather adequate data to qualify *Aspilia pluriseta* plant species as a cover crop in the arid and semi-arid environments

ii. In recent years, plant cell and tissue culture techniques have developed into very powerful tools for the large-scale propagation of plants of interest. Although *Aspilia* can be vegetatively propagated from soft woody, stem cuttings other quicker methods should be explored for rapid multiplication of *Aspilia pluriseta* for commercial exploitation.

iii. There is need to conduct field replication of this research study on major cereals and legume crops in the arid and semi-arid areas of Gakurungu, Tunyai and Kanyuambora

iv. There is need to evaluate mycorrhizal propagules in the rhizosphere of *Aspilia pluriseta* plant for commercial use in the agricultural sector. This study found out that the rhizosphere of *Aspilia pluriseta* has 35 distinct mycorrhiza species. Empirical data is required on the most versatile and infective mycorrhiza species for use in agricultural systems. Gaps exist on the minimum amount of mycorrhiza that need to be applied per specific crop enterprise to be effective as well as developing the most effective consortia of mycorrhiza to cause production revolution.

v. Plants' root exudate composition vary between different plant species. Exudate composition changes in the same plant at different ages or when grown under different environmental conditions. Exudation levels of particular constituents are not always the same along the plant root axis. Plant root exudates alter the physical and chemical conditions of the rhizosphere by changing pH levels and mineral availability via desorption and chelation, and also influence the growth and interactions of numerous microorganisms that populate the rhizosphere. *Aspilia pluriseta* produces root exudate that require to be defined in terms of their role in mycorrhizal network and follower crop productivity.

6.3.2 Recommendation for further work

i. There is need for comparative studies on common agroforestry shrubs like *Lantana camara, Leucania leucocephala* and other shrubs in the arid and semi-arid climates that could be harbouring some of the essential mycorrhizal fungi microbiome that can be exploited to enhance agricultural productivity.

ii. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs which, after root colonization, exert widely accepted benefits to a wide range of host-plant species. Mass production of contaminant-free AM fungi has remained a bottleneck for application in agriculture for decades. However, using the monoxenic cultivation system, allowing the realization of large-scale

production under strictly controlled conditions, the use of some of the most common mycorrhizal species identified in the rhizosphere of *Aspilia pluriseta* could be a breakthrough for commercial mycorrhiza inoculum production.

6.3.3 Recommendation to the national and county governments

A review of policy to include the use of *Aspilia pluriseta* in land rehabilitation, conservation and re-seeding programmes in dryland ares.

6.3.4 Recommendation to farmers

i. Farmers are encouraged to use mychorrizal inoculated gadam sorghum to improve on their sorghum crop yields. Using soils from *Aspilia pluriseta* or inoculating gadam sorghum seed with *Aspilia* soils improves yield by about 16%.

ii. Instead of leaving the land bare and vulnerable between shifts of cultivation, farmers are advised to leave *Aspilia pluriseta* plants growing. This rejuvenates the land ready for the subsequent season.

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APPENDICES

Appendix 1: Plates showing *Aspilia pluriseta* shrub and the root fungus

Plate 1.1. Aspilia pluriseta Schweif. shrub growing in the wild in Tunyai, Kenya during the wet season (Photo taken by James Muchoka on 10th November, 2017)

Plate 1.2. Roots of a newly uprooted Aspilia pluriseta exposing the white mycelia (Photo taken by James Muchoka on 10th November, 2017)

Appendix 2: Soil Sampling at the sites

Plate 3.1. Collection of soil samples at Kanyuambora, 00021’00” S, 37028’30” E (Photo by James Muchoka, 12th September 2017)

Plate 3.2. Soil samples collection at Tunyai site, 00010’00” S, 37050’00” E (Photo by James Muchoka, 12th September 2017);

Plate 3.3. Soil samples collection at Gakurungu, 00012’00” S, 37051’00” E (Photo by James Muchoka, 13th September 2017);

Plate 3.4. Silt loam soil sampling along the rhizosphere of Aspilia pluriseta in Tunyai site (Photo by James Muchoka, 28th September 2017)

(Persons whose photos appear in this chapter gave express permission to be photographed and used in this document)

Appendix 3: Layout of treatment factors for experiment one

Soil type	Soil cover	Depth	Inoculations
Silty Clay	Asp	0-20	1
Silty Clay	Asp	0-20	2
Silty Clay	Asp	21-40	1
Silty Clay	Asp	21-40	2
Silty Clay	Asp	41-60	1
Silty Clay	Asp	41-60	2
Silty Clay	No Asp	0-20	1
Silty Clay	No Asp	0-20	2
Silty Clay	No Asp	21-40	1
Silty Clay	No Asp	21-40	2
Silty Clay	No Asp	41-60	1
Silty Clay	No Asp	41-60	2
silt Loam	Asp	0-20	1
silt Loam	Asp	0-20	2
silt Loam	Asp	21-40	1
silt Loam	Asp	21-40	2
silt Loam	Asp	41-60	1
silt Loam	Asp	41-60	2
silt Loam	No Asp	0-20	1
silt Loam	No Asp	0-20	2
silt Loam	No Asp	21-40	1
silt Loam	No Asp	21-40	2
silt Loam	No Asp	41-60	1
silt Loam	No Asp	41-60	2
Sandy loam	Asp	0-20	1
Sandy loam	Asp	0-20	2
Sandy loam	Asp	21-40	1
Sandy loam	Asp	21-40	2
Sandy loam	Asp	41-60	1
Sandy loam	Asp	41-60	2
Sandy loam	No Asp	0-20	1
Sandy loam	No Asp	0-20	2
Sandy loam	No Asp	21-40	1
Sandy loam	No Asp	21-40	2
Sandy loam	No Asp	41-60	1
Sandy loam	No Asp	41-60	2

Appendix 4: Test factors and variables for greenhouse experiments

Factor	G7	H7	NL7	LL7	SC14	H14	NL14	LL14	SC21	H21	NL21	LL21	SC28	H28	NL28
Silty Clay	83.3b	6.2a	1.9b	5.4a	83.3b	19.3a	2.7a	15.8a	83.3b	48.3a	2.9a	37.1a	84.4b	94.7a	3.1a
Silt Loam	96.9a	6.3a	1.9ab	4.5b	96.9a	19.1a	2.8a	12.8b	94.8a	46.9a	3.0a	30.7b	94.8a	93.4a	3.0a
Sandy Loam	90.2ab	5.2b	2.0a	3.8c	90.2ab	16.0b	2.8a	10.9c	90.2ab	39.1b	2.8b	25.5c	90.2ab	72.3b	3.1a
LSD	7.2	0.6	0.2	0.5	7.2	1.8	0.2	1.6	7.6	3.6	0.1	3.7	7.6	6.5	0.1
Block 1	83.3b	6.2ab	1.8b	5.3a	83.3b	19.6a	2.8ab	15.6a	83.3b	48.8a	3.0a	37.5a	84.7b	96.9a	3.1a
Block 2	91.7ab	6.7a	2.0a	4.9ab	91.7ab	19.5a	2.7b	14.9a	91.7ab	48.4ab	2.9a	33.1b	91.7ab	90.8ab	3.0b
Block 3	98.6a	5.7b	2.0a	4.3b	98.6a	17.7a	2.9a	11.7b	95.8a	44.3b	3.0a	28.6b	95.8a	86.0b	3.1a
Block 4	86.9b	5.0c	1.9ab	3.6c	86.9b	15.7b	2.7b	10.5b	86.9b	37.6c	2.8b	25.2c	86.9b	73.4c	3.0b
LSD	8.3	0.7	0.2	0.6	8.3	2	0.2	1.9	8.7	4.2	0.1	4.3	8.8	7.5	0.1
NoAp	83.8b	5.3b	1.9b	4.3b	83.8b	17.1b	2.7a	12.8a	83.8b	43.6a	2.9a	31.0a	84.4b	84.2a	3.0a
Ap	96.5a	6.5a	2.0a	4.8a	96.5a	19.1a	2.8a	13.6a	95.1a	45.9a	2.9a	31.2a	95.1a	89.3a	3.1a
LSD	5.9	0.5	0.1	0.4	5.9	1.4	0.1	1.3	6.2	3	0.1	3	6.2	5.3	0.1
0-20 cm	95.8a	7.1a	2.1a	5.4a	95.8a	20.8a	2.9a	15.6a	95.8a	50.5a	3.0a	35.8a	95.8a	93.7a	3.2a
21-40 cm	90.6ab	5.8b	1.8b	4.1b	90.6ab	18.2b	2.9a	12.0b	90.6a	44.0b	3.0a	27.8b	90.6a	84.2b	3.0b
41-60 cm	84.0b	4.8c	1.8b	4.1b	84.0b	15.4c	2.4b	11.9b	81.9b	39.7c	2.8b	29.7b	82.9b	82.4b	3.0b

LSD	7.2	0.6	0.2	0.5	7.2	1.8	0.2	1.6	7.6	3.6	0.1	3.7	7.6	6.5	0.1
SOi	83.8b	5.3b	1.9b	4.3b	83.8b	17.1b	2.7a	12.8a	83.8b	43.6a	2.9a	31.0a	84.4b	84.2a	3.0b
SNi	96.5a	6.5a	2.0a	4.8a	96.5a	19.1a	2.8a	13.6a	95.1a	45.9a	2.9a	31.2a	95.1a	89.3a	3.1a
LSD	5.9	0.5	0.1	0.4	5.9	1.4	0.1	1.3	6.2	3	0.1	3	6.2	5.3	0.1
Mean	90.1	5.9	1.9	4.5	90.1	18.1	2.7	13.2	89.4	44.8	2.9	31.1	89.8	86.8	3

Factor	**LL28**	**SC35**	**H35**	**NL35**	**LL35**	**SC42**	**H42**	**NL42**	**LL42**	**SC49**	**H49**	**NL49**	**LL 49**
Silty Clay	74.4a	82.3b	113.6a	3.6a	93.7a	79.8b	115.3b	3.8b	93.8b	80.3b	115.3b	3.7b	94.0b
Silt Loam	69.2a	94.8a	119.1a	3.7a	93.7a	94.8a	124.5a	4.0a	102.9a	94.8a	124.5a	4.0a	102.3a
Sandy Loam	48.2b	90.2a	100.5b	3.5a	74.0b	86.1ab	120.9a	3.7b	94.8b	85.5b	127.7a	3.9a	104.9a
LSD	7.6	7.8	5.5	0.2	5.5	8.8	3.8	0.2	4.6	8.8	3.3	0.2	4
Block 1	76.0a	81.9b	114.6a	3.6bc	91.2ab	78.6b	116.8b	3.8bc	91.2b	79.3b	116.8b	3.7b	91.6b
Block 2	69.3a	91.7a	115.0a	3.7ab	96.9a	91.7a	118.4b	3.9ab	101.3a	91.7a	118.3b	3.9ab	101.4a
Block 3	59.9b	95.8a	115.5a	3.8a	87.0b	90.6a	124.6a	4.0a	101.1a	90.6a	127.5a	4.0a	105.0a
Block 4	50.6c	86.9ab	99.1b	3.4c	73.5c	86.8ab	121.1ab	3.6c	94.9b	86.0ab	127.3a	3.8b	104.5a
LSD	8.8	9	6.3	0.2	6.3	10.2	4.4	0.2	5.3	10.2	3.8	0.2	4.6
NoAp	61.8a	83.1b	112.5a	3.6a	88.9a	79.4b	120.6a	3.8a	97.8a	78.6b	121.2a	3.8a	99.3a
Ap	66.1a	95.1a	109.6a	3.6a	85.4a	94.4a	119.9a	3.8a	96.5a	95.1a	123.8a	3.8a	101.9a
LSD	6.2	6.3	4.5	0.1	4.5	7.2	3.1	0.1	3.8	7.2	2.7	0.1	3.3
0-20 cm	70.9a	95.8a	115.9a	3.8a	89.3a	91.5a	123.3a	4.0a	98.2ab	91.5a	125.5a	4.0a	101.5a
21-40 cm	57.8b	90.6a	109.0b	3.5b	84.0a	88.5ab	119.0b	3.9a	94.1b	89.0ab	121.6b	3.9a	98.5a
41-60 cm	63.2b	80.8b	108.3b	3.4b	88.1a	80.7b	118.3b	3.6b	99.1a	80.2b	120.3b	3.7b	101.9a

LSD	7.6	7.8	5.5	0.2	5.5	8.8	3.8	0.2	4.6	8.8	3.3	0.2	4
SOi	61.8a	83.1b	112.5a	3.6a	88.9a	79.4b	120.6a	3.8a	97.8a	78.6b	121.2a	3.8a	99.3a
SNi	66.1a	95.1a	109.6a	3.6a	88.4a	94.4a	119.9a	3.8a	96.5a	95.1a	123.8a	3.8a	101.9a
LSD	6.2	6.3	4.5	0.1	4.5	7.2	3.1	0.1	3.8	7.2	2.7	0.1	3.3
Mean	64	89.1	111.1	3.6	87.1	86.9	120.2	3.8	97.1	86.9	122.5	3.8	100.6

Factor	**SC56**	**H56**	**NL56**	**LL56**	**SC63**	**H63**	**NL63**	**LL63**	**SC70**	**H70**	**NL70**	**LL70**
Silty Clay	80.3b	115.3b	3.8b	94.0b	80.3b	115.3b	4.0b	94.0b	80.3b	115.3b	4.0c	94.1b
Silt Loam	94.8a	124.5a	4.1a	102.9a	93.8a	124.5a	4.4a	102.9a	93.8a	124.5a	4.6a	102.9a
Sandy Loam	85.0b	127.7a	4.0a	104.9a	85.0ab	127.7a	4.1b	104.9a	84.6b	127.7a	4.2b	104.9a
LSD	9	3.3	0.2	4	9	3.3	0.2	4	9.1	3.3	0.2	4
Block 1	79.3b	116.8b	3.8b	91.6b	79.3b	116.8b	4.1bc	91.6b	79.3b	116.8b	4.1b	91.7b
Block 2	91.7a	118.3b	4.0a	101.4a	91.7a	118.3b	4.4a	101.4a	91.7a	118.3b	4.4a	101.4a
Block 3	90.6a	127.5a	4.1a	105.0a	89.2ab	127.5a	4.2ab	105.0a	89.2ab	127.5a	4.4a	105.0a
Block 4	85.3ab	127.3a	3.9ab	104.5a	85.3ab	127.3a	4.0c	104.5a	84.7ab	127.3a	4.1b	105.5a
LSD	10.4	3.8	0.2	4.6	10.4	3.8	0.2	4.6	10.5	3.8	0.2	4.6
NoAp	78.3b	121.2a	3.9a	99.3a	77.6b	121.2a	4.2a	99.3a	77.3b	121.2a	4.3a	99.4a

Ap	95.1a	123.8a	3.9a	101.9a	95.1a	123.8a	4.2a	101.9a	95.1a	123.8a	4.2a	101.9a
LSD	7.3	2.7	0.1	3.3	7.3	2.7	0.2	3.3	7.4	2.7	0.2	3.3
0-20 cm	91.5a	125.5a	4.1a	101.5a	91.5a	125.5a	4.6a	101.5a	91.5a	125.5a	4.6a	101.6a
21-40 cm	88.5ab	121.6b	4.0a	98.5a	88.5a	121.6b	4.3b	98.5a	88.5a	121.6b	4.4b	98.5a
41-60 cm	80.1b	120.3b	3.7b	101.9a	79.1b	120.3b	3.7c	101.9a	78.6b	120.3b	3.9c	101.9a
LSD	9	3.3	0.2	4	9	3.3	0.2	4	9.1	3.3	0.2	4
SOi	78.3b	121.2a	3.9a	99.3a	77.6b	121.2a	4.2a	99.3a	77.3b	121.2a	4.3a	99.4a
SNi	95.1a	123.8a	3.9a	101.9a	95.1a	123.8a	4.2a	101.9a	95.1a	123.8a	4.2a	101.9a
LSD	7.3	2.7	0.1	3.3	7.3	2.7	0.2	3.3	7.4	2.7	0.2	3.3
Mean	86.7	122.5	3.9	100.6	86.4	122.5	4.2	100.6	86.2	122.5	4.3	100.7

Factor	**SC77**	**H77**	**NL77**	**LL77**	**SC84**	**H84**	**NL84**	**LL84**	**Y84**
Silty Clay	80.3b	116.4c	4.4c	94.1b	80.3b	115.3b	4.3c	94.1b	19.2c
Silt Loam	93.8a	124.5b	5.3a	102.9a	93.8a	124.5a	5.3a	102.9a	28.1a
Sandy Loam	84.6b	127.7a	4.6b	104.9a	84.6b	127.7a	4.6b	104.9a	21.9b
LSD	9.1	3.2	0.2	4	9.1	3.3	0.2	4	1.2
Block 1	79.3b	118.4b	4.6b	91.7b	79.3b	116.8a	4.6b	91.7b	19.9c
Block 2	91.7a	118.3b	5.0a	101.4a	91.7a	118.3b	5.0a	101.4a	25.1a
Block 3	89.2ab	127.5a	4.9a	105.0a	89.2ab	127.5a	5.0a	105.0a	25.7a
Block 4	84.7ab	127.3a	4.4b	104.5a	84.7ab	127.3a	4.4b	104.5a	21.6b
LSD	10.5	3.7	0.2	4.6	10.5	3.8	0.2	4.6	1.4
NoAp	77.3b	122.0a	4.8a	99.4a	77.3b	121.2a	4.8a	99.4a	21.8b
Ap	95.1a	123.8a	4.7a	101.9a	95.1a	123.8a	4.7b	101.9a	24.3a
LSD	7.4	2.6	0.2	3.3	7.4	2.7	0.1	3.3	1

0-20 cm	91.5a	125.5a	5.3a		101.6a	91.5a	125.5a	5.3a	101.6a	24.3a
21-40 cm	88.5a	122.8ab	4.8b		98.5a	88.5a	121.6b	4.8b	98.5a	25.3a
41-60 cm	78.6b	120.3b	4.2c		101.9a	78.6b	120.3b	4.2c	101.9a	19.6b
LSD	9.1	3.2		0.2	4	9.1	3.3	0.2	4	1.2
SOi	77.3b	122.0a	4.8a		99.4a	77.3b	121.2a	4.8a	99.4a	21.8b
SNi	95.1a	123.8a	4.7a		101.9a	95.1a	123.8a	4.7b	101.9a	24.3a
LSD	7.4	2.6		0.2	3.3	7.4	2.7	0.1	3.3	1
Mean	86.2	122.9		4.7	100.7	86.2	122.5	4.7	100.7	23.1

Key:
Records were taken at the intervals of 7 days
G7-Emergence/Germination at day 7
H7-Height at day 7
NL7-Number of leaves at day 7
LL7-Length of the longest leaf at day 7
SC14-Stand count at day 14
Y84-Plant yield at day 84;
SOi-Seed inoculated
SNi-Seed not inoculated

Appendix 5: Interaction between test factors on growth parameters of gadam sorghum

The SAS System 19:23 Saturday, September 11, 2021 1

The GLM Procedure

Class Level Information

Class	Levels	Values
BLOCK	4	1 2 3 4
SOIL	3	1 2 3
SITE	2	1 2
DEPTH	3	1 2 3
INO	2	1 2

Number of observations 144

The SAS System 19:23 Saturday, September 11, 2021 2

The GLM Procedure

Dependent Variable: SC35 SC35

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	17586.80556	1034.51797	3.23	<.0001
Error	126	40312.50000	319.94048		
Corrected Total	143	57899.30556			

R-Square	Coeff Var	Root MSE	SC35 Mean
0.303748	19.88966	17.88688	89.93056

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.000000	.	.	.
SOIL*DEPTH	4	694.444444	173.611111	0.54	0.7047
SOIL*INO	0	0.000000	.	.	.

SITE*DEPTH	0	0.000000	.	.
SITE*INO	0	0.000000	.	.
SOIL*SITE*DEPTH	4	1041.666667	260.416667	0.81 0.5185
SOIL*SITE*INO	0	0.000000	.	.
SITE*DEPTH*INO	0	0.000000	.	.
DEPTH*INO	0	0.000000	.	.

The GLM Procedure

Dependent Variable: H35 H35

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	23836.43653	1402.14333	12.84	<.0001
Error	126	13761.93117	109.22168		
Corrected Total	143	37598.36769			

R-Square	Coeff Var	Root MSE	H35 Mean
0.633975	9.410620	10.45092	111.0545

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.000000	.	.	.
SOIL*DEPTH	4	4585.222413	1146.305603	10.50	<.0001
SOIL*INO	0	0.000000	.	.	.
SITE*DEPTH	0	0.000000	.	.	.
SITE*INO	0	0.000000	.	.	.
SOIL*SITE*DEPTH	4	1846.740430	461.685108	4.23	0.0030
SOIL*SITE*INO	0	0.000000	.	.	.
SITE*DEPTH*INO	0	0.000000	.	.	.
DEPTH*INO	0	0.000000	.	.	.

The GLM Procedure

Dependent Variable: NL35 NL35

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	17	12.38888889	0.72875817	4.13 <.0001
Error	126	22.25000000	0.17658730	
Corrected Total	143	34.63888889		

R-Square	Coeff Var	Root MSE	NL35 Mean
0.357658	11.68187	0.420223	3.597222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.00000000	.	.	.
SOIL*DEPTH	4	2.11111111	0.52777778	2.99	0.0214
SOIL*INO	0	0.00000000	.	.	.
SITE*DEPTH	0	0.00000000	.	.	.
SITE*INO	0	0.00000000	.	.	.
SOIL*SITE*DEPTH	4	1.11111111	0.27777778	1.57	0.1855
SOIL*SITE*INO	0	0.00000000	.	.	.
SITE*DEPTH*INO	0	0.00000000	.	.	.
DEPTH*INO	0	0.00000000	.	.	.

The GLM Procedure

Dependent Variable: LL35 LL35

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	23189.04413	1364.06142	10.35	<.0001
Error	126	16610.11940	131.82634		
Corrected Total	143	39799.16353			

R-Square	Coeff Var	Root MSE	LL35 Mean
0.582652	13.17487	11.48157	87.14744

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.000000	.	.	.
SOIL*DEPTH	4	2577.467947	644.366987	4.89	0.0011
SOIL*INO	0	0.000000	.	.	.

SITE*DEPTH	0	0.000000	.	.
SITE*INO	0	0.000000	.	.
SOIL*SITE*DEPTH	4	391.131007	97.782752	0.74 0.5653
SOIL*SITE*INO	0	0.000000	.	.
SITE*DEPTH*INO	0	0.000000	.	.
DEPTH*INO	0	0.000000	.	.

The GLM Procedure

Dependent Variable: Y84 Y84

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	3717.321181	218.665952	41.14	<.0001
Error	126	669.773750	5.315665		
Corrected Total	143	4387.094931			

R-Square	Coeff Var	Root MSE	Y84 Mean
0.847331	9.996761	2.305573	23.06319

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.0000000	.	.	.
SOIL*DEPTH	4	186.3552778	46.5888194	8.76	<.0001
SOIL*INO	0	0.0000000	.	.	.
SITE*DEPTH	0	0.0000000	.	.	.
SITE*INO	0	0.0000000	.	.	.
SOIL*SITE*DEPTH	4	180.6291667	45.1572917	8.50	<.0001
SOIL*SITE*INO	0	0.0000000	.	.	.
SITE*DEPTH*INO	0	0.0000000	.	.	.
DEPTH*INO	0	0.0000000	.	.	.

The GLM Procedure

Dependent Variable: BLOCK BLOCK

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	176.0000000	10.3529412	326.12	<.0001

Error	126	4.0000000	0.0317460
Corrected Total	143	180.0000000	

R-Square	Coeff Var	Root MSE	BLOCK Mean
0.977778	7.126966	0.178174	2.500000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SOIL*SITE	0	0.00000000	.	.	.
SOIL*DEPTH	4	3.33333333	0.83333333	26.25	<.0001
SOIL*INO	0	0.00000000	.	.	.
SITE*DEPTH	0	0.00000000	.	.	.
SITE*INO	0	0.00000000	.	.	.
SOIL*SITE*DEPTH	4	3.77777778	0.94444444	29.75	<.0001
SOIL*SITE*INO	0	0.00000000	.	.	.
SITE*DEPTH*INO	0	0.00000000	.	.	.
DEPTH*INO	0	0.00000000	.		

Appendix 6: pH, Phosphates and Phosphorus evaluation

The GLM Procedure

Class Level Information

Class	Levels	Values
Soil	3	1 2 3
Location	2	1 2
Depth	3	1 2 3

Number of observations 18

The GLM Procedure

Dependent Variable: pH1 pH1

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	1.26277778	0.25255556	9.67	0.0007
Error	12	0.31333333	0.02611111		
Corrected Total	17	1.57611111			

R-Square	Coeff Var	Root MSE	pH1 Mean
0.801198	2.618009	0.161589	6.172222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	0.44777778	0.22388889	8.57	0.0049
Location	1	0.46722222	0.46722222	17.89	0.0012
Depth	2	0.34777778	0.17388889	6.66	0.0113

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	0.46722222	0.46722222	2.09	0.2854
Depth	2	0.34777778	0.17388889	0.78	0.5628

The GLM Procedure

Dependent Variable: pH2 pH2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.85111111	0.17022222	6.96	0.0029
Error	12	0.29333333	0.02444444		
Corrected Total	17	1.14444444			

R-Square	Coeff Var	Root MSE	pH2 Mean
0.743689	2.581880	0.156347	6.055556

Source	DF	Type III SS	Mean Square	F Value	Pr > F

Soil	2	0.22111111	0.11055556	4.52	0.0344
Location	1	0.26888889	0.26888889	11.00	0.0061
Depth	2	0.36111111	0.18055556	7.39	0.0081

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	0.26888889	0.26888889	2.43	0.2592
Depth	2	0.36111111	0.18055556	1.63	0.3798

The GLM Procedure

Dependent Variable: Phos1 Phos1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13548.78611	2709.75722	2.79	0.0675
Error	12	11655.40333	971.28361		
Corrected Total	17	25204.18944			

R-Square	Coeff Var	Root MSE	Phos1 Mean
0.537561	36.83614	31.16542	84.60556

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1252.817778	626.408889	0.64	0.5420
Location	1	6479.013889	6479.013889	6.67	0.0240
Depth	2	5816.954444	2908.477222	2.99	0.0881

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	6479.013889	6479.013889	10.34	0.0846
Depth	2	5816.954444	2908.477222	4.64	0.1772

The GLM Procedure

Dependent Variable: Phos2 Phos2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	15284.99111	3056.99822	3.08	0.0511
Error	12	11900.04000	991.67000		
Corrected Total	17	27185.03111			

R-Square	Coeff Var	Root MSE	Phos2 Mean
0.562258	36.84091	31.49079	85.47778

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1155.367778	577.683889	0.58	0.5735
Location	1	8862.242222	8862.242222	8.94	0.0113
Depth	2	5267.381111	2633.690556	2.66	0.1109

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	8862.242222	8862.242222	15.34	0.0594
Depth	2	5267.381111	2633.690556	4.56	0.1799

The GLM Procedure

Dependent Variable: Phr1 Phr1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1029.518889	205.903778	1.30	0.3270
Error	12	1900.952222	158.412685		
Corrected Total	17	2930.471111			

R-Square	Coeff Var	Root MSE	Phr1 Mean

0.351315	39.98443	12.58621	31.47778	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	308.6144444	154.3072222	0.97	0.4055
Location	1	1.6200000	1.6200000	0.01	0.9211
Depth	2	719.2844444	359.6422222	2.27	0.1458

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	1.6200000	1.6200000	0.01	0.9277
Depth	2	719.2844444	359.6422222	2.33	0.3002

The GLM Procedure

Dependent Variable: Phr2 Phr2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1045.060556	209.012111	1.30	0.3252
Error	12	1922.168889	160.180741		
Corrected Total	17	2967.229444			

R-Square	Coeff Var	Root MSE	Phr2 Mean
0.352201	41.50347	12.65625	30.49444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	365.0811111	182.5405556	1.14	0.3523
Location	1	4.2050000	4.2050000	0.03	0.8740
Depth	2	675.7744444	337.8872222	2.11	0.1640

Tests of Hypotheses Using the Type III MS for Soil as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	4.2050000	4.2050000	0.02	0.8933

Depth	2	675.7744444	337.8872222	1.85	0.3508

The GLM Procedure

t Tests (LSD) for pH1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.026111
Critical Value of t	2.17881
Least Significant Difference	0.166

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location
A	6.33333	9	2
B	6.01111	9	1

The GLM Procedure

t Tests (LSD) for pH2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.024444
Critical Value of t	2.17881
Least Significant Difference	0.1606

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location
A	6.17778	9	2

B 5.93333 9 1

The GLM Procedure

t Tests (LSD) for Phos1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	971.2836
Critical Value of t	2.17881
Least Significant Difference	32.01

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location
A	103.58	9	1
B	65.63	9	2

The GLM Procedure

t Tests (LSD) for Phos2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	991.67
Critical Value of t	2.17881
Least Significant Difference	32.344

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location

t Grouping	Mean	N	Location
A	107.67	9	1
B	63.29	9	2

The GLM Procedure

t Tests (LSD) for Phr1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	158.4127
Critical Value of t	2.17881
Least Significant Difference	12.927

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location
A	31.778	9	2
A			
A	31.178	9	1

The GLM Procedure

t Tests (LSD) for Phr2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	160.1807
Critical Value of t	2.17881
Least Significant Difference	12.999

Means with the same letter are not significantly different.

t Grouping	Mean	N	Location

A	30.978	9	2
A			
A	30.011	9	1

The GLM Procedure

t Tests (LSD) for pH1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.026111
Critical Value of t	2.17881
Least Significant Difference	0.2033

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	6.30000	6	3
A			
A	6.26667	6	1
B	5.95000	6	2

The GLM Procedure

t Tests (LSD) for pH2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.024444
Critical Value of t	2.17881
Least Significant Difference	0.1967

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	6.15000	6	1
A			
A	6.11667	6	3
B	5.90000	6	2

The GLM Procedure

t Tests (LSD) for Phos1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	971.2836
Critical Value of t	2.17881
Least Significant Difference	39.204

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	96.18	6	1
A			
A	80.78	6	3
A			
A	76.85	6	2

The GLM Procedure

t Tests (LSD) for Phos2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	991.67

Critical Value of t 2.17881
Least Significant Difference 39.613

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	96.75	6	1
A			
A	80.83	6	3
A			
A	78.85	6	2

The GLM Procedure

t Tests (LSD) for Phr1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 12
Error Mean Square 158.4127
Critical Value of t 2.17881
Least Significant Difference 15.833

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	35.267	6	3
A			
A	33.450	6	2
A			
A	25.717	6	1

The GLM Procedure

t Tests (LSD) for Phr2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	160.1807
Critical Value of t	2.17881
Least Significant Difference	15.921

Means with the same letter are not significantly different.

t Grouping	Mean	N	Soil
A	34.483	6	3
A			
A	32.800	6	2
A			
A	24.200	6	1

The GLM Procedure

t Tests (LSD) for pH1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.026111
Critical Value of t	2.17881
Least Significant Difference	0.2033

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	6.36667	6	1
B	6.10000	6	3
B			
B	6.05000	6	2

The GLM Procedure

t Tests (LSD) for pH2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.024444
Critical Value of t	2.17881
Least Significant Difference	0.1967

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	6.25000	6	1
B	6.00000	6	3
B			
B	5.91667	6	2

The GLM Procedure

t Tests (LSD) for Phos1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	971.2836
Critical Value of t	2.17881
Least Significant Difference	39.204

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	108.57	6	1
A			
B A	79.98	6	3

B

B 65.27 6 2

The GLM Procedure

t Tests (LSD) for Phos2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	991.67
Critical Value of t	2.17881
Least Significant Difference	39.613

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	108.38	6	1
A			
B A	80.77	6	3
B			
B	67.28	6	2

The GLM Procedure

t Tests (LSD) for Phr1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	158.4127
Critical Value of t	2.17881
Least Significant Difference	15.833

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	40.267	6	1
A			
A	28.500	6	2
A			
A	25.667	6	3

The GLM Procedure

t Tests (LSD) for Phr2

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	160.1807
Critical Value of t	2.17881
Least Significant Difference	15.921

Means with the same letter are not significantly different.

t Grouping	Mean	N	Depth
A	38.967	6	1
A			
A	27.833	6	2
A			
A	24.683	6	3

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