ENHANCEMENT OF COLONISATION OF SOYBEAN ROOTS BY ARBUSCULAR MYCORRHIZAL FUNGI USING VERMICOMPOST AND BIOCHAR

NAME: LEAH WATHIRA NJUNGE

REG NO: I575/84397/2012

A thesis submitted in partial fulfilment for the award of Masters of Science degree in Mycology

of the University of Nairobi

SCHOOL OF BIOLOGICAL SCIENCES

COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES

UNIVERSITY OF NAIROBI

2018

DECLARATION

I hereby declare that this project is my own original work, has been developed under the guidance of my supervisors and that all material cited has been duly referenced.

                                                                           Date

…………………………………………..   …………………

Leah Wathira Njunge

 I575/84397/2012

This thesis has been submitted for examination with our approval as University supervisors.

                                                                           Date

………………………………………….   …………………

Dr. Peter. M Wachira

School of Biological Sciences

University of Nairobi

                                                                           Date

………………………………………….   …………………..

Prof. Sheila Okoth

School of Biological Sciences

University of Nairobi

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Application of organic matter and use of mycorrhiza have been recommended as ways of improving plant growth and increasing yields. The aim of the study was to investigate the effect of organic amendments on colonisation of soybean roots by arbuscular mycorrhizal fungi. The study consisted of a field experiment of seven treatments with 3 replications in a complete randomized block design. The treatments were biochar (B), vermicompost (V), mycorrhiza (M), biochar and vermicompost (BV), biochar and mycorrhiza (BM), biochar, vermicompost and mycorrhiza (BVM), mycorrhiza and vermicompost (MV), and the control plot which had no treatments (NT). Uncultivated fallow land was subdivided into 21 sub-plots separated by 1m wide paths. Soil samples were collected for characterization of soil chemical properties and estimation of the population of mycorrhizal spores. The different amendments were applied to their respective subplots. Soybean seeds were then planted in each plot. At flowering time, roots were screened for percentage mycorrhizal colonisation and dry mass of sample soybean plants from each plot was taken. At harvest time, soil samples from each plot were collected and the dry weight of plants from each plot and harvested soybeans was taken. There was mean increase of 54% in levels of phosphorous and 15 % of carbon in the soil after application of amendments while levels of nitrogen decreased in all treatment plots. There was a significant (P<0.05) increase in arbuscular mycorrhizal fungi spores after application of the amendments. The colonization percentage of arbuscules in roots was highest (15%) in the BV plots while the control (NT) plots had the lowest colonization percentage of 1%. The highest dry weight of both shoots and roots were recorded in the B plot which also had a significantly higher yield of harvested soybean seeds than the control NT with a mean of 171g. Plots treated with mycorrhiza had a significantly lower (P<0.05) yield of soybean seeds with a mean of 58g. From this study it was concluded that the organic amendments enhanced the activity of the already-present mycorrhizal fungi in the soil, without requiring the introduction of commercial mycorrhizae and that biochar enhances microbial activity which stimulates crop productivity. It was recommended that organic amendments should be considered as agents for improving soil nutrient content and thus improve plant growth and yield.

Key words: Arbuscular mycorrhizal fungi, organic amendments, soybean, vermicompost, biochar.

CHAPTER ONE

INTRODUCTION

1.1 Background

Soybean is a legume crop that has been cultivated for about 4000 years (Chianu *et al.,* 2008). It is one of the leading agricultural crops grown globally with production estimated at an average of 208 million metric tons per year. In spite of the high global demand for soybean, the contribution of Africa to the total production of soybeans is 0.4-1%. In Africa, soybean is mainly produced in Nigeria, South Africa, Uganda and Zimbabwe (Chianu *et al.,* 2008). Compared to other soybean producers in Africa, Kenya is ranked very low in production. Production of soybeans in Kenya is estimated at between 5,000-10,000 metric tonnes in a year. In spite of this, demand is estimated at between 50,000 and 70,000 metric tonnes per year (Chianu *et al.,* 2008). The deficit is offset by imports from mainly Brazil, the US and Uganda. In 2008, Kenya spent a total of 27.54 million USD in soybean imports; foreign exchange that could have been used in other development projects (Abuli, 2016).

One of the major constraints to crop production in Kenya, including soybean production is low soil fertility that leads to low yields. This is due to poor agronomic practices such as lack of proper crop rotation and intercropping practices and inefficient use of both organic and inorganic fertilisers, leading to nutrient depletion or poor nutrient balance in the soil (Chianu *et al.,* 2008). Major nutrients required for soybean growth include phosphorus, nitrogen, potassium, and presence of indigenous strains of nitrogen fixing bacteria (Abuli, 2016).

Although soybean, like other legumes, are nitrogen fixers, soil must have available nitrogen for the crops to use before they can begin to fix their own nitrogen. Phosphorous is also necessary in the nitrogen fixation process. Potassium has been found to help in activation of enzymes, translocation and disease and pest resistance, leading to higher yields due to better plant health (McGrath *et al.,* 2013). Bio-intensive farming has been suggested as a way of improving soil fertility in Kenya. This refers to using organic methods to improve soil quality. This is because it is safer to the environment as it does not disrupt the nutrient balance in the soil and organic fertilisers are more accessible than inorganic fertilisers financially (Alakonya *et al.,* 2014). Examples of organic methods of soil improvement include use of arbuscular mycorrhizal fungi, which assist the plant to take up unavailable nutrients, especially phosphorous; and organic soil amendments like vermicompost and biochar, which improve the quality of the soil.

According to Brundrett (2009), mycorrhiza is a symbiotic association essential for both partners, between a soil fungus and a root of a vascular plant, which is primarily responsible for nutrient transfer. The most well-known associations are arbuscular mycorrhizal (AM) fungi, which form symbiotic relationships with 80% of terrestrial plants. Due to this relationship, AM fungi have been proposed as a way of enhancing nutrient uptake by plants, especially phosphorous. One of the most essential macronutrients for plant growth is phosphorous. Phosphorous availability in the soil leads to improvement in leaf area, root growth, stalk and stem vigour, crop maturity and yield; and resistance to pests and diseases. Phosphorous also plays a major role in important processes like photosynthesis, energy transfer and storage, cell enlargement and cell division (Brundrett, 2009).

However, despite its presence in soil in organic form, and its addition by farmers in inorganic form, it still remains a limiting nutrient. This is because phosphorous becomes tightly bound with calcium, aluminium or iron, leaching to its precipitation, and forming phosphorous depleted zones near the contact areas of roots and soil. Inoculation of soybean and maize crops with AM fungi has been reported to enhance growth of the crops. This was attributed to better uptake of phosphorous by the plants (Turk *et al.,* 2006). Moreover, use of AM fungi to enhance nutrient uptake by plants could reduce the amount of mineral fertiliser required by growing plants (Orlando, 2003). This would reduce the damage caused to the soil by heavy use of mineral fertilisers.

Organic soil amendments have been proposed as alternatives to synthetic fertilisers for the improvement of soil quality. Soil amendments directly enhance nutrient uptake by plants by improving the structure and aggregation of the soil, balancing the pH, among other functions (Orlando, 2003). An example of an organic amendment that can be used to improve soil is biochar. Biochar is a porous, fine grained substance with a similar appearance to charcoal produced by slow combustion of biomass under oxygen-limited conditions for the purpose of using it as a soil amendment. Due to differences in the process of making biochar, biochar has higher porosity and adsorption capacity than charcoal. Although biochar has little plant nutrient content, its high surface area and porous structure increase the soil surface area. It also provides a habitat for beneficial soil microorganisms, aids in water retention and reduces leaching out of nutrients. All of these functions increase availability of nutrients to plants (Schahczenski, 2010). Vermicompost is a humus-like substance formed from the bio-

oxidation and stabilisation of organic material by the joint action of earthworms and microorganisms (Lazcano *et al.,* 2008).

1.2 Problem Statement

Poor soil fertility is the major cause of low crop yields in Kenya. Many farmers try to remedy this by applying inorganic fertilisers to the soil. However, because of the high cost of these inputs, they end up applying insufficient quantities, leading to negative nutrient balance in the soil. As a consequence of poor yields, many farmers experience lower household incomes and food insecurity. This has an overall negative effect on the Kenyan economy. On the other hand, practices like over-tillage and overuse of synthetic fertilisers in other cases can lead to destruction of soil structure and formation of soil crusts. Cultivation of nitrogen fixing legumes, application of organic soil amendments and use of mycorrhiza have been recommended as remedies to these problems. There is need to promote use of organic practices of farming such as use of AM fungi to aid in nutrient uptake in plants, and soil amendments to improve soil quality and as a result increase crop yields. Organic soil improvement methods are more affordable and safer for the environment than inorganic fertilisers in the long run.

1.3 Justification

The major soils in the study area have weak soil structure and sandy loamy to loamy texture. Soil pH ranges between 6 and 7, and gets higher with depth. Organic soil amendments have been proposed as alternatives to synthetic fertilisers for the improvement of soil quality. Soil amendments indirectly enhance nutrient uptake by plants by improving the structure and aggregation of the soil, and balancing the pH, among other functions. In addition, arbuscular mycorrhizal fungi have been proposed as a way of enhancing nutrient uptake by plants. In particular, the importance of AM fungi symbiosis in legume plants has been attributed to the high requirement for phosphorous in the nodulation and nitrogen fixation processes (Orlando, 2003). As nitrates are one of the least occurring nutrients in Kenyan soils, cultivation of legumes like soybean will further reduce the need to use nitrate-adding fertilizers to improve soil fertility, as they improve soil fertility by fixing atmospheric nitrogen into the soil (Chianu *et al.,* 2008). It has been found that physiology of soybean roots is altered by colonisation of the plant roots by arbuscular mycorrhizal fungi, with improved phosphorous nutrition being the best known of these effects (Gabor, 1997). Inoculation with mycorrhiza was reported to enhance growth of soybean plants and maize, a result that was attributed to better uptake of

phosphorous by the plants (Turk *et al.,* 2006). In addition, use of arbuscular mycorrhiza to enhance micronutrient uptake by plants could reduce the amount of mineral fertiliser required by growing plants (Orlando, 2003). This would reduce the damage caused to the soil by heavy use of mineral fertilisers. There is therefore a need to establish whether addition of two different types of organic amendments, biochar and vermicompost, to improve soil quality enhances colonisation of soybean roots by AM fungi.

1.4 Objectives
General objective

To enhance mycorrhizal colonisation of soybean roots using biochar and vermicompost for enhanced soybean

Specific Objectives
1. To determine the occurrence and diversity of arbuscular mycorrhizal fungi in the study area.
2. To evaluate the effect of soil amendments (biochar and vermicompost) on soil chemical properties.

3. To determine the effect of selected organic amendments (biochar and amendments) on the colonisation of soybean roots by AM fungi in soybean plants.

1.5 Hypotheses
1. Arbuscular mycorrorrhizal fungi occur in the study area.

2. Addition of organic soil amendments enhances soil physical and chemical properties.

3. Addition of organic amendments enhances mycorrhizal colonisation of soybean roots.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mycorrhizal Fungi

Mycorrhizae are highly evolved, mutualistic associations between soil fungi and the roots of vascular plants. A recent broad definition of mycorrhizal relationship is given by Brundrett (2009): "Mycorrhiza is a symbiotic association essential for both partners, between a soil fungus and a root of a vascular plant, which is primarily responsible for nutrient transfer. "Soil fungi in this association include Zygomycetes, Ascomycetes and Basidiomycetes. There are at least seven types of recognised mycorrhizal association, depending on the type of fungus involved and the structures produced by the resulting morphological patterns. The most well-known associations are vesicular-arbuscular mycorrhiza (VAM, or simply AM fungi), ectomycorrhizas (ECM), in which fungi form a mantle around roots and a hartig net between root cells, orchid mycorrhizae, where fungi produce coils within the roots of orchid plants and ericoid mycorrhiza, which involve formation of hyphal coils in the outer cells of narrow hair roots of plants belonging to the class Ericales (Brundrett, 2009).

2.1.1 Arbuscular Mycorrhizal Fungi

It is now known that mycorrhizal fungi are a major component of soil microflora in most ecosystems, with Arbuscular Mycorrhizal (AM) fungi forming symbiotic relationship with 80% of terrestrial plant (Jansa, 2003). Arbuscular mycorrhizal fungi form a monophyletic group in the phylum Glomeromycota. Glomeromycotan fungi are obligate symbionts. This means that they cannot be grown in the absence of their host plant roots (Jansa, 2003). This phylum comprises about 200 described species, traditionally described by spore wall features. Genera and families have been described using mode of spore formation, while species have been distinguished using the layered structure of the spore walls.

Arbuscular mycorrhizal fungi derive their name from the tree-like structures they form within root cells of the host plant called arbuscules or hyphal coils. Some species of AM fungi also produce storage organs called vesicles and are termed 'Vesicular Arbuscular Mycorrhizal' (VAM) fungi. These fungi have three sources of inoculum, or propagules, for colonisation of host plant roots: hyphae, infected root fragments and spores. The best defined are the spores, and they are the most reliable for morphological identification of species. Spore formation in Glomeromycotan fungi is asexual. Spores produced are relatively large, ranging from 40-800µm, have layered walls, and contain several hundreds to thousands of nuclei. Sexual

reproduction in AM fungi is unknown, but parasexuality has been demonstrated (Jansa, 2003). Glomeromycotan fungi lack regular septa (Redecker and Raab, 2006).

Spores of AM fungi contain numerous nuclei and a large amount of storage lipids and polysaccharide. Under suitable conditions, the spores germinate to form the primary mycelium, which only grows when stimulated by the presence of host roots. The primary mycelium hyphae penetrate into the root cells into the root cortex layer, where they establish intraradical infectious structures. Depending on the fungal species, hyphae, hyphal coils or arbuscules might be formed inside the roots. Following penetration, extraradical mycelium forms in the soil, several centimetres around the roots, and undergoes extensive branching, increasing the surface area of contact with soil particles (Brundrett, 2009).

Mycorrhiza develop in the rhizosphere from propagules-spores and hyphae- which form a link between soil and plant roots; namely spores, hyphae and rhizomorphs. Signaling events occuring in the rhizosphere lead to growth of fungal hyphae and contact with the root surface. This leads to colonisation of the plant root by the mycorrhizal fungi. Colonisation of the plant root by arbuscular mycorrhizal fungi leads to formation of hyphopodia or appressoria on the root surface, inter and intracellular hyphae, coils and arbuscules, which form inside cortical cells. Arbuscules are the main point of nutrient exchange between the symbionts (Figure 1).
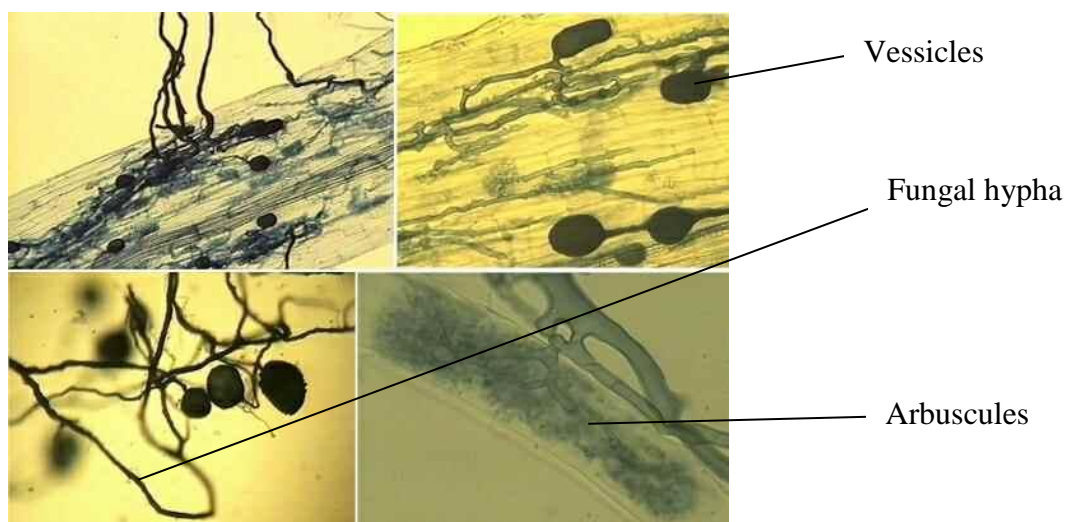

Figure 1: Arbuscular mycorrhizal fungi in root cells showing hyphae, vessicles and arbuscules. (Source: http://archive.bio.ed.ac.uk/jdeacon/FungalBiology/chap13_1.htm)

2.2 Benefits of Mycorrhizal Fungi

Mycorrhizal fungi have been reported to benefit plants by generally enhancing nutrient uptake, especially with regards to phosphorous, and other micronutrients like zinc, copper and manganese (Brundrett, 2009). They do this through the fungal hyphae which explore the rhizosphere extensively, accessing nutrients that would otherwise be unavailable to the plant roots. In return, the fungi receive plant carbohydrates that are important for completing the fungal life cycle (Brundrett, 2009). It has been found that AM fungi have active phosphate transporters which take up organic phosphate from the soil and facilitate its delivery to the plant. Plants aslo possess mycorrhiza- specific phosphate transporters which receive phosphorous from the plant and deliver it to plant cells (Bonfante and Anca, 2009). Other benefits related to improved phosphorous nutrition include improved resistance to pests and diseases, improved osmotic adjustment under water and salinity stress, stimulation of production of growth regulating substances and increased photosynthesis (Ortas, 2010). Mycorrhizal fungi have also been found to have genes involved in uptake of organic and inorganic nitrogen. In addition, molecular and physiological data have shown that nitrogen transporters in plants get activated during mycorrhization (Bonfante and Anca, 2009).

Previous studies on arbuscular mycorrhizal fungi in Kenya have focused mostly on effects of land use on mycorrhizal populations in field soils and root colonisation. For example, Mathimaran *et al.,* (2007) observed that crop rotation with maize and crotalaria did not have a significant influnce on the density and species diversity of AM fungi spore communities in field soil. Muchane *et al.,* (2012) found that AMF communtity in the study area was adversely affeced by human disturbance in semi arid regions. They observed that agricultural practices using high leves of fertiliser and monocultures had negative effects on AMF diversity in savannah eosystems as compared to subsistence farming with more than one crop which maintained AMF diversity. They suggested promotion of use of biofertilisers to restore AMF populations in the study area and crop diversification to improve soil chemical and biological properties which would in turn improve crop production (Muchane *et al.,* 2012).

In a study on influence of land use types on occurence of arbuscular mycorrhizal fungi in the high altitude regions of Mt. Kenya, (Jefwa *et al.,* 2009), seventeen species were descibed from different land use types, with *Acaulospora* and *Glomus* forming the highest proportion whereas low abundance of *Gigaspora* and *Scutellospora* was recorded. This was attributed to

the fact that *Glomus* and *Acaulospora* species are more responsive to soil disturbance and hence underwent more sporulation than *Gigaspora* and *Scutellospora* due to practices like application of fertilisers, weeding and tillage. It was found that land use types dominated by perennial plants had the highest root colonisation. This was attributed to continuous growth of roots and less soil disturbance due to cultivation. On the other hand coffee, which is perennial but is often frequently weeded and intercropped with other food crops and hence undergoes more disturbance had low AMF colonisation, indicating disturbance of infective propagules by human activities. It was seen that disturbance had negative effects on mycorrhizal diversity due to disruption of nutrient transport in hyphal networks. Soil chemical characterisics were also found to influence spore abundance, with increases in carbon, nitrogen and potassium leading to an incease in spore abundance while increased phosphorous and acidity led to a decrease in spore abudance (Jefwa *et al.,* 2009).

In a similar study (Soka *et al.,*2015), AM hyphal densities were found to be higher in wildlife grazed system than in livestock grazed system. This was attributed to overgrazing by livestock which decreased carbon inputs to the soil, thus depriving the AM fungi of their source of carbon. Low AM hyphal densities were found in cultivated soils, indicating that tillage causes physical damage of hyphal networks. Crop diversification and non tillage farming were suggested as a means of improving mycorrhizal symbiosis and soil chemical properties, which would in turn lead to improved plant productivity in poor soils.

2.3 Soybean
Soybean (*Glycine max*) is a legume that grows in tropical, sub-tropical and temperate climates. It is a small grain, creamy in colour, with some black varieties. Each pod is hairy and contains two to three seeds. Soybean plant grows to a height of about 60 -120cm, and matures within 3-6 months, depending on the climate, location and variety. It requires a warm climate and can grow at low to medium altitudes. Soybean can grow at altitudes ranging between 0-2200m and under a rainfall regime of between 300-1200mm. However, it grows best in warm climates and low to medium altitudes. In Kenya it is mostly intercropped with maize, and has been reported to increase maize yields by upto 25%. (Mathu *et al.,* 2012). The most commonly used soybean varieties in Kenya are Duiker, Nyala and Gazelle. The leading porducer of soybean is the Western region of Kenya, particularly, Butere, Kakamega, Mumias, Siaya, Homa Bay, Nyamira, Kisii, Busia and Bungoma Counties. This area accounts for about 50% of soybean grown by smallholder farmers. The other major areas producing

soybean in Kenya are in Central Kenya particularly Kirinyaga, Embu, Tharaka Nithi and Meru Counties, accounting for 11-12% of soybean grown by smallholder farmers. Out of all the soybean grown in Kenya, 80% is used in the livestock industry while about 20% is used for human consumption. About 60% of the livestock feed used in Kenya, particularly dairy, poultry and pig feed, consists of soybean products. Due to lack of adequate supply from local farmers, this industry depends largely on imports for their soybean supply. The main large scale buyers of soybean products for human consumption in Kenya are Bidco and Proctor and Allan, which use soybean grains in production of oil (Chianu *et al.,* 2008).

2.3.1 Health Benefits of Soybean

Soybean has numerous benefits to human health that should be exploited. While other beans contain about 20% protein, soybean contains 40% protein. It has been found that daily consumption of small amounts of soybean lowers risk of cancer of the breast, colon and prostate gland, and also lowers the recurrence rate. The high levels of glucosycermide found in soybean are thought to be the reason for the cancer preventive qualities of soybean (Chianu *et al.,* 2008).

Soybean is beneficial to lactose-intolerant people and eases the symptoms of menopause. Unlike animal protein which contains saturated fat, soybean contains unsaturated fat, which reduces the risk of heart ailments. Since they contain high amounts of essential amino acids, soy proteins are superior to other vegetable proteins. Soybean contains cellulose, pectin and phytic acid. Cellulose is useful in digestion and is a deterrent to rectal cancer. Mature soybean seeds have been found to contain vitamins such as thiamine, niacin, riboflavin, cholin and vitamins A and K, which are necessary for normal growth and development. In addition, the mineral content of soybean, which consists of potassium, magnesium, calcium, zinc and copper, contribute to the overall requirements for children and pregnant women. Soybean isoflavones cause production of fewer and smaller fat cells, and as a consequence reduce obesity. These isoflavones also prevents osteoporosis in women during their menopause years (Chianu *et al.,* 2008).

Frequent consumption of soybean also helps minimise risk of coronary heart disease through control of cholesterol, blood pressure, vascular function and direct effects on the cells of the artery wall. The protein and fibre in soybean can prevent high blood sugar levels and help in controlling blood sugar levels in patients with non-insulin-dependent Diabetes Mellitus. Products derived from soybean are cholesterol-free, high in phosphorous, calcium and fibre.

Products like soybean oil, which is widely used as margarine, cooking oil, shortening and a salad dressing is highly digestible, with high levels of polyunsaturated fatty acids with no cholesterol (Chianu *et al.,* 2008).

2.3.2 Nitrogen Fixation in Soybean

Many legume plants have the ability to fix unavailable atmospheric nitrogen from the air to the soil through a symbiotic relationship with nitrogen fixing bacteria in their root nodules (Coskan and Dogan, 2011). Soybeans are known to be one of the most effective nitrogen fixing legumes (Tinsley, 2009). Nitrogen fixing bacteria associated with soybean, *Bradyrhizobium japonicum,* infect the roots through infection threads, leading to the formation of root nodules in which the bacteria live (Coskan and Dogan, 2011). These bacteria fix nitrogen by reducing atmospheric nitrogen to amino acids and protein. The bacteria supply reduced nitrogen to the plant while the plant supplies organic compounds and components of nitrogenase enzyme, which is used to reduce atmospheric nitrogen, to the bacteria (Coskan and Dogan, 2011).

Nitrogen fixing capacity of soybean is a great advantage to soil fertility as it increases the amount of available nitrogen in the soil. When intercropped with other crops, the surplus nitrogen left in the soil after soybean has been harvested benefits the subsequent crop. In addition, soybean can be grown in areas where soil mining has taken place as a result of continuously cultivating nutrient demanding crops which deplete the soil of its nitrogen. It can also be planted by resource- poor farmers who cannot afford to buy inorganic fertilisers to improve nitrogen content in the soil (Mathu *et al.,* 2012).

2.4 Soil Amendments

The need for conservation of the environment, has led to development of numerous sustainable agricultural practices. Use of mycorrhiza as biological fertiliser is one. Use of organic soil amendments to improve soil structure and properties so as to further reduce the need for inorganic fertilizers is another. Soil amendments affect plant growth indirectly by improving the physical and biological properties of soil, for example aeration, water retention and enhancing microbial activity and diversity. The soil amendments used in this study were biochar and vermicompost.

2.4.1 Biochar

This is a porous, fine-grained substance, with a similar appearance to charcoal, produced by slow combustion of biomass under oxygen-limited conditions, for the purpose of using it as a soil amendment (Verheijen *et al.,* 2010). It can be produced at temperatures of between 400-700° C from materials like wood chips, sugarcane waste, manure and other farm wastes (Elmer and Pignatello, 2011). Concern about greenhouse gas emission and its presence in the atmosphere has led to research into means of reducing its content in the atmosphere. Combustion of fossil fuels to produce energy and decomposition of soil organic matter by soil microorganisms are two of the causes of emission of carbon dioxide from the soil to the atmosphere. On the other hand, use of biochar as a fuel is 'carbon negative'. This means that the carbon in biomass that would have been combusted to produce energy or decomposed is instead transformed into stable carbon structures in form of biomass which remain sequestered in soil for many years (http://www.biochar-international.org/biochar/carbon) Carbon sequestration or storage in soil has been suggested as one of the ways of reducing carbon dioxide emmission into the atmosphere. Biochar components are decomposed much more slowly than other organic matter in the soil (between 100-1000 years). Because of this, biochar has been suggested for use as a possible carbon sink, because carbon input into soil is more than carbon output through soil microbial respiration (Verheijen *et al.,* 2010). This is shown in the figure below (Figure 2).

The carbon cycle versus the biochar cycle

The carbon cycle

Photosynthesis

Biomass

Biomass decomposes or burns 99% of carbon released as CO₂

Most carbon returns to atmosphere
Green plants use solar energy to remove CO_2 from the atmosphere via photosynthesis and store it as chemical energy in biomass. When biomass decomposes or burns, this process is reversed and nearly all CO_2 returned to the atmosphere.

The biochar cycle

Photosynthesis   Atmospheric CO_2
50% Less Returns

Biomass   50% of carbon   Pyro-lysis   Biochar   Energy

Syn-gas or bio-oil

Up to 50% of carbon stays in the soil
Pyrolysis destroys the structure of the biomass. One half of the carbon is converted to woodgas and the other half remains in the created char. If the char is buried in the soil as biochar, most of the carbon stays there and is sequestered as biochar.

Source: adapted from Wilson (2013), based on Biochar Solutions Inc. (2011)

Figure 2: Diagram showing a comparison between how carbon is used up in the carbon cycle and in the biochar cycle. (Source: adapted from Wilson (2013), based on Biochar Solutions Inc. (2011).

Biochar has also been put forward as a sustainable soil improvement agent. With regards to plant nutrient content, biochar has little. However, its high surface area and porous structure increase the soil surface area, provides a habitat for beneficial soil microorganism, aids in water retention and reduces leaching out of nutrients. These functions increase availability of nutrients to plants. In addition, studies show that biochar also reduces acidity in soils and provides for greater pH balance (Schahczenski, 2010). The specific mechanisms in which biochar influences symbiosis between arbuscular mycorrhizal fungi and host plants remain largely unknown. Several theories have been put forward to explain how biochar affects AM colonization in plants. Elmer and Pignatello (2011) proposed that alteration of soil properties leading to greater nutrient availability for plant roots, and stimulation of soil microbial populations that favour AM colonization are two effects of biochar on mycorrhizal colonization of plant roots.

In Kenya, a number of projects on use of biochar and biochar-producing stoves are being carried out in Bungoma, Western Kenya. The African Christians Organisation Network (ACON) has been working there with the aim of providing environmentally sustainable methods of cooking and farming. The organisation has empowered residents of this area by promoting use of biochar producing stoves for cooking, and using biochar as a soil amendment to improve soils in the area. Field tests that were done on the effect of using biochar as a soil amendment found that soil treated with biochar retained moisture better, enhanced plant growth and led to higher yields. (http://www.biochar-international.org/profile/ACON/Kenya). In another study on the effects of biochar on soils carried out in Kisumu, Soderberg (2013) found that carbon, magnesium and calcium content in soil significantly increased in plots treated with biochar. There was also a significant increase in soil pH of biochar treated plots.

2.4.2 Vermicompost

Accumulation of solid waste in the environment has become a point of concern, due to the pollution it causes. Practices like dumping, land filling and incineration are increasingly becoming unpopular due to their adverse effects in the environment. Because of this, biotechniques for the disposal of solid wastes are being developed. Vermicomposting is one

of these. Vermicompost is a humus-like substance formed when organic matter is broken down by the joint action of earthworms and microorganisms (Lazcano *et al.,* 2009). It is different from compost, which is a product formed from the aerobic decomposition of organic waste like animal droppings, crop wastes and even municipal wastes (Sinha, 2009). Vermicomposts are highly porous, well aerated, well drained and have good water holding capacity. They also contain important nutrients like nitrogen, phosphorous and potassium. Increased biomass and plant height has been attributed to these properties of vermicomposts (Darzi *et al.,* 2012). Waste materials that can be used in vermicomposting include food waste, paper cardboard, manure, biosolids and agricultural waste (Aalok, 2008).

Two phases are recognised in vermicomposting: the active phase which involves processing of the waste by the earthworms, modifying its physical state and microbial composition (Lazcano *et al.,* 2008). In this phase, the substrate is ground, mixed and aerated by the movements of the earthworms. The substrate is also changed biochemically by being digested in the intestines of the earthworms (Ndegwa *et al.,* 2000). The second phase is the maturation phase which consists of displacement of the earthworms to fresher layers of waste that is still undigested, and the microbes take over the decomposition of the waste (Lazcano *et al.,* 2008). Vermicomposting also leads to the formation of two products: earthworm biomass, which can further be processed to make earthworm meal for farm animals; and vermicompost (Figure 3), which makes good quality horticultural compost, is homogenous, has reduced levels of contaminants and holds nutrients for a longer period (Ndegwa *et al.,* 2000).

Figure 3: (a) Worms in vermicompost production unit. (b) Solid vermicompost (Source: www.realimpact.or.ke)

Vermicomposting is a faster way of obtaining compost from organic waste compared to the traditional microbial composting process. Vermicomposts and composts have different physical and chemical properties, and hence different effects on plant growth. This is due to differences in how they are produced (Lazcano *et al.,* 2009). Vermicomposts require less production time than composts. They are also finer in structure and retain nutrients for a longer time. In addition, unlike composts which are more abundant in ammonium, vermicomposts contain high amounts of nitrates, which are a more readily available form of nitrogen. Vermicomposts also have a more abundant supply of important nutrients like potassium and phosphorous and provide a more diverse microbial community than ordinary composts (Sinha, 2009). These properties of vermicompost give it an edge over ordinary composts.

Vermicomposting in Kenya is being caried out by both small scale and large scale farmers. A number of small scale farmers grow the earthworms and make the vermicompost using household and farm waste (http://www.nation.co.ke/business/seedsofgold/My-worms-my-money/-/2301238/2389514/-/qg9g4bz/-/index.html). Some large scale farms have also taken up the practice, making vermicompost for their own use and selling the surpus.

With regard to use as an organic soil amendment, low concentration of the humic acids in the amendment is said to stimulate metabolism of beneficial microorganisms, nutrient uptake and improvement of soil conditions (Gutierrez *et al.,* 2008). Silva *et al.,* (2012) suggested that the absorption and transport functions in fungal mycelia of AMF are favoured by humic substances like fulvic acids that result from the decomposition of organic fertilizers (Silva *et al.,* 2012). Cavender *et al.,* (2003) suggested that vermicompost stimulated fungal development directly or indirectly through its promotion of host root growth. Other theories that have been brought forward include provision of a large surface area for retention of nutrients in readily available forms, and presence of biologically active substances such as plant growth regulators (Cavender *et al.,* 2003).

In a study on effect of vermicomposting on kale production in Central Kenya, Savala *et al.,* (2003) observed that the farmers there did not practise nutrient recycling by reusing house

and farm waste as soil amendments. They found that vermicompost enhanced kale growth and soil conditions better than normal compost and inorganic fertiliser (Savala *et al.,* 2003). They also found that the vermicompost prepared from indigenus earthworms performed just as well as that prepared from *Eisenia foetida*, which is the most commonly used earthworm.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The field experiments were conducted in Naivasha, Kenya, located about 1700m above sea level in the Rift Valley region (Figure 4). Average annual rainfall occurs in bimodal peaks from March-May and October-December and is about 1100mm. Maximum temperatures are about 25°C in the dry season in January and February (www.icipe.org). The area has seven major landscape units: lacustrine plain, volcanic plain, highland, high plateau, low plateau, step-faulted plateau & volcanic lava-flow plateau. The study area (Sulmac farm) falls under the lacustrine plain. The area has almost flat to gently undulating topography, deep soils, dark olive brown to olive brown, loamy sand to loam, and weak to very weak subangular blocky structure. The dominant soil types include Eutric Cambisols, Areni-Vitric Andosols, Pach-Sodic Phaeozems, Calcic Cambisols and Cheomic Cambisols. The sandy nature of the topsoil in the area affects the water and nutrient holding capacity of the soils (Girma *et al*., 2001).

Figure 4: Location of Naivasha town in a map of Kenya. The study was carried out in the Sulmac area in the lacustrine plain of Naivasha. (www.getbirding.com)

3.2 Study Design

The field experiment consisted of seven treatments used as soil amendments, in plots on which soybean was cultivated. The treatments were as follows: Biochar (B), Vermicompost (V), Mycorrhiza (M), Biochar+Mycorrhiza (BM), Vermicompost+Mycorrhiza (MV), Biochar+Vermicompost+Mycorrhiza (BVM), and control or no treatment (NT). All the treatments were arranged in a complete randomised block design with three replicates (Figure 5).

TREATMENTS

Replications		40m							
1	NT	B	MV	BVM	V	BV	M	BM	
2	BM	M	BV	BVM	NT	MV	V	B	
3	BM	B	BVM	NT	V	BV	M	MV	12m
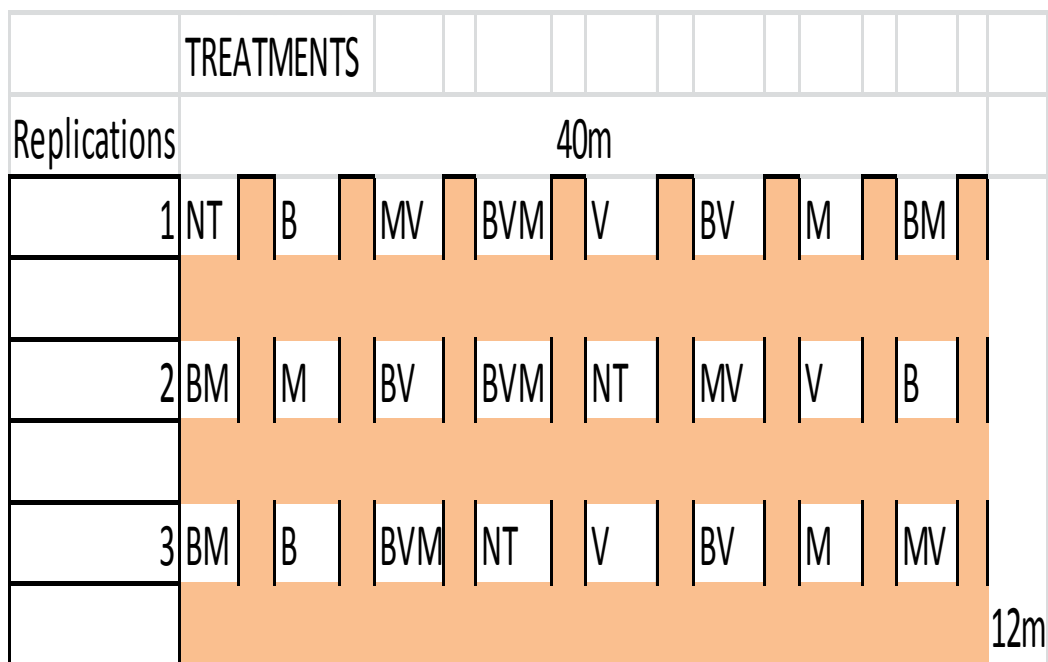
	1m wide footpath in between blocks
	each block is 3x2m
B	Biochar
V	Vermicompost
M	Mycorrhiza
BM	Biochar+Mycorrhiza
VM	Vermicompost+Mycorrhiza
VB	Vermicompost+Biochar
BVM	Biochar+Mycorrhiza+Vermicompost
NT	No treatment

Figure 5: Layout of plots treated with seven different treatments for the study.

Land measuring 40m by 12m was cleared. Sixty soil samples weighing 500g were collected using a soil auger, from a depth of 1cm to 20cm from different random spots in each experimental plot. The plot was then sub- divided into 24 sub-plots each measuring 3m by 2m. The sub-plots were separated by paths measuring 1m wide (Figure 6).

Figure 6: Pictures displaying sowing of soybean seeds and germinated shoots.

Individual holes were dug for sowing the soybean seeds at a spacing of 30 cm by 30 cm. Biochar used was obtained from farm and forest waste and characterised in Table 1. It was applied to these holes and mixed with the soil and was applied at the rate of 6.75kg per plot or 11.25t/ha, in plots which require biochar alone (B), Biochar+Mycorrhiza (BM), biochar, mycorrhiza and vermicompost (BVM) and biochar and vermicompost (BV).

The mycorrhizal treatment (Rhizatech[(R)]) was supplied by Dudutech Ltd., at the rate of 2.25l per plot or 46.875l/ha. Rhizatech contains spores, colonised root fragments, and other arbuscular mycorrhizal fungi mycelial fragments at a concentration of 50 propagules per cubic centimetre of the product. The fungal components are contained in a granular carrier. The treatment was applied to the hole before sowing the seeds in plots requiring mycorrhiza alone (M), biochar and mycorrhiza (BM), vermicompost and mycorrhiza (MV) and biochar,

vermicompost and mycorrhiza (BVM). Vermicompost (Vermitech$^{(R)}$), an organic fertiliser made from digestion of organic matter by *Eisenia andrei* worms, was also provided by Dudutech Ltd. The vermicompost was applied to the soil at the time of sowing at the rate of 6.75kg per plot of 11.25t/ha in the plot requiring vermicompost alone (V), vermicompost and mycorrhiza (MV), vermicompost and biochar (BV) and all combinations: biochar, vermicompost and mycorrhiza (BVM). Chemical characteristics of the vermicompost used are shown below (Table 1). Soybean seeds were planted in each treatment plot at the rate of 90 seeds per plot with 2 seeds in every hole, with a spacing of 30cm in between the rows. The field was maintained under overhead sprinkler irrigation. Weeding was done manually.

Table 1: Chemical characteristics of treatments used during study

SAMPLE DESCRIPTION	pH	N (%)	C (%)	K(Cmol/kg)	P(ppm)
VERMICOMPOST	8.5	1.54	13.18	40	1130
BIOCHAR	9.15	1.33	6.58	2	50

3.3 Sampling of Soil and Plant Tissues

At flowering time, ten soybean plants in each plot were randomly selected and carefully uprooted so as to obtain the roots. Roots were cut off from the shoot with some of the surrounding soil, wrapped in damp paper and sealed in labelled polythene bags. These were analysed for root colonisation. At harvest time, soybean pods were harvested, weighed and spread out to dry. After one week, the soybean seeds were manually extracted from the dry pods and weighed. Ten sample plants were carefully uprooted and the shoot and root weight taken. Ten soil samples were also collected from each treatment plot for analysis of chemical properties and characterisation of mycorrhizal spores.

3.4 Samples Preparation

3.4.1 Spore Extraction

The soils were pre-soaked before processing, then mixed with water, stirred and decanted through 710 and 45 micron sieves. This process was repeated several times, and any lumps present were broken between washes. The water passing through the sediment on the 45 micron sieve was collected into four 50ml centrifuge tubes, which were balanced by weight

and centrifuged for 5 minutes at 1750 rpm. Water from the tubes was decanted out and floating debris discarded as well. 48% sucrose was added to the pellet in the tube, and the substance mixed thoroughly and balanced by weight, before centrifuging for 15 seconds at 1750 rpm. The sucrose solution was carefully decanted through a small 45 micron sieve. This was done by passing water through the sediment on the sieve. Spores retained on the sieve were rinsed thoroughly with water to wash out the sucrose. The spores were transferred into a small Petri dish for examination using a dissecting microscope. Spore types were distinguished using spore colour, size and attachment of hyphae on spore surface. Colours were identified using the Edinburgh Botanic Gardens colour chart for fungi. Specimens for each morphotype were examined further under a compound microscope and described using spore wall characteristics, type of spore wall, number and size of layers and reaction to Meltzer's reagent. These features were compared and matched with species from the INVAM database (http://invam.wvu.edu/the-fungi/species-descriptions) and the University of Agriculture, Poland, Department of Plant Pathology database. (http://www.zor.zut.edu.pl/Glomeromycota/Species%20descriptions%20of%20AMF.html). Spores were enumerated for each morphotype.

3.4.2 Soil Analysis

Soil samples collected were analysed for total carbon, nitrogen, pH and available phosphorous at soil laboratories at Kenya Agricultural and Livestock Research Organisation (KALRO). Organic carbon was determined calorimetrically after H_2SO_4-dichromate oxidation at 150^0C for 30 minutes (Mathimaran *et al.,* 2012). Total Nitrogen was determined by Kieldahl digestion with sulphuric acid and selenium as a catalyst and was estimated calorimetrically (Mathimaran *et al.,* 2012). Soil pH was measured in acqueous suspension (1:2.5 w:v), while phosphorous was extracted with 0.5M $NaHCO_3$+0.01M ethylenediaminetetraacetic acid (EDTA) pH 8.5 modified Olsen using a 1:10 soil/solution ratio (Mathimaran *et al.,* 2012).

3.4.3 Root Analysis

Roots were soaked in water to loosen attached soil particles, and washed in a strong flow of water over a 2mm mesh sieve, with a 0.25mm mesh sieve placed underneath so as to ensure that no root fragments are lost. The roots were cut into 1 cm fragments, carefully cleaned with tap water and washed using deionised water. They were then transferred to modified syringes. A modified syringe consists of a normal syringe with the needle section removed

and plastic mesh (0.5mm) cut from a tea strainer placed across the mouth of the tube holding the plunger. Thus when the plunger is pushed down, any liquid in the tube is forced out while solid material is trapped on the mesh.

The syringes were arranged in beakers containing 2.5 % potassium hydroxide (KOH) and the plunger pulled back to let some of the liquid into the tube so as to mix with the roots. The beakers were then autautoclaved at 121°C for 15 minutes. The 2.5 % KOH was then expelled from the modified syringes, leaving the roots on the mesh. The roots were then rinsed in water by passing water through the syringes. The syringes containing the root fragments were then placed in beakers containing alkaline hydrogen peroxide so as to bleach them, and again rinsed in water. The root fragments were then placed in 1% hydrochloric acid (HCL) for one hour, and subsequently stained in 0.05% trypan blue in acidic glycerol by autoclaving at 121°C for 3 minutes and finally washed out into a Petri dish.

The roots were cut into pieces approximately 5mm in length. Fifteen drops of glycerol were placed on a slide, and fifteen root fragments were laid out, one on each drop. Another slide was placed on top of the previous one to protect the root fragments. The fragments were then scored under a compound microscope for extent of root colonisation. Thirty fragments from each sample were observed.

The method described by Machua (2002) was used to score occurrence of mycorrhiza in the soybean roots. Scoring was done by observing the occurrence of mycorrhizal hyphae, vesicles and arbuscules and comparing with the chart shown in figure 7. Presence of mycorrhizal hyphae was given by a number between 0-5. This was followed by a number preceded by the letter A and ranging between 0-3 indicating presence of arbuscules. For example, a root fragment described as 4A3 would have >50% mycorrhizal presence and abundant arbuscules. These parameters were entered into Mycocalc software to calculate the parameters F%, (frequency of mycorrhiza in root system) M% (intensity of Mycorrhizal colonization in root system), m% (intensity of the mycorrhizal colonisation in the root fragments), a% (arbuscule abundance in mycorrhizal parts of root fragments) and A% (arbuscule abundance in root system). The parameters analysed were (F %), (M %), and (A %).
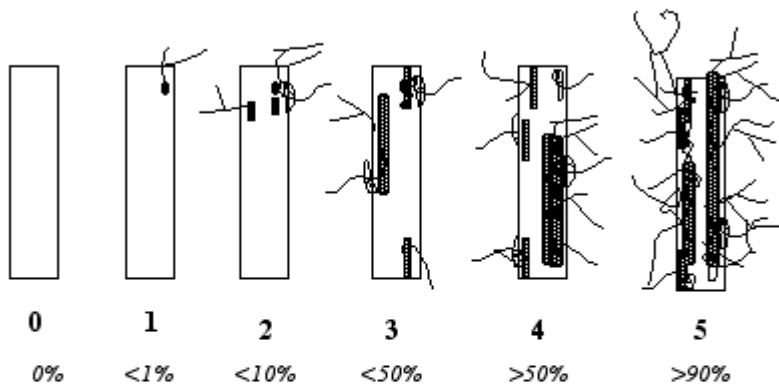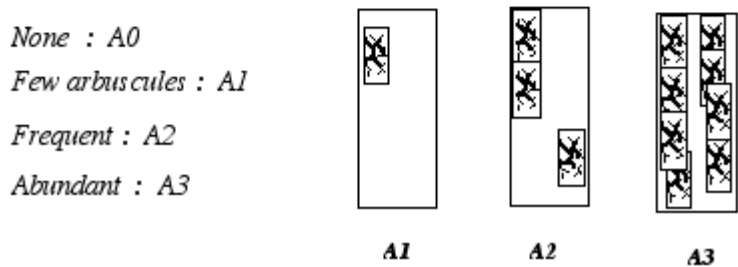
Figure 7: Chart showing how to score mycorrhizal colonization of infected roots (Machua, 2002).

3.5 Data Analysis

After fulfilling the assumption of normality and homogeneity of variances, the data was subjected to analysis of variance (ANOVA) to test the differences in AM colonisation and spore populations. Mean separation was done by Fisher's least significant difference (LSD) at the 0.05 level of probability (Muchane *et al.,*2012). The analysis was carried out using Genstat software.

CHAPTER FOUR

RESULTS

4.1 Characterisation of the Study Area

At the beginning of the experiment, soil phosphorous content was 159 ppm. The soil pH level was at 7.38, while nitrogen content was 0.51%. Organic carbon content of the soil before application of treatments was recorded at 0.84% as indicated in Table 2.

Table 2: Soil, vermicompost and biochar chemical characteristics before the experiment.

SAMPLE DESCRIPTION	pH	N (%)	C (%)	P(ppm)
SOIL	7.38	0.51	0.84	159
VERMICOMPOST	8.5	1.54	13.18	1130
BIOCHAR	9.15	1.33	6.58	50

A total of 2390 isolates were characterized as arbuscular mycorrhizal fungi (AMF) before application of amendments. They were grouped into four genera and ten species. *Scutellospora* was the most diverse genus with a total of four species, followed by *Glomus* with a total of three species, with *Acaulospora* and *Gigaspora* having one species each. The most frequent species was *Glomus etunicatum* with a mean occurrence of 23.09 spores followed by *Glomus mosseae* and *Glomus intraradices,* with a mean occurrence of 17.74 spores and 17.03 spores respectively in that decreasing order (Table 3).

Table 3: Frequency of arbuscular mycorrhizal fungi spores isolated from the soil before application of treatments (*Glomus etunicatum, Glomus mosseae, Glomus intraradices, Scutellospora nigra, Scutellospora verrucosa, Acaulospora denticulata, Scutellospora calospora, Gigaspora albida, Scutellospora pellusida*).

Isolate	Rank	No. of isolates	Mean	Percentage

Glomus etunicatum	1	531	23.09	22.2
Glomus mosseae	2	408	17.74	17.1
Glomus intraradices	3	398	17.03	16.7
S.nigra	4	292	12.70	12.2
S. verrucosa	5	274	11.91	11.5
A. denticulata	6	250	10.87	10.5
S. calospora	7	123	5.35	5.1
Gigaspora. albida	8	73	3.17	3.1
S. pellusida	9	41	1.78	1.7

4.2 Effect of soil amendments on soil chemical characteristics

The phosphorous content in the soil increased after addition of soil amendments. The V plot had the highest phosphorous content at 262 mg/kg, followed by the BVM plot and the B plot (Table 4). The M plot had the lowest phosphorous content at 215 mg/kg. The soil pH at the end of the experiment increased in some treatment plot and reduced in others. The B plot had the highest pH of 7.46, followed by that of the BV and BVM plots. The M plot and the NT plot had the lowest pH of 7.17 and 7 respectively (Table 4).

Percentage nitrogen content in the soil decreased after application of amendments, with the highest nitrogen content being recorded in the MV treatment plot at 0.14%. The lowest percentage nitrogen was recorded in the V, B and NT plots, at 0.098%. Carbon content in the soil increased as a result of application of amendments. The BVM plot had the highest carbon content of 1.08 (Table 4). The lowest carbon content of 0.86 was found in the M plot (Table 4).

Table 4: Soil chemical characteristics before and after application of amendments.

	P(Mg/Kg)	Ph	%N	%C
Before treatments	159	7.38	0.51	0.84
After treatments	P(Mg/Kg)	pH	%N	%C
M	215	7.17	0.112	0.86

B	250	7.46	0.098	0.9
MV	247	7.36	0.14	0.95
BV	243	7.41	0.1	1.03
BVM	250	7.4	0.112	1.08
NT	245	7	0.098	0.99
BM	236	7.28	0.112	0.91
V	262	7.36	0.098	1.03

There was a 64.78% increase of phosphorous content in the V plot. This was followed by the BVM plot and the B plot, with an increase of 57.23%. The M plot had the lowest phosphorous content but was still 37.11% higher than the initial soil phosphorous content. The B plot had an increase of 1.08% from the initial pH, followed by that of the BV plot with an increase of 0.41% and BVM with an increase of 0.27% in that decreasing order. The MV plot had a 72.54% drop from the initial nitrogen in the soil. The V, B and NT plots, had an 80.78% drop from initial percentage nitrogen. The BVM plot had 28.22% higher carbon content than the initial carbon content in the soil, followed by both BV and V plot which had a 22.62% increase. The lowest increase in carbon content was in the M plot at 2.38% (Table 5).

Table 5: Percentage change in soil chemical characteristics after application of amendments.

Treatment	P(Mg/Kg)	pH	%N	%C
B	57.23	1.08	-80.78	7.14
BV	52.83	0.41	-80.39	22.62
BVM	57.23	0.27	-78.04	28.57
V	64.78	-0.27	-80.78	22.62

MV	55.34	-0.27	-72.55	13.1
BM	48.43	-1.36	-78.04	8.33
M	37.11	-2.85	-78.04	2.38
NT	54.09	-5.15	-80.78	17.86

4.3 Effect of soil amendments on mycorrhizal spores

A significant difference ($P<0.05$) was observed on the population of arbuscular mycorrhizal fungi (AMF) spores as a result of different soil amendments. The highest number of AMF spores was isolated from the plot amended with vermicompost (V), with 1,348 isolates, followed by biochar (B) with 1,325 isolates, and the MV plot with 1,242 in that decreasing order (Figure 8).
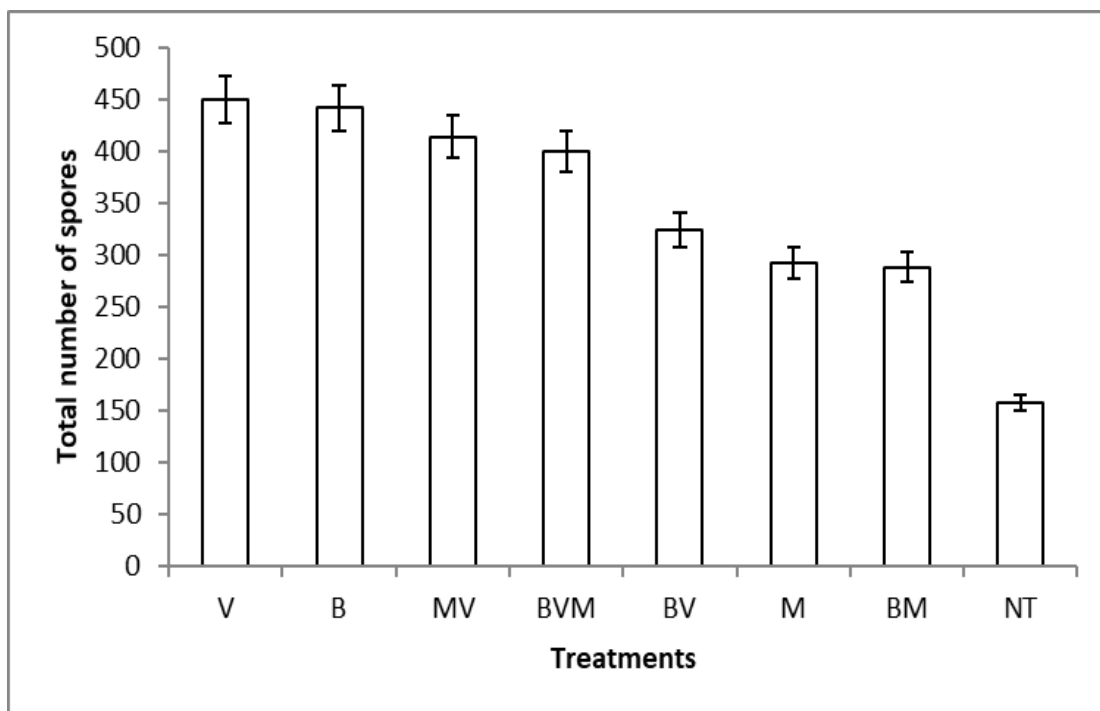

Figure 8: Total occurence of Arbuscular Mycorrhizal fungi across different organic soil amendments applied. Vermicompost (V), Biochar (B), Mycorrhiza+Vermicompost (MV),

Biochar+Mycorrhiza+Vermicompost (BVM), Biochar+Vermicompost (BV), Mycorrhiza (M), Biochar+Mycorrhiza (BM), No Treatment (NT)

Glomus etunicatum, was the most frequently isolated species with a mean of 117 spores, followed by *Glomus intraradices* at 79.6 spores, *Glomus mosseae* with 61.7 spores in that decreasing order. The least frequently occurring species was *Gigaspora albida* with a mean of 3.04 spores. The most frequently occurring genus was *Scutellospora*, with 4 species, while the least frequently occurring genus was *Gigaspora* (Table 5). Figure 9 shows the different genera isolated after the study while table 6 shows the characteristics of arbuscular mycorrhizal species identified.
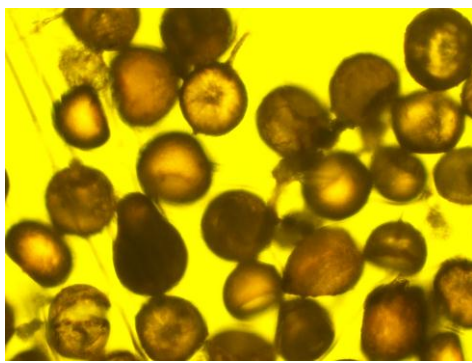
(a)
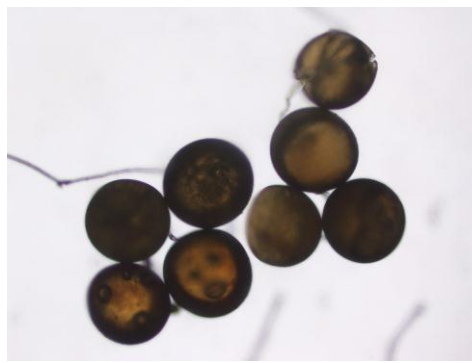
(b)
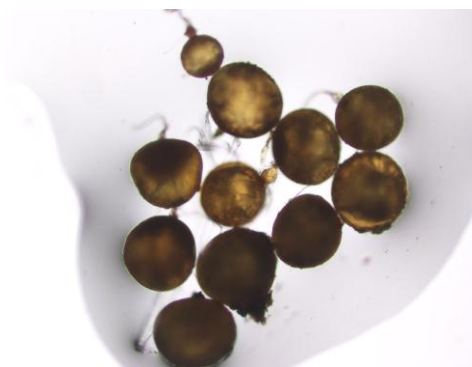
(c)

Figure 9: Arbuscular mycorrhizal spores isolated at the end of the experiment at genus level. (a) *Glomus* sp (b) *Scutellospora* sp (c) *Gigaspora* sp

Table 6: Diversity of arbuscular mycorrhizal fungi isolated after the study

Isolate	Rank	No. of isolates	Mean	Proportion	Cum frequency	P value (P=0.05)
Glomus etunicatum	1	2797	117	33.7	33.7	0.307

Glomus intraradices	2	1910	79.6	23	56.7	0.018
Glomus mosseae	3	1481	61.7	17.8	74.6	0.073
Acaulospora denticulata	4	766	31.9	9.2	83.8	0.021
Scutellospora nigra	5	605	25.2	7.3	91.1	0.032
Scutellospora verrucosa	6	409	17	4.9	96	0.197
Scutellospora calospora	7	166	6.92	2	98	0.563
Scutellospora pellusida	8	90	3.75	1.1	99.1	0.06
Gigaspora albida	9	73	3.04	0.9	100	0.026

Table 7: Morphological characteristics of arbuscular mycorrhizal spores isolated after application of amendments

ISOLATE	CHARACTERISTICS
Glomus etunicatum	Colour: pale yellow to yellow Shape: subglobose to globose, sometimes ovoid subtending hypha Size: 75-135 μm diameter Spore wall: two layers
Glomus mosseae	Colour: pale yellow to golden yellow Shape: subglobose to globose. Size: between 80-280 μm diameter subtending hypha Spore wall: 3 layers
Glomus intraradices	Colour: pale yellow to greyish yellow, greenish tint Shape: globose to subglobose Size: 30-120 μm diameter Spore wall: 3 layers
Scutellospora calospora	Colour: Pale yellow with a greenish tint to yellow-brown with greenish tint in older spores. Shape: from subglobose to ellipsoid to oblong, at times irregular Size: 120-220 μm diameter
Scutellospora pellucida	Colour: Hyaline/white in most recently formed spores to yellow-brown (0-5-40-0) in older spores (especially those from field soils). Shape: subglobose, often elliptical or strongly oblong. Size: 120-240 μm diameter Bulbous subtending hypha
Scutellospora nigra	Colour: Dark red-brown to black

	Shape: mostly globose Size: 240-520 μm. Subtending hypha
Acaulospora dentisculata	Colour: Pale orange-brown to dark orange-brown. Shape: Globose to sub-globose Size: 120-180 μm
Gigaspora albida	Colour: Cream with pale green tint Shape: Globose to subglobose Size: 200 – 280 μm Subtending hypha

Glomus etunicatum was ranked first in frequency of occurrence both before and after application of amendments. The population of *Glomus intraradices* surpassed that of *Glomus mosseae,* which ranked second as the most frequently occurring isolate before the experiment. *Acaulospora denticulata* also occurred more frequently than *Scutellospora nigra* at the end of the experiment. The population of *Scutellospora pellusida* was also higher than that of *Gigaspora albida* at the end of the experiment (Figure 10).
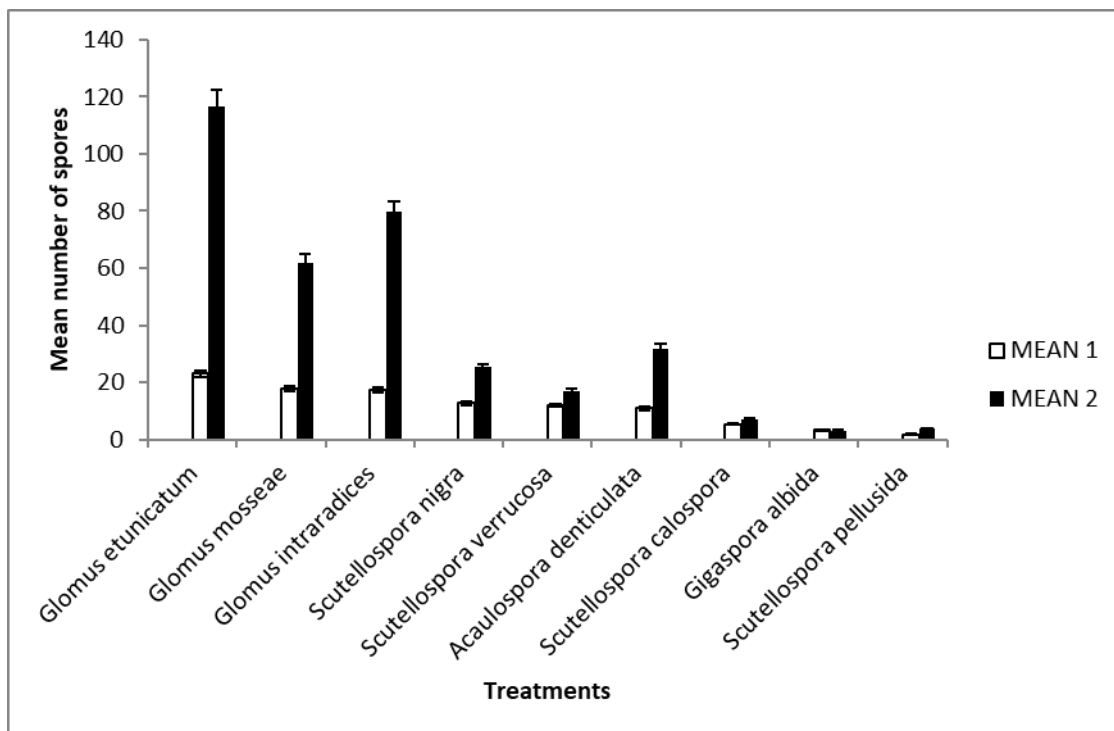

Figure 10: Mean occurrence of Arbuscular Mycorrhizal Fungi before and after the experiment. G.e: *Glomus etunicatum*, G.m: *Glomus mosseae*, G.i: *Glomus intraradices*, S.n:

Scutellospora nigra, S.v: *Scutellospora verrucosa*, A.d: *Acaulospora denticulata*, S.c: *Scutellospora calospora*, Gi. a: *Gigaspora albida*, S. p: *Scutellospora pellusida*.

4.4 Effects of soil amendments on root colonisation

Root samples collected after the experiments exhibited colonisation by arbuscular mycorrhizal fungi hyphae and formation of vesicles and arbuscules (Figure 11). No significant difference (P=0.926) was observed on both the frequency (F %) and intensity (M %) of mycorrhiza in the root system as a result of applying different soil amendments on different plots. However, all treatments had significantly (P<0.05) higher levels of colonization than those of the control. A significant difference (P<0.05) was observed on the percentage of arbuscules in the root system as a result of applications of different soil amendments (Table 7).

(a)   (b)
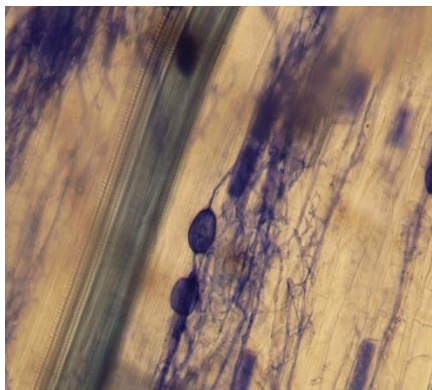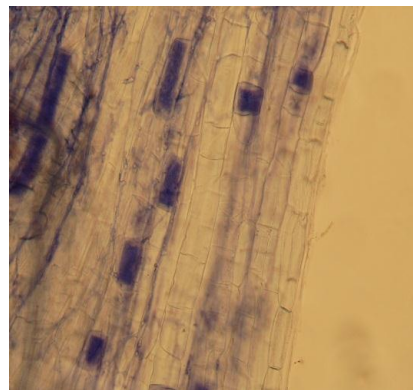
Figure 11: (a) Vessicles of arbuscular mycorrhizal fungi in roots at flowering time (b) Arbuscules of arbuscular mycorrhizal fungi in roots at flowering time.

Table 8: Mean occurrence of mycorrhiza in root system of soybean plants (F%‑ Frequency of mycorrhiza in root system, M%‑ Intensity of mycorrhizal colonisation in root system, m%‑ Intensity of mycorrhizal colonisation in root fragments, a%‑ Arbuscule abundance in mycorrhizal parts of root fragments, A%‑ Arbuscule abundance in root system. Treatments with different letters within columns are significantly different.

TREATMENT	F%	M%	m%	a%	A%
MV	92.2a	22.7a	24.6ab	42.0ab	9.5ab
B	88.9a	23.4a	27.4ab	50.4a	11.9ab
BVM	86.7a	22.6a	26.0ab	43.6ab	10.2ab
BV	83.3a	25.3a	30.2a	56.0a	14.7ab
V	80.0a	23.7a	29.1a	54.4a	14.2a
BM	76.7a	21.8a	28.6a	55.6a	12.0ab
M	75.6a	21.3a	27.2ab	52.8a	14.0a
NT	56.7b	5.8b	11.0b	20.5b	1.2b
Mean	80	20.8	25.5	46.9	10.5
LSD (0.05)	17.3	15.1	17.2	29.4	12.3
CV (%)	12.4	41.3	38.5	35.8	66.7

4.5 Effect of soil amendments on soybean plants and yield

At harvest time, the B plot had the highest percentage change in shoot weight, at 235.29% up from the weight taken at flowering time, followed by the BM plot and the M plot, in that decreasing order. The highest percentage change in root weight was recorded in the V plot, followed by the BM plot and the M plot in that decreasing order (Table 9).

Table 9: Percentage changes in shoot and root weight of soybean plants at the end of the study.

	Shoot weight			Root weight		
Treatment	Flowering (kg)	Harvest (kg)	%change	Flowering (g)	Harvest (g)	%change
MV	0.49	0.76	55.1	70.1	74.2	5.85
BM	0.38	1	163.16	51.71	73.5	42.14
V	0.4	0.95	137.5	51.04	88.7	73.79
B	0.34	1.14	235.29	78.6	86.23	9.71
NT	0.56	0.87	55.36	70.05	77.07	10.02
M	0.52	1.12	115.38	51.39	70.4	36.99
BVM	0.46	0.7	52.17	71.75	78.53	9.45
BV	0.52	0.75	44.23	77.36	66	-14.68

At harvest time, the B plot recorded the heaviest weight in yield of soybean seeds, at a mean of 171.28g, followed by the V plot and the BVM plot. Compared to the other treatment plot, the block treated with M recorded the lowest yield of soybean seeds at 58.17g (Figure 12).
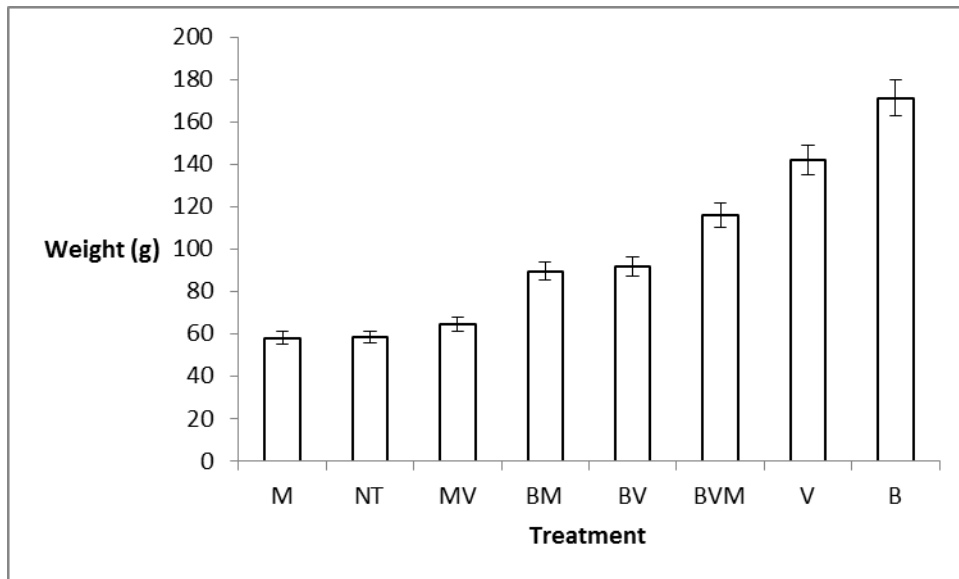

Figure 12: Yield of harvested soybean seeds across treatments.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

5.1 Discussion

From this study, phosphorous content in the soil increased after application of soil amendments. Plots that were treated with vermicompost registered high levels of phosphorous. This could be due to the fact that the vermicompost used as an amendment had high levels of phosphorous. Jain *et al.,* (2012) reported that application of vermicompost increased the N, P and K content of the soil. The results in this study also concur with Mau and Utami (2014), who found that application of biochar and AM fungi significantly increased total soil P content. They suggested that the increase in phosphorous was related to the supply of high surface area provided by biochar which created a better habitat for the development and activities of soil micro-organisms, which in turn increased the availability of soil phosphorous.

Plots treated with the biochar recorded higher levels of pH than the control plot. This could be attributed to the high initial pH recorded on the biochar that was used in the study. These results agree with Laufer and Tomlinson (2013) who found that application of biochar as a soil amendment increased soil pH. Plots treated with vermicompost and mycorrhiza recorded pH lower than the initial pH. The addition of the amendments probably produced a favourable environment for development of micro-organisms, whose metabolic processes resulted in an increase in acidity in the soil. These results agree with Jain *et al.,* (2012) who also reported a reduction in soil pH after application of vermicompost and mycorrhiza to soils in orchards.

The nitrogen content across all treatments generally reduced. This was attributed to the fact that no nitrogen fertiliser was added to the soil prior to planting. The land used in the study had no history of legume production. This meant that the plants utilised the available nitrogen prior to fixing nitrogen to the soil, leading to further depletion of the nitrogen already

available in the soil (Mathu et al., 2012). In addition, according to Lehmann (2003), biochar has been known to decrease nitrogen availability. Elmer and Pignatello (2011) reported a reduction in soil nitrogen after application of biochar. Warnock *et al.,* (2010) proposed that biochar may in the short term reduce ammonification either in nitrogen availability due to immobilization during initial decomposition of the nitrogen- poor biochar or by a reduction in carbon cycling. Carbon content in the soil increased as a result of application of amendments.This could be due to the fact that the amendments provided a suitable environment for development of micro-organisms, whose metabolic activities led to an increase in carbon. Jain *et al.,* (2012) reported an increase in soil carbon content after application of vermicompost. In another study, application of biochar to soils led to an increase in soil organic carbon, among other nutrients (Laufer and Tomlinson, 2013).

The addition of organic amendments to the soil enhanced mycorrhizal sporulation. Moreover, the addition of commercial strains of mycorrhiza did not result in an increase in sporulation. The amendments may have enhanced development of both indigenous and introduced mycorrhizal fungi while the commercial strains introduced in some of the blocks may have taken time to adjust to the environment. These results agree with Mau and Utami (2014) who found that a plot treated with biochar alone recorded the highest abundance of spores, compared to plots treated with a combination of biochar and mycorrhiza, and attributed this to the slow adjustment of the introduced mycorrhizal species to the environment. The results of this study also agree with Coelho *et al*., (2012), who found that substrates treated with vermicompost produced the highest number of mycorrhizal spoeres compared with substrates treated with coir dust and Topstrato[TM]. They attributed this to the high levels of phosphorous in the substrate provided by vermicompost, which played an improtant role in production of AM fungi propagules. Another study by Parmar *et al.,* (2011) showed that vermicompost was the best substrate for the production of mycorrhizal spores. This was also attributed to increased supply of minerals by vermicompost.

In terms of diversity, the genus *Glomus* recorded the highest spore count while *Gigaspora* recorded the lowest. It should be noted that the commercial mycorrhiza added to some of the plots contained *Glomus* sp. This, coupled with the presence of *Glomus* species in the soil before addition of the amendments, could explain the high spore count recorded. These results are consistent with a study carried out by Silva *et al.,* (2012) on the occurrence of AM fungi after organic fertilization, who found that *Glomus* species had the highest presence,

presumably because species in this genus generally predominate in a pH of 6.0 to 8.0. The soil pH during this experiment ranged from 7.0 to 7.41. In addition, *Glomus* has the capacity to adapt to different organic matter contents in the soil. Furthermore, genera such as *Glomus* and *Acaulospora* produce small spores and are able to survive by adapting their sporulation patterns under unfavourable conditions like aridity (Silva *et al*., 2012). The results of this study also agree with Coelho *et al*., (2012), who found that members of the Gigasporaceae family generally produce few spores and are incapable of colonising from hyphal fragments.

In this study, the population of *Acaulospora* sp exceeded that of the initial population of *Scutellospora* sp. It seems that some species respond better to organic fertilisation than other species. According to Coelho *et al.,* (2012), this could be due to the addition of phosphorous to the soil by the organic amendments, because this nutrient has an important role in the regulation of production of AMF propagules. In addition, organic amendments reduce soil density and increase soil porosity, both of which are advantageous to sporulation.

These results concur with Oehl *et al.,* (2010), who reported a high frequency of occurence of *Acaulospora* species and a low frequency of occurence of *Gigaspora* and *Scutellospora* in areas with organic fertilisation. Contrary to the results of this study, Mathimaran *et al.,* (2012) found that spore densities in the soil were not affected by addition of phosphorous. Silva *et al.,* (2012), reported negative responses of AM fungi due to the incorporation of organic residues to high nutrient content of these materials, presence of phytotoxic substances, specific composition of the residue and pressure of pathogens.

In this study, mycorrhizal spore diversity at the end of the experiment did not reflect root colonization by mycorrhiza. The root colonization by AM fungi in the BV plot was significantly higher than in that the V plot. It was also higher than that in the B plot, although the difference was not significant. Gai *et al.,* (2009) observed that spore populations do not directly reflect the AM fungal communities actually colonising plant roots. Coelho *et al.,* (2012) also observed that colonization produced by the AMF in their experiment was not related with the number of infective propagules. Reasons given for this include long dormancy period of some species like *Acaulospora longula*, production of very few spores e.g. by *Gigaspora*, inability to colonise roots using hyphal fragments for example by *Gigaspora*, and use of hyphae instead of spores in the root colonization process by some species.

In another experiment (Oehl *at al.,* 2010) it was noted that mycorrhizal root colonization and spore numbers do not necessarily reflect the AMF populations on the soil. This is because freshly formed spores are not readily distinguished from spores formed earlier in the season. Some species also not sporulate, and thus would not be detected in the field samples. In addition, species that may not sporulate in the field may sporulate in pot trap cultures. This could explain the absence of new species in the field samples collected after the experiment. The trap culture method is recommended for the purpose of detecting species that may not sporulate under field conditions but can do so in trap pots. In the above-mentioned experiment, species that were not found in spore analysis from a number of field sites, sporulated subsequently in the corresponding trap cultures. This was due to the different environmental conditions in the trap pots as compared to that in the fields (Mathimaran *et al.,* (2012). However, because environmental conditions in the pot cultures are different from those in the field, relative species abundance and diversity observed in the pots may also differ from spore diversity in the field soils. Consequently, measures of diversity estimated in the pot cultures should be treated carefully as they may have limited relevance to the situation in the field (Mathimaran *et al.,* 2012). Use of recently developed molecular methods in combination with morphological tools to present a more complete picture of AMF communities in the plant roots and soils has also been suggested (Gai *et al.,* 2009; Oehl *et al.,* 2010). Still, this method is also limited due to the expenses involved and the lack of adequate primers to cover the whole range of AM fungi and the difficulties involved in assigning sequences to taxonomic units (Gai *et al.,* 2009).

All the plots treated with soil amendments resulted in significantly ($P<0.05$) higher root colonisation than the NT plot. The B and BM plots came fourth and fifth respectively, while the BVM plot had the second lowest root colonisation rates. This indicates that the soil amendments enhanced the activities of the already-present mycorrhiza species in the soil without requiring the addition of commercial mycorrhiza. The results from this study show that the net effect of biochar on root colonisation was negative. These results agree with Warnock *et al.,* (2010) who reported that application of biochar at different rates resulted in neutral to decreased AM fungi abundance, which was measured by percent root colonisation and/ or extraradical hyphae production. The study suggested that biochar's capacity to adsorb signaling compounds and act as a sink could decrease ability of mycorrhizal fungi to colonise plant roots. Permanent removal of signal molecules from soils could result in a net decrease in the number of signal molecules reaching mycorrhizal hyphae and spores. This leads to a

decrease in hyphal growth and spore germination and ultimately, fungal abundance. In addition, biochar could also adsorb compounds toxic to mycorrhizal fungi (Warnock *et al*., 2010). However, contrary to the results in this study, positive effects on AM fungi root colonisation as a result of application of biochar to soil have been reported. Solaiman *et al*., (2010) found that AM fungi colonisation increased significantly in the biochar treatment for wheat grown in well-watered and periodic water stressed environments. Elmer and Pignatello (2011), found that biochar had a positive linear effect on percent root colonization by arbuscular mycorrhiza.

The results of this study show that vermicompost improved colonisation of soybean roots by mycorrhiza. It could be said that the combination of biochar and vermicompost provided favourable conditions for colonization of the soybean roots by arbuscular mycorrhiza species that were already present in the soil. This result is consistent with previous findings by Silva *et al.,* (2012), who suggested that transport and absorption of nutrients in mycelia of AM Fungi were favoured by humic substances like fulvic acids that result from the decomposition of organic fertilisers. Cavender *et al.,* (2013) found that vermicompost stimulated colonization of sorghum roots after mycorrhizal inoculation in peat medium. The results of this study differ with Copetta *et al*., (2012) who found that mycorrhizal colonisation and arbuscule formation significantly decreased with the increase of green compost in soil. In addition, Gutierrez-Miceli *et al.,* (2008) found that high concentrations of vermicompost inhibited mycorrhization of maize roots, and attributed this to the hormone-like characteristics of humic acids and their effects on soil properties. Coelho *et al*., (2012) suggested that some substances present in organic composts could have a phytotoxic effect and/ or inhibit the development of AM fungi.

The highest soybean harvest was recorded in the biochar and vermicompost treatment plots respectively. This indicated that in addition to enhancing colonisation of soybean roots by mycorrhiza, soil amendments also enhanced plant growth and yield. In previous study, application of biochar increased yield of maize over the control plot by 2.2 tonnes per hectare (Laufer and Tomlinson, 2013). Shishehbor *et al.,* (2013) also reported that biological yield was greater when vermicompost was applied along with azotobacter and arbuscular fungi, while according to Copetta *et al.,* (2012), the best dry weight yield occurred at compost rates of 75% and AM fungi application. Contrary to the results in this study, Cavender *et al.,* (2003) suggested that while vermicompost stimulated fungal development, as much as 20%

of the total carbon assimilated by the sorghum plants may have been taken up by the mycorrhizal fungi. They concluded that the effect of vermicompost on mycorrhizal colonization was harmful, rather than beneficial, to plant growth. Contrary to the results in this study, Warnock *et al.,* (2010) reported that plant biomass production was not significantly affected by addition of biochar. The mycorrhiza plot recorded the lowest harvest of soybean seeds compared to the blocks treated with biochar and / or vermicompost. This could mean that these amendments enhanced the activities of the beneficial microorganisms in the soil, including indigenous mycorrhiza, by improving the soil properties, and creating a conducive environment for their development, thus leading to higher yields. In their study, Copetta *et al.,* (2012), the best dry weight yield occurred at compost rates of 75% and AM fungi application.

5.2 Conclusions and Recommendations

The following are the conclusions from the study:

1. The study showed that application of organic amendments to soil increased abundance of arbuscular mycorrhizal fungi in the soil by providing favourable conditions for growth and sporulation of indigenous mycorrhiza in the soil.

2. The study also showed that application of organic amendments increased carbon and phosphorous content, thus providing nutrients for the growth and development of mycorrhizal spores and soybean plants.

3. It also showed that application of amendments to soil enhances colonisation of soybean roots by arbuscular mycorrhizal fungi. From the study, it was found that the amendments enhanced the activities of AM fungi that are indigenous to the soil without the need to apply additional commercial strains. Plots that were treated with biochar and vermicompost showed greater colonisation than plots treated only with mycorrhiza. Organic amendments can thus be used to improve colonization of plant roots by arbuscular mycorrhizae without the need to add commercial strains to the soil.

4. The amendments both encouraged plant growth through improvement of soil characteristics and enhancement of the action of indigenous mycorrhiza. The highest yield of soybean was in the plots treated with biochar and vermicompost respectively. It is evident that organic amendments and biochar have a positive impact on soil microbial organisms. They can improve the soil environment to aid the action of indigenous organisms, and also have a positive impact on general plant health through provision of additional nutrients and improving the soil structure, which in turn leads to increased plant growth and yield.

It was recomended that:

1. Organic amendments should be considered as agents for promoting grwoth and development of indigenous mycorrhiza in the soil.

2. Organic amendments should be considered as agents for improving soil nutrient content and thus improve plant growth and yield.

REFERENCES

Aalok, A., Tripathi, A. K., & Soni, P. (2008). Vermicomposting: A Better Option for Organic Solid Waste Management, *24*(1), 59–64.

Alakonya, A.E., Runo, S. N., Monda, E. O., & Sinha, N. R. (2014). Application and intercropping of maize and sorghum with *Lupinus albus* inhibits *Striga hermonthica* emergence and Arbuscular Mycorrhizal Fungal Colonisation. *Agricultural Science Research Journal, 4* (*1*), 1-9.

Abuli, S.J. (2016) A brief history of soybean production in Kenya *Research Journal of Agriculture and Environmental Management. Vol. 5(2),*58- 64.

Bethlenfalvay, G., Schreiner, R. P., & Mihara, K. L. (1997). Mycorrhizal Fungi Effects on Nutrient Composition and Yield of Soybean Seeds. *Journal Of Plant Nutrition*, *20*(4&5), 581–591.

Brundrett, M. (2009). Mycorrhizas in Natural Ecosystems. *Advances In Ecological Research*, *21*, 171–313.

Cavender, N., Atiyeh, R., & Knee, M. (2003). Vermicompost stimulates mycorrhizal colonization of roots ol Sorghum hicolor at the expense of plant growth. *Pedobiologia*, *47*, 85–89.

Chianu, J. N., Ohiokpehai, O., Vanlauwe, B., Adesina, A., De Groote, H., & Sanginga, N. (2009). Promoting a Versatile but yet Minor Crop: Soybean in the Farming Systems of Kenya. *Journal of Sustainable Development in Africa*, *10*(4), 324–344.

Coelho, I., Pedone-Bonfim, M., Silva, F., & Maia, L. (2014). Optimization of the production

of mycorrhizal inoculum on substrate with organic fertilizer. *Brazilian Journal of Microbiology*, *45*(4), 1173–1178.

Copetta, A., Bardi, L., Bertolone, E., & Berta, G. (2011). Fruit production and quality of tomato plants (Solanum lycopersicum L.) are affected by green compost and arbuscular mycorrhizal fungi. *Plant Biosystems*, *145*(1), 106–115.

Darzi, M. T., Seyedhadi, M. H., Rejali, F. (2012). Effects of the application of vermicompost and phosphate solubilizing bacterium on the morphological traits and seed yield of anise (*Pimpinella anisum* L.) *Journal of Medicinal Plants Research, 6*(2), 215-219.

da Silva, L. G., Martins, L. M. V., & da Silva, F. S. B. (2014). Arbuscular mycorrhizal symbiosis in the maximisation of the concentratiion of foliar bimolecules in pomegranate (*Punica granatum* L.) seedlings. *Journal of Medicinal Plant Research*, *8*(28), 953–957.

Elmer, W. H., & Pignatello, J. J. (2011). Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. *Plant Disease*, *95*(8), 960–966.

Gai, J. P., Christie, P., Cai, X. B., Fan, J. Q., Zhang, J. L., Feng, G., & Li, X. L. (2009). Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau.

Girma, A., Rossiter, D., Siderius, W., & Henneman, I. (2001). *Soils of the Lake Naivasha Area, Kenya* (Technical Report) (pp. 1–34). the Netherlands: International Institute for Aerospace Survey & Earth Sciences.

Gutie´rrez-Miceli, F. A., Moguel-Zamudio, B., Abud-Archila, M., Gutie´rrez-Oliva, V. ., & Dendooven, L. (2008). Sheep manure vermicompost supplemented with a native diazotrophic bacteria and mycorrhizas for maize cultivation. *Bioresource Technology*, *99*, 7020–7026.

Jain, M. C., Sharma, M. K., Bhatnagar, P., Meena, M., & Yadav, R. K. (2012). Effect of Mycorrhiza and Vermicompost on Properties of Vertisol Soil and Leaf Content of Nagpur Mandarin (*Citrus reticulata* Blanco). *Asian Journal of Horticulture*, *7*(2), 528–532.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I. R., & Frossard, E. (2003). Soil Tillage Affects The Community Structure Of Mycorrhizal Fungi In Maize Roots. *Ecological Applications*, *13*(4), 1164–1176.

Laufer, J., & Tomlinson, T. (2013). *Biochar Field Studies: An IBI Research Summary* (Research Summary) (pp. 1–10). International Biochar Initiative.

Lazcano, C., Arnold, J., Tato, A., Zaller, J. G., & Domínguez, J. (2009). Compost and vermicompost as nursery pot components: effects on tomato plant growth and morphology. *Spanish Journal of Agricultural Research*, *7*(4), 944–951.

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota - A review. *Soil Biology & Biochemistry*, *43*, 1812–1836.

Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E., & Jansa, J. (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agriculture, Ecosystems and Environment*, *119*, 22–32.

Mathu, S., Hermann, L., Pypers, P., Matiru V., Mwirichia R., Leseur D. (2012). Potential of indigenous *bradirhizobia* versus commercial inoculants to improve cowpea (*Vigna unguiculata* L. Walp.) and green gram (*Vigna radiata* L. Wilczek.) yields in Kenya. *Plant Science and Plant Nutrition 58(6), 750-763*.

Mau, A. E., & Utami, S. R. (2014). Effects of biochar amendment and arbuscular mycorrhizal fungi inoculation on availability of soil phosphorus and growth of maize. *Journal Of Degraded And Mining Lands Management*, *1*(2), 69–74.

McGrath, C., Wright, D., Mallarino, A. P., & Lenssen, A. W., (2013). Soybean nutrient needs. Agriculture and Environment Extension Publications, 189.

Ministry of Environment and Mineral Resources,Kenya. (2011). *Kenya National Profile to Assess Chemicals Management* (National Profile) (pp. 1–143). Nairobi: Ministry of Environment and Mineral Resources.

Muchane, M. N., Muchane, M., Mugoya, C., & Masiga, C. (2012). Effect of land use system on Arbuscular Mycorrhiza fungi in Maasai Mara ecosystem, Kenya. *African Journal of Microbiology Research*, *6*(17), 3904–3916.

Ndegwa, P. N., Thompson, S. A., & Das, K. C. (2000). Effects of stocking density and feeding rate on vermicomposting of biosolids. *Bioresource Technology*, *71*, 7–12.

Nepolean, P., Jayanthi, R., Vidhya Palavi, R., Balamurugan, A., Kuberan, T., Beulah, T., & Premkumar, R. (2012). Role of Biofertilisers in Increasing Tea Productivity. *Asian Pacific Journal of Tropical Biomedicine*, S1443–S1445.

Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., & Wiemken, A. (2003). Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. *Applied And Environmental Microbiology*, *69*(5), 2816–2824.

Parmar, N., Gami, B., & Patel, B. (2013). Evaluation of Soil Compositions and Hosts for Sporulation of Vesicular Arbuscular Mycorrhiza (VAM). *Journal of Agricultural Science and Applications*, *2*(2), 67–71.

Quilambo, O. A. (2003). The vesicular-arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology*, *2*(12), 539–546.

Redecker, D., & Raab, P. (2006). Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia*, *98*(6), 885–895.

Savala, C. N. E., Kithome M., & Woomer, P.L. (2003). Vermicomposting for kale

production. *African Crop Science Proceedings*, (6), 489-496.

Savci, S. (2012). An Agricultural Pollutant: Chemical Fertilizer. *International Journal of Environmental Science and Development*, *3*(1), 77–80.

Schahczenski, J. (2010). Biochar and Sustainable Agriculture. National Sustainable Agriculture Information Service. Retrieved from www.attra.ncat.org/attra-pub/PDF/biochar.pdf

Selden, P., DuPonte, M., Sipes, B., & Dinges, K. (2005). Small-Scale Vermicomposting. *Home Garden*, *45*, 1–4.

Shishehbor, M., Madani, H., & Ardakani, M. (2013). Effect of vermicompost and biofertilizers on yield and yield components of common millet (Panicum miliaceum). *Annals of Biological Research*, *4*(2), 174–180.

Sinha, R. K. (2009). Earthworms vermicompost: a powerful crop nutrient over the conventional compost & protective soil conditioner against the destructive chemical fertilisers for food safety and security. *American-Eurasian Journal of Agricultural and Environmental Sciences, 5*(S), 1-55.

Sohi, S., Loez-Capel, E., Krull, E., & Bol, R. (2009). Biochar, climate change and soil: A review to guide future research (No. 05/09) (p. 64). Australia.

Solaiman, Z., Sarcheshmehpour, M., Abbott, L., & Blackwell, P. (2010). Effect of biochar on arbuscular mycorrhizal colonisation, growth, P nutrition and leaf gas exchange of wheat and clover influenced by different water regimes. In *Soil Solutions for a Changing World*. Brisbane, Australia: 19th World Congress of Soil Science.

Soleimanzadeh, H. (2010). Effect of VA-Mycorrhiza on Growth and Yield of Sunflower (Helianthus annuus L.) at Different Phosphorous Levels. *International Journal of Chemical and Biological Engineering*, *3*(3), 159–162.

Sousa, C. da S., Menezes, R. S. C., Sampaio, E. V. de S., Oehl, F., Maia, L. C., Garrido, M.

da S., & Lima, F. de S. (2012). Occurrence of arbuscular mycorrhizal fungi after organic fertilization in maize, cowpea and cotton intercropping systems. *Acta Scientarum*, *34*(2), 149–156.

Turk, M. ., Assaf, T. ., Hameed, K. ., & Al-Tawaha, A. . (2006). Significance of Mycorrhizae. *World Journal of Agricultural Sciences*, *2*(1), 16–20.

Verheijen, F. G. ., Jeffery, S., Bastos, A. C., van der Velde, M., & Diafas, I. (2009). *Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions* (No. EUR 24099 EN) (pp. 1–149). Luxembourg: Office for the Official Publications of the European Communities.

Warnock, D., Lehmann, J., Kuyper, T., & Rillig, M. (2007). Mycorrhizal responses to biochar in soil – concepts and mechanisms. *Plant Soil*, *300*, 9–20.

Warnock, D., Mummey, D., McBride, B., Major, J., Lehmann, J., & Rillig, M. (2010). Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Applied Soil Ecology*, *46*, 450–456.