MICROBIAL PROFILES OF SMALL HOLDER FARMS UNDER DIFFERENT CROPPING SYSTEMS ALONG RIVER ISIUKHU WATERSHED OF KAKAMEGA COUNTY

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A Thesis Submitted in Partial Fulfillment for The Requirement of the Award of Master of Science Degree in Microbiology of Masinde Muliro University of Science and Technology.

October 2020

DECLARATION

This thesis is my original work prepared with none other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled "Microbial Profiles of Small Holder Farms Under Different Cropping Systems Along River Isiukhu Watershed of Kakamega County".

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DEDICATION

This thesis is dedicated to my loving parents Christopher Matindu and Betty Masitsa and to my siblings Onesmus, Christabel and Gloria.

ACKNOWLEDGEMENT

I thank the Almighty God, above all, for granting me good health, strength and determination to accomplish the work. I am grateful to my supervisors Dr. Francis N. Muyekho and Dr. Anthony Wawire Sifuna for their constant guidance, mentorship and support that led to success of this work. Much appreciation also goes to Dr. Greg Thorn and Dr. Irena Creed of University of Western Ontario and Dr. Dennis Omayio of Biological Sciences department, Masinde Muliro University of Science and Technology for their continuous moral support and encouragement. I am thankful to Mr. Willy Aganyanya of Biological Sciences department, Masinde Muliro University of Science and Technology and Ferdinant Anyika of University of Nairobi, Department of Soil Science for their technical and material support during soil analysis. I would like to acknowledge the funding support from Queen Elizabeth II Scholarship. More appreciation goes to Ms. Rael Adhiambo for the assistance in data collection throughout the study.

ABSTRACT

The River Isiukhu watershed is endowed with rich agricultural soils. However, the region is characterized with high population density and therefore farm land is under enormous pressure as it is put under continuous production of crop to meet the ever growing food demand and source of livelihoods. On the other hand, crop rotations to rejuvenate the soils have been limited by the small farm sizes, while application of soil fertility amendments has been hindered by the high cost of inputs, and therefore communities depending on agriculture productivity have continued to suffer from low crop yields. Additionally, how farming practices within the region impact on the soil physicochemical structure and microbial community is least understood. Farmers have therefore continued to employ the same farming practices overtime without considering the effects of the practices on soils in the region. This study aimed at determining the soil microbial profiles, physicochemical parameters and understanding the relationship between soil physicochemical properties and microbial profiles under different cropping system. Three cropping systems commonly practiced by farmers in the study area namely, maize and bean intercrops, pure sugarcane fields, napier grass fodder fields were evaluated, whereas a site within the Kakamega tropical forest was considered to represent an undisturbed site. For each system, soil microbial community composition was determined using Illumina Miseq sequence data of 16S rRNA gene and ITS region for bacteria and fungi respectively. Soil organic carbon and total nitrogen were determined using Walkley-Black method, Kjeldahl digestion method respectively. The highest levels of soil organic carbon (37.03 Mg/ha) and total nitrogen (3.27 Mg/h) were recorded in maize and beans intercrop system while the least, 27.15 Mg/ha and 2.38 Mg/ha, respectively from napier cropping system. The results showed the presence of 20 bacterial phyla and 3 fungal phyla across all the treatments. *Acidobacteria* (30.2*%)*, *Proteobacteria* (19.6%), *Verrucomicrobia* (12.3%) and *Actinobacteria* (7.8%) were found to be the main dominant phyla in all the treatments. Under fungal community structure, *Ascomycetes* (65.4%) and *Basidiomycetes* (19.2%) were the main dominant phyla. The relative abundance of *Proteobacteria* was recorded highest in forest and lowest in sugarcane. *Acidobacteria* on the other hand was recorded highest in maize and beans intercrop and lowest in forest. Under bacteria, Chao1 diversity index was less in sugarcane and more under forest. The Shannon diversity index was greater in forest and least under sugarcane cropping system. When comparing the fungi diversity, Chao 1 diversity index was more in sugarcane cropping system and less under maize and beans intercrop. The Shannon diversity index was great under sugarcane and least under napier cropping system. In this study *Acidobacteria* and *Chloroflexi* were positively correlated with SOC while *Basidiomycetes* was found to have a negative correlation. Total nitrogen (TN) was positively correlated to *Planctomycetes* and *Ascomycetes*. Only *Acidobacteria* showed a positive correlation with pH. These findings reveal that the different cropping systems practiced in the area influence soil carbon, total nitrogen, pH and microbial soil profiles. The findings further show that the soils are only marginally acidic, but soils under napier appear to have the least levels of fertility and microbial populations and structure. This study therefore provides an insight of how different cropping systems influence soil microbial profiles and physicochemical properties and it could be used in the development of sustainable land management approaches with reference to cropping systems within the watershed.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
DNA	Deoxyribonucleic acid
FAME	Fatty Acid Methyl Ester
GDP	Gross Domestic Product
PCR	Polymerase Chain Reaction
PLFAs	Phospholipid Fatty Acids
RFLP	Restriction Fragment Length Polymorphism
SOC	Soil Organic Carbon
TN	Total Nitrogen
ITS	Internally Transcribed Spacer

CHAPTER ONE

INTRODUCTION

1.1 Background

Microorganisms forms an essential component in the cycling of material and energy transformation process with respect to soil ecosystem (Zhang *et al*., 2019) and soil microbial profiles therefore forms a representation of a vital aspect to be put into consideration when studying the impacts of different cropping systems on soil microbial profiles. Soil microbes have shown to have a close relationship to health and stability of the soil ecosystem and are proven to be driven by numerous factors and external condition changes like change in land use, cultivation, soil physicochemical characteristics and management measures (Zhang *et al*., 2019; Zhou *et al*., 2017a). Microbial profiles of soil and diversity are therefore often used as pointers to variations in soil quality (Bucher and Lanyon, 2005).

The worldwide approval of soil maintenance practices in farming is therefore critical in reversing degradation, maintaining biodiversity and fertility of soil. According to Food and Agriculture Organization (FAO) soil degradation in various parts of the world results from inappropriate farming methods and incorrect usage of pesticides and fertilizer (FAO, 2012). Anthropogenic activities also influence both how the microbial communities' functions and biodiversity of these microbes and therefore possibly result in microbial roles reduction and species loss (FAO 2012; Brown *et al*., 2002).

The River Isiukhu watershed is an area in western Kenya that consists of a remnant of Equatorial rain forest and provides suitable agricultural soils and weather, nevertheless,

the watershed serves as an important agricultural area growing maize, beans, tea, sugar cane being the major crops (Wood *et al.*, 2015; Amadalo *et al.*, 2003). Researches have revealed that cropping systems may influence the community structure of soil microbes and organic matter content and contribute to loss of fertility (FAO, 2012). Interestingly, although communities within the watershed have over the time engaged in different agricultural practices, less is known about the soil microbial profiles and diversity and how cropping systems have influenced the soil microbial distribution and possibly influencing soil fertility in this agroecosystem. Additionally, in the River Isiukhu watershed there exists limited information on soils nutrition balance, furthermore, how the nutrients are sustained within the agricultural soils under different agricultural practice is not clearly understood. Such information is important as it can be used to support soil fertility improvement with a view of increasing farm yields and food availability in the region. Deforestation, intensification of agriculture and land degradation have resulted in loss of soil microbial community diversity, damaging to resilience and sustained productivity.

Since the soil biota is affected in different ways by the various types of agricultural practices and systems, it is necessary to understand how this practices influence soil microbial diversity in an important agro-ecosystem like the River Isiukhu which supports a large agricultural community in Western Kenya. This study therefore aimed at understanding the relationship between different crops system, physicochemical parameters; and soil microbial diversity.

1.2 Statement of the Problem

Changes in microbial profiles and functions have been hypothesized to alter ecosystem processes like availability of nutrients and decomposition of plant litter (McGuire and Treseder, 2010) and it has been suggested that changes in land use, including different cropping systems affects the microbial breakdown organic matter and litter which leads to regulation of soil nitrogen and carbon balance within the terrestrial ecosystems (Brackin *et al*., 2013). Microorganisms in soil therefore possess an essential role in conservation of soil quality and health since varied microbes participate in key soil functions.

Although many studies have attempted to understand soil fertility changes based on different agricultural systems practiced in the region, little is known about variations among the microbial profiles colonizing these agricultural soils and the role played by the farming practices in influencing the microbial and physicochemical variations. This study therefore aimed at investigating the effects caused by various cropping systems on the taxonomic diversity of microorganisms colonizing smallholder agricultural soils within the River Isiukhu watershed and their relationship between soil physiochemical parameters namely total nitrogen and organic carbon and thus being important for better understanding of how these cropping systems affects the soil microbial profiles.

1.3 General Objective

To determine soil microbial profiles of small holder farms under different cropping systems along river Isiukhu watershed of Kakamega County.

1.4 Specific Objectives

1. To characterize the soil microbial profiles in small holder farms under different cropping systems along river Isiukhu watershed of Kakamega County.
2. To determine soil physicochemical properties (SOC, TN, pH and texture) in small holder farms under different cropping systems.
3. To determine the relationship between soil physicochemical parameters (SOC, TN and pH) and soil microbial profiles along River Isiukhu watersheds.

1.5 Hypothesis

1. Cropping systems practiced in small holder farms along River Isiukhu watershed do not influence soil microbial profiles.

2. Cropping systems practiced in small holder farms along River Isiukhu watershed do not influence the physicochemical parameters of the soil.

3. There is no relationship between soil physicochemical parameters and microbial profiles of soils under different cropping systems along the River Isiukhu watershed.

1.6 Significance of Study

Due to the small land farms owned by farmers in the river Isiukhu watershed, monoculture is the most practiced farming method in the region, with most farmers growing mainly maize and sugarcane, and therefore there is a need to understand how these practices influence microbial profiles and diversity within the ecosystem, since soil microbial diversity and profiles are known to be pointers of soil quality changes. The knowledge obtained on the microbial profiles in soils of the watershed can help in understanding their

functions in soil, and develop appropriate technologies for microbial maintenance and restoration in sites with altered structures. Physicochemical parameters of soils have been shown to be affected by cropping systems, and therefore may have an effect on microbial diversity in agricultural soils. This study therefore provided an insight of how different physicochemical properties influence soil microbial profiles within the river Isiukhu watershed and further demonstrated how the cropping systems influenced total nitrogen and carbon levels in the farms studied.

CHAPTER TWO

LITERATURE REVIEW

2.1 Soil Microbial Profiles Under Different Cropping Systems

Soil microorganisms are essential for productivity of soil and cycling of nutrients in both agricultural and natural ecosystems since they aid in absorbance of nutrients by plants by colonizing their roots and organic matter decomposition for provision of soil nutrients and improvement of the soil structure. As a result of intensification of human disturbance, most of the world's tropical forests have been either afforested, reforested or converted to plantations (Wang *et al*., 2017). Several studies have suggested that monocultures support microbial communities, with some researches pointing out that monocultures of maize, wheat or soybean may result to reduction of metabolic diversity or the decline of fungal species (Depret *et al*., 2004, Meriles *et al*., 2009). Vegetation cover affects structure of soil microbial profiles since the variations in plant species composition always contributes to the changes in litter quantity and quality, thus altering cycling processes and content of soil nutrients (Miki *et al*., 2010).

Bacteria accounts for the largest proportion of the soil microbial community and several researches documented that continuous cropping increases fungi in soil, which intensifies soil borne diseases but fungi play an essential role in the breakdown of recalcitrant complexes in corn residues at later stages (Zhang *et al*., 2014). Land cover, which includes different cropping systems is the determinant factor that affects organic matter content of the soil thus playing a role in microbial profiles regulation accordingly (Moon *et al*., 2016). Bakker *et al*., 2012 found out that numerous bacteria flourish in the rhizosphere of

different crops as a result of nutrient supply from the respective plant residues and these results revealed that plant species currently in season plays a great role in determination of bacterial community than the pre-crop species. Continuous cropping inhibits plant growth and causes soil-borne diseases as reported by Liu *et al*., (2014). Under intercropping system, roots of various plant species forms direct association with each other which further affects root exudation thereby altering the microbial structure, activity and diversity (Zhou *et al.,* 2011, Broeckling *et al*., 2008). Several reports have revealed close association between underground microbial diversity and the aboveground crop diversity (Williamsa *et al*., 2014).

2.2 Effects of Land Use on Soil Microbial Communities

Several studies showed that different land use regulates structure of soil bacterial communities (Osborne *et al*., 2011; Wallenius *et al*., 2011) and these communities have shown to be more sensitive to land use changes as compared to soil physicochemical characteristics (Romaniuk *et al*., 2012). Microbes exists all over the soil profile; however, they are more abundant in soil surface, plants' rhizosphere and around macropores (Fierer *et al.,* 2007). Soil microbes are assumed to take part in critical roles that facilitate the response of ecosystem to anthropogenic environmental changes (Zak *et al.,* 2011). Soil microbial profiles are therefore regarded as architects of soils (Rajendhran and Gunasekaran, 2008) since most ecosystem services are closely associated to the activities of these microbes and their linked functional characteristics (Torsvik and Ovreas, 2002).

Microbial populations play both active and passive roles in enhancing soil fertility. The microbial community is most beneficial to the grower when it is diverse, abundant and active. The limitation of knowledge on microbial profiles and function in soil is as a result

methodological and taxonomic limitations associated with the study of these microrganisms (Kirk *et al*., 2004). However, many factors generally influence the activity and species composition of microbes including physicochemical characteristics of the soil, vegetation and temperature. Shifts in the soil microbial profiles are strong pointers of biological activity of soil and quality of terrestrial agroecosystems (Edmeades, 2003). The knowledge of how soil management leads to changes observed in microbial community structures is essential for optimized management practices, since soil microbes constitutes a central regulatory soil processes. A research done by Vitousek *et al.*, 2009, revealed that some of the microbial driven process like decomposition determines the rate at which nutrients are retained or lost in the ecosystem managing agriculture for production of crops while minimizing the loss of nutrient to the environment.

The major drivers of soil microbial profiles are soil and plant types (Meliani *et al*., 2012). Plant species affect microbial community profiles due to differences in the plants canopy cover, their rooting depth and the quantity and quality of litter (zak *et al*., 2003). The soil biota is affected in different ways as a result of various types of agricultural practices leading to either a negative or positive response depending on the part of soil biota that is affected; fungal or bacterial.

A study by Tolli and King, (2005), revealed that various practices like fertilizer treatment, land use alteration and plant covers increased the abundance of soil bacterial communities. Several other studies have shown that tilled soils may either contain greater or less diversity of bacteria than non-tilled soils (Ferreira *et al*., 2000). Variations in the community profiles have been as a result of changes in agronomic practices like no-tillage (Drijber *et al*., 2000), rotation of crop and inoculations of microbes (Roesti *et al*., 2006).

Monoculture production versus crop rotation have yielded varying results with regards to impacts of continuous monoculture on microbial community. Crop rotation and no-till practice have been largely accepted in various agricultural set ups and is usually believed that the practices potentially increase microbial activity and biomass (Feng *et al*., 2003). Monoculture selects for less diverse microbial communities. Diminishment of soil degradation can be achieved by avoiding monoculture and encouraging the practice of crop rotation or associations which should include at least three varied crops (FAO, 2012). Unfortunately, monoculture prevails world-wide leading to depletion of organic matter content, resulting to disposition to diseases and increased rates of weed infestation. On the other hand, crop rotations coupled with the use of green manure breaks pathogen cycles and enhance soils physicochemical characteristics, including soil organic matter (Boddley *et al*., 2010).

Diverse soil microorganisms can be induced by distinct soil and vegetation properties due to the creation of varied microhabitats that support diverse collection of species as shown by Zak *et al*., (2003). Studies have evidently shown that environmental conditions and practices of land management greatly shape soil microbial profiles (Steenwerth *et al*., 2002). Several researchers have demonstrated that fertilization highly affects the composition, population and roles of soil microbes and fertilizer alterations have increased soil microorganism's activities (Mandal *et al*., 2007). Some researchers have however illustrated that fertilizers, both organic and organic have had moderately slight or no impacts on microbial activities and diversity of the soil (Treseder, 2008).

Brimecombe *et al*., 2000, in their study showed that plants apply strong impact on the soil microbial community structure through decay of litter and rhizodeposition. They also demonstrated that the relation between the species of plant and microbe's diversity in the

soil of the rhizosphere is strict and as a result of co-evolution. Most microbial activities occur in the few centimeters of the soil surface (Babujia *et al*., 2010) and thus several reports demonstrate that absence of soil disturbance and plant residues in no till system also favors improved microbial activity and biomass (Babujia *et al*., 2010; Silva *et al*., 2013). The reaction of soil microbes to the disturbances caused as a result of changes in management and crop may result to variations in range and activity of soil biota.

2.3 Microbial Communities in an Ecosystem

The physicochemical study of parameters is essential for growth of plants and soil management. Soil carbon storage is facilitated by microorganisms that use organic matter of soil as their source of carbon thus regulation of composition and size of soil microbial profiles achieved through complex relations with plants (Butler *et al*., 2004) and litter substrate quality (Myers *et al*., 2001).

Soil microbes participate in crucial role of process of nutrient cycling. Carbon and nitrogen do not form part of mineral composition and are limited in soils, emphasizing the importance of understanding them from the microorganisms' perspective. Enrichment of soil nitrogen and carbon can be achieved by appropriate land management methods known to either raise the levels of organic matter input, lowers soil organic matter mineralization or both (Paustian *et al*., 2000; Follet, 2001). Contents of nitrogen and carbon in soil play crucial role in the sustaining quality of the soil, crop production and environmental quality as a result of their impacts on soil biological and physicochemical characteristics. Lombard *et al*., (2011), demonstrated that soil matrix together with physicochemical features possesses a great effect on the dynamics of soil microbial profiles.

Soil microbes are essential in maintaining and sustaining agro-ecosystems by controlling the cycling rate of carbon and nitrogen thus posing directly increasing fertility of plant and soil nutrition. Bacterial and fungal activities primarily drive soil carbon decomposition while only 10-15% of soil carbon is directly linked to the action of fauna (Hopkins and Gregorich, 2005). Soil microbes are also significantly affected by management of crops since different plant species impact the cycling of nutrients and therefore affecting the functioning and structure of soil microbial profiles (Carrera, 2007). Changes in the land use alters the below ground ecosystem, often leading to the depletion of soil carbon.

Soil microbial activity highly depend on each other and researches demonstrate that growth and activity of microbes are normally restricted by carbon, nitrogen and phosphorus availability (Vineela *et al*., 2008).

2.4 Determination of Soil Microbial Profiles

Two main approaches have been used in the study of soil microbial communities – convectional plating of the cultivable microbes and the molecular techniques that are independent of cultivation. Standard culture techniques used in the characterization of microbial ecology usually involves the isolation and characterization of microbes by use of commercial growth media (Kirk et al. 2004) but their main drawback is that more than 99% of the microbe's present are not cultivable using usual culturing techniques (Hugenholtz 2002).

Molecular methods on the other hand provides general insights into the genetic heterogeneity of the soil microbial profiles and allows the identification of specific microbes without isolation. These culture-independent methods include the evaluation of

whole genomes or certain genes like 18S and 16S rRNA for eukaryotes and prokaryotes respectively. The usage of 16S phylogenic marker is however frequently criticized because of its heterogeneity that exists among operons that belong to similar genome (Acinas *et al*, 2004) or its lack of resolution at species category (Pontes *et al*, 2007). Irrespective of those shortcomings, it is however still considered as a 'gold standard' for the identification of bacterial since it is sequenced rapidly and easily as documented by Spiegelman *et al*, (2005). The 16S rRNA has also been extensively employed in determination of microbial diversity since the genes are functionally and structurally preserved and pose highly and variable conserved regions (Hugenholtz, 2002). The method however dependents highly on the success of DNA isolation, presence of DNA restriction or amplification inhibitors, primer choice and discriminating analysis power (Kowalchuk *et al*., 2006).

The modern development of high-throughput sequencing technologies has given room for a profound understanding of the microbial diversity in different soils around the world (Zarraonaindia *et al*., 2015). High throughput methodologies that are DNA characterized like sequencing and sequence alignment, are used directly to determine composition and variation in microbial species with low richness (Shi *et al*., 2014; Zhao *et al*., 2014).

NGS sequencing technologies are used for deeper investigation of microbial communities and are essential in the presentation of unbiased view of phylogenetic and functional diversity of microbial profiles in the environment (Zwolinski 2007). Next Generation Sequencing techniques(NGS) are the current standards for the generation of genomic data, which produces rapid information at low costs (Metzker 2010) and recovery of DNA sequence data directly from environmental samples have been made possible (Sogin *et*

al., 2006). NGS possesses high degree of parallelism and uses reaction volumes that are smaller compared to the convectional Sanger sequencing, leading to generation of large amounts of data, lesser sequencing time and reduced costs although at the expense of shorter read lengths and increased error rates (Wang *et al*., 2012). With comparison to the conventional Sanger sequencing, NGS methods uses smaller reaction volumes and has high degree of parallelism which enables usage of low costs to offer large amounts of data at shorter sequence time with shortcomings of high rates of error and shorter read lengths. Among the NGS methods, Illumina Miseq sequencing platform is the most efficient and widely used technology worldwide (Lindahl *et al.*, 2013) because of its low error rate and cost per million bases although it needs diagnostic regions that are short, about 300 base pairs for its effectiveness.

2.5 Soil Physicochemical Properties Under Different Cropping Systems

In sub-Saharan Africa, conversion of natural ecosystems into agricultural lands has been taking place tremendously, with significant portions under production of crops like maize. The freshly cultivated soils may be nutrient rich due to soil aeration inducing the mineralization of organic nutrients. However, it has been shown that these soils can lose their efficiency within 25 years under continuous cultivation without organic/inorganic fertilizer application as a result of nutrients loss and structural stability loss (Ushio *et al.*, 2010). Fertility depletion in tropical soils that dominates Africa is known to be mainly as a result of continuous cropping with lack of proper replacement of nutrients (Oyamo *et al*., 2016). Researches are therefore required to explore the potential changes occurring in soil under cultivation of natural ecosystems.

Successful agriculture demands for the sustainable utilization of soil resource, since soils are known to easily lose their quantity and quality in a shorter period of time due to several reasons and therefore a success in soil management in maintaining the quality of soil will depend on the understanding of soil response to different agricultural practices overtime. Assessment of impacts of management systems on conditions of the soil is therefore essential for understanding and monitoring management effects practices on properties of soil and sustainability of soil productivity (Martyniuk *et al.*, 2015).

The implementation of suitable land management methods and land use planning would help in both restoring the degraded soil physicochemical quality and ensuring stable and sustainable productivity (Ovela and Philip, 2014). Few reports exist on the effects of continuous cropping on the nutrient stocks and properties of soil under small holder farmers' conditions in the developing countries. The quality of soil is therefore becoming an important resource in raising productivity of crops in order to meet the food security in developing countries (Negasa *et al.*, 2017). Yesilonis *et al.*, (2016) found out that suitable cropping systems plays an essential role in improvement of organic matter stock and also in maintaining of the soil nutrients which are important for both plants and the soil microorganisms. Nonetheless, there are limited evidences that have evaluated characteristics of soil under various cropping systems that are managed by smallholder farmers within the River Isiukhu watersheds of Kakamega, western Kenya. The existing literatures have also proved that more is needed in understanding the impacts of continuous cropping systems on soil quality indicators for suitable measures that improve proper crop production to be taken.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was done along river Isiukhu watersheds within Kakaemga county (0°07′30″ and 0°15′ N and between 34°20′32″ and 35°57′30″ E, at an altitude of 1250–2000 meters above sea level). This watershed covers an area of approximately 8400km^2. The key economic activity of the local inhabitants is agriculture with livestock and cash crops such as sugar cane, beans, maize and tea (Ojiem, 2006). The annual rainfall ranges between 1000mm and 2100mm. Mean day temperature ranges from 28$^{\mathbf{0}}$ C to 32^0 C. Southern part is characterized by mainly poorly drained clay ferral soils and the northern part is endowed with well drained sandy clay arcisols (Sombroek *et al*., 1982). The study site is categorized as a humid forest agroecological zones and the climate presents a growing period of 300-350d for upland crops.

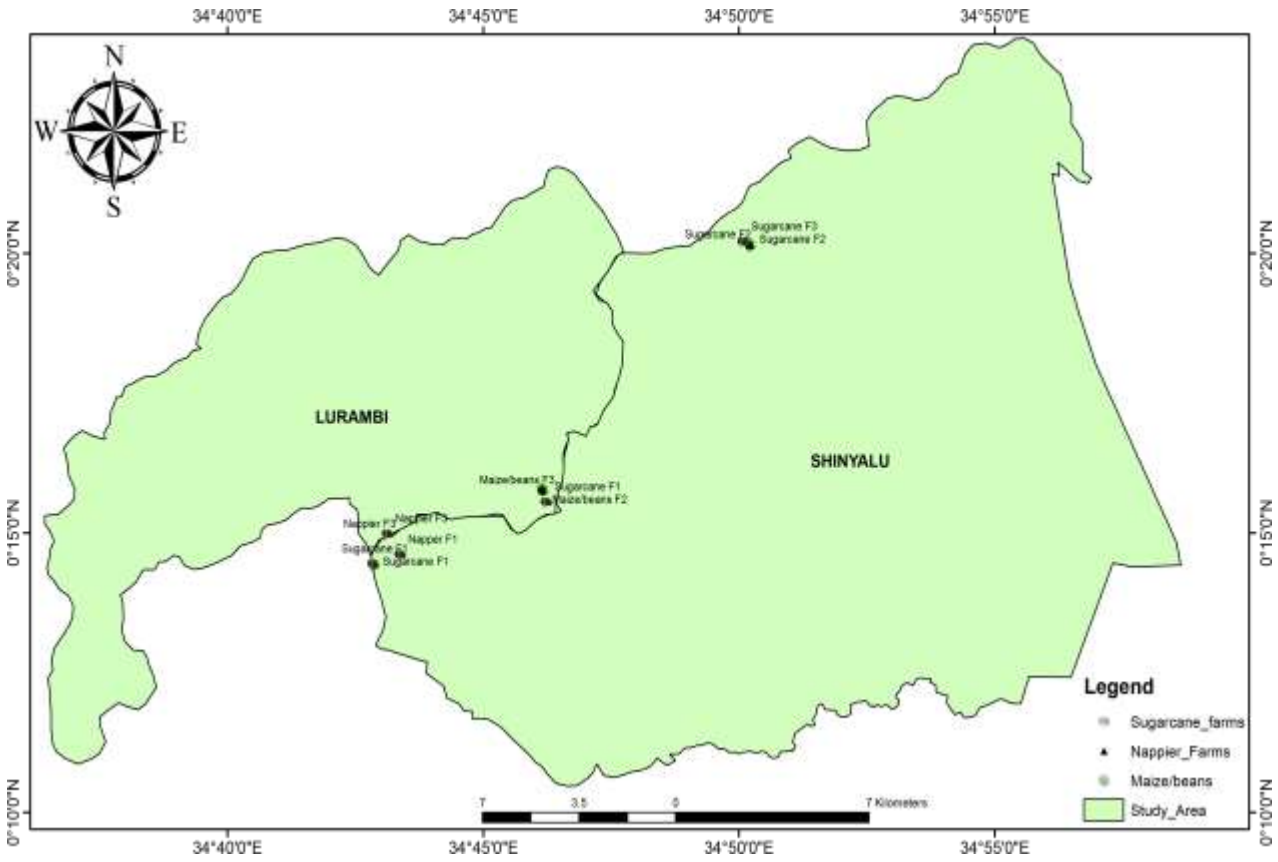


Figure 3. 1: Map of the Study Area.

3.2 Study Design

Purposive sampling was used in which the treatments consisted of different cropping systems (maize and bean intercrops, sole sugarcane and Napier grass fields) and the different treatment were chosen basing on the most common practices carried out by farmers according to different cropping systems. The crops had been planted continuously for a minimum of five years on the same field. These agricultural practices were assessed alongside long term wild forest coverage site. The sampling areas included Ikolomani, Township, Bukhaywa and Magakha (forest), located in Ikolomani, Lurambi and Shinyalu sub-counties respectively.

3.2.1 Soil Sampling

A total of 450 samples were collected in different cropping systems (30 farms x 5 sampling points per farm x 3 sampling replicates/ sessions. From each sampling area, 3 farms for each cropping system were selected, however only one site was considered for forest samples.

Prior to soil sample collection, an area of 10m by 10m was selected on each of the 30 farms sampled. Soils samples were obtained using a 5cm internal diameter soil auger by drilling into the ground.

Soil samples were obtained from 0 - 20cm layer after removal of surface vegetation and litter, 1 at the centre and 4 at 4 equidistant points at the perimeter of 10m x 10m area. The samples were stored in zip lock bags and taken for laboratory analysis at Masinde Muliro University of Science and Technology where plant residues were removed.

Single soil samples collected from each cropping system were pooled and mixed thoroughly to form one sample for each site, hence leading to in 84 composite samples. The soil was then air dried and then passed through a 2mm sieve prior to analysis. Sub-samples were then analyzed for physicochemical parameters. The remaining soil was stored at -20^0 C until DNA extraction.

3.2.2 Estimation of Soil Bulk Density

Soil bulk density were obtained as defined by Anderson and Ingram (1993). The core rings used for soil sample collection were pressed firmly on the soil by use of soil auger from 0 – 5 cm depth. The excess soil was scraped off from the core rings once the soil has been collected and the lids fastened to secure the soil samples within the core rings. The samples were then pre-weighed and dried at 105^0 C in an oven for 24 hours and the final mass that

was weighed and noted. Bulk density was then obtained as the dry mass of soil divided by the volume using the following formula:

Bulk density (g cm^{-3}) = (W2 g - W1 g)/ V cm^3

Where:

W1= weight of the core rings

W2= weight of dry soil samples + weight core rings

V = Volume of the core ring

3.2.3 Estimation of soil organic carbon

The samples were air dried for a period of 24 hours, crushed and sieved via a 2 mm sieve. Organic carbonncontent was obtained by use of Walkley-Black method (Walkley and Black, 1943).

About 2.0 g of dried soil was weighed into a 250ml graduated conical flask. Titration of two blank samples (no soil) was done prior to proceeding with any unknown samples for standardization of Ferrous Sulfate ($FeSO_4$) solution. Potassium dichromate ($K_2Cr_2O_7$) solution was put in 250 ml conical flask containing the 2g soil using a pipette. The contents of the flask were then mixed carefully by flask rotation for all the soil samples to be wet. 20ml of concentrated sulfuric acid (H_2SO_4) was then carefully added to the flask, under a fume hood and mixed gradually. The flasks were then left to settle for 5 min in a fume hood after which distilled water was topped up in each flask to constitute a final volume of roughly 125 ml, followed by a gentle swirl to allow for mixing.

The samples were then cooled to room temperature and the volume rechecked after half an hour. Thereafter, 5 drops of Phenolphthalein complex were added then titrated with

Ferrous Sulfate solution. The samples were then mixed by stirring using a glass rod and titrated until the color changed to reddish-brown from green. Volumetric readings were recorded to the nearest X.X ml.

The content of organic carbon in the sample was obtained as follows:

Organic carbon (%) = (B – S) x 0.006)/m) x 100

Where:

B = ferrous solution volume used in the blank titration,

S = ferrous solution volume used in the sample titration;

m = sample mass in grams used in the analysis.

The total soil organic carbon was then obtained as the product of the percentage carbon content, layer thickness (20cm) and bulk density expressed in g/cm^2. The total soil organic carbon was then scaled to Mg per unit hectare thus giving the estimates of SOC in units of Mg/ha (Omoro et al., 2013).

3.2.4 Estimation of soil total nitrogen

To determine soil total nitrogen, samples were air dried for 1 day, crushed and passed through a 2mm sieve. Total Nitrogen content was evaluated by use of the Kjeldahl Digestion method (Anderson and Ingram, 1993). A soil sample of 0.3g was transferred into digestion tube followed by addition of 4.4 ml of digestion mixture. The mixture was then digested by heating at a temperature of 360^oC for 2 hours. The digest content was cooled and transferred to a 50 ml volumetric flask and topped up with distilled water to 50 ml.

An aliqot of 10ml of the sample solution was then transferred to the reaction chamber (steam distillation apparatus), and 10ml of 1% NaOH added. The content was then steam-

distilled in 5ml of 1% boric acid to which 4 drops of the mixed indicator. The distillation process was continued for 2 more minutes from the time the indicator turned green. The distillate was then obtained and titrated with standard HCl. Steam was allowed to pass through the apparatus for 30 minutes and the blank of the steam checked by obtaining 50ml distillate and titrating with standard HCl. Total nitrogen in the sample was then calculated as:

$$\% \text{ of N in the sample} = \frac{(a-b) \times 0.1 \times V \times 100}{1000 \times w \times al}$$

Where:

a = HCl titre volume for the blank

b = HCl titre volume for the sample

v = final volume of the digestion

w = sample weight

al = aliquot of the solution taken for analysis

Total nitrogen was obtained as the product of percentage nitrogen content, layer thickness (20cm) and bulk density expressed in g C cm-2. TN was then scaled to Mg per unit hectare thus giving their estimates in units of Mg/ha (Omoro *et al.*, 2013).

3.2.5 Determination of soil pH

Soil sample pH was determined as described by Rhoades (1982) in ratio of soil: distilled water suspension (1: 1). 10g of soil was placed in an open container and 10ml of distilled water added. The mixture was stirred for 5-10 seconds and then let to settle for 15 minutes. The pH meter electrode was then immersed into the soil suspension and the pH value recorded.

3.2.6 Determination of Soil Texture

The soil texture was obtained by hydrometer method (Bouyoucous G.J 1936) where by 50g of air dried < 2mm soil (M1) was weighed and placed into 400ml beaker and the soil saturated by the use of distilled water after which 10ml of calgon solution was added and this was allowed to settle for 10 minutes. The suspension was moved to the dispersing cup and the volume made to the mark in the cup with distilled water. The suspension was shaken overnight on reciprocating shaker after which it was transferred into a graduated cylinder. Hydrometer was inserted into the suspension and water added to 1130ml followed by the removal of the hydrometer. The cylinder was tightly covered with a fitting rubber bung and the suspension mixed by carefully inverting the cylinder 10 times and the time recorded. Three (3) drops of amyl alcohol were added quickly to the soil suspension to get rid of the foam and the hydrometer gently placed into the column after 20 minutes. At 40 seconds, the hydrometer readings were taken and the temperature of the suspension measured. This reading was denoted as R1. The mixing of the solution for 10 times was repeated and the cylinder allowed to settle for 2 hours after which both hydrometer and the temperature readings were noted denoted as R2. The percentage of sand, silt and clay were obtained as follows:

$$\% \text{ sand} = (M1\text{-}R1/M1) \times 100$$

$$\% \text{ clay} = (R2/M1) \times 100$$

$$\% \text{ silt} = 100 - (\text{sand } \% + \text{clay } \%)$$

3.3 Sample Preparation and DNA Extraction

Soil for DNA extraction was pooled according to the different cropping systems and thoroughly homogenized to form 8 composite samples. Soil weighing 50g each from

individual farms of all soils based on the different cropping systems were pooled together and mixed from which total genomic DNA was extracted from 0.25g of soil using Powerlyzer PowerSoil DNA Isolation Kit (MOBIO Laboratories Inc California- USA) following the manufacturer's procedure. A soil sample of 0.23g was was added to PowerLyzer Glass Bead Tube and 750 µl of bead solution added and vortexed gently so as to mix. 60 µl of solution C1 was then added and vortexing briefly done. The supernatant was transferred to a clean 2ml collecting tube, 250 µl of solution C2 added, vortexed for 5 seconds and incubated at 4^0 C for 5 minutes. The tubes were then centrifuged at room temperature for 1 minute at 10000xg. A volume of 600 µl of the supernatant was transferred to a 2ml collecting tube and 200 µl of solution C3 added before being vortexed briefly and incubated at 4^0 C for 5 minutes. Centrifugation was done at room temperature for 1 minute at 10000xg and 750 µl of the supernatant was transferred into a clean 2ml collection tube followed by addition of 1200 µl of solution C4 to the supernatant and vortexing was done for 5 seconds. A volume of 675 µl was loaded onto the spin filter and centrifuged at 10000xg for 1 minute at room temperature. The flow through was castoff and additional 675 µl of the supernatant was loaded onto the spin filter and centrifuged at 10000xg for 1 minute at room temperature. The remaining supernatant was loaded onto the spin filter and centrifuged at 10000xg for 1 minute at room temperature and then 500 µl of solution C5 was added and centrifugation was done at room temperature for 30 seconds at 10000xg and the flow through was discarded. The tube was centrifuged again at room temperature for 1 minute at 10000xg. The spin filter was carefully placed in a clean 2ml collection tube and 100 µl of solution C6 added to the centre of the white filter membrane. Centrifugation was done at room temperature for 30 seconds at 10000xg. The spin filter was discarded and the DNA in the tube preserved for downstream processing.

3.3.1 PCR Amplification of 16S rRNA

The quality and relative quantity of the extracted DNAs in the samples was determined using NanoDrop technique (NanoDrop 2000, Thermo Scientific) and kept at -20^0 C until Polymerase Chain Reaction amplification (PCR). PCR amplification was done using 2 set of primers U516F/806R targeting bacterial and archael diversity and LSU200A-F/LSU476A-R targeting fungal (Ascomycota) diversity, with each primer modified with diagnostic barcodes to distinguish samples and adaptors for the Illumina system (Asemaninejad *et al*., 2016) as shown in Table 3.1.

Table 3. 1: PCR Primers Used in Amplification

Primer	Sequence	Reference
U516F	CCAGCMGCCGCGGTAA	Caparaso *et al*., 2010
U806R	GGACTAACHVGGGTWTCTAAT	
LSU200A-F	AACKGCGAGTGAAGCRGYACSAT	Asemaninejad *et al*.,
LSU200A-R	CACTSTACTTGTKCGC	2016

The PCR mixture total volume of 25 µl contained 12.5 µl of Accustart II PCR Toughmix mastermix (molecular graded magnesium chloride, dNTPs, Accustart II hot start Taq DNA polymerase, ToughMix additives and stabilisers), 1.5 µl of forward and reverse primers, DNA loading volumes of 4 µl, 0.5 loading dye and 5 µl of nuclease free water. Negative controls were done using reaction mixture lacking DNA template but with 9 µl nuclease free water. The thermal cycling scheme used were: 94°C for 1 min, 29 cycles of 94°C for 30 s 55°C for 30 s, 72°C for 18 s and stored at 4°C. Products of from the soil samples were checked for successful amplification via gel electrophoresis by use of 1% agarose gel in 1 x TAE buffer. Amplified DNAs were submitted for paired end sequencing in an Illumina Miseq sequencer at the London Regional Genomics Centre (Robarts Research Institute, London Canada.

3.3.2 Illumina Sequencing Analysis

Illumina MiSeq sequencing of the fungal ITS1 and the 16S rRNA genes was done to determine the structure of the soil fungal and bacterial community, respectively. The raw sequences obtained were processed and analyzed by use of QIIME1 Pipeline version 1.9.0. Quality control of the Miseq Illumina sequencing reads was done using NGSToolkit v2.3 (Patel and Jain 2012). Filter parameters were set at cut-off quality (Phred) score of 20 with a cut-off read length for high quality set at 70%. High quality reads were written into a separate file. The merging of high quality reverse and forward reads were done using pandaseq (Andre *et al.*, 2012).

The fasta reads that were merged were quality filtered and reads assigned to their samples source and metadata mapping file using Qiime script split_libraries_fastq.py (Bokulich *et al.*,2013, Caporaso *et al*., 2010). Reads that were too short after quality truncation were discarded. For further analysis, the high quality sequences were clustered to operational taxonomic units (OTUs) using an open-reference OTU picking protocol in Qiime pipeline (Caporaso *et al.*, 2010, Rideout *et al.*, 2014). To achieve that the python script pick_otus.py was used with the setting enable_rev_strand_match set to true (Rideout *et al.*, 2014) and against the Greengenes database for bacteria 16S and ITS (DeSantis *et al.*, 2006). Representative sequences from each OTUs were picked to generate the table out table biom and checked for chimera with QIIME via ChimeraSlayer (Caporaso *et al.*, 2010). A new chimera free out table biom was generated for downstream analyses. The chimera free out table biom containing taxonomy and metadata information was used to run alpha diversity analysis. The OTUs were grouped into various taxonomic levels

(phylum, class, order, family and genus) using Qiime workflow script (Caporaso *et al.*, 2010).

3.4 Statistical Analysis

One-way Analysis of Variance (ANOVA) was used in the determination of the impacts of cropping system on measured soil physicochemical parameters. Differences between individual means were tested by Tukey HSD post hoc test. OTUs were grouped into different taxonomic levels (phylum) and diversity plots were visualized using Emperor. The relationship between the abundance of specific microbial taxonomic groups with soil characteristics was assessed by Spearman's correlation coefficient. All the analysis above was performed using SPSS software Version 20.0. In all the analysis, statistical significance was considered at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1: Soil Microbial Profiles Under Different Cropping Systems

A total of 2,449,276 reads were obtained from the 14 samples sequenced. The reads represented 13,360 sequence variant species including 119 archae, 5814 bacteria, 718 fungi and 1460 were unclassified. The sequences were submitted to NCBI for accession numbers and the following accession numbers were given; SAMN 16521379, SAMN 16521380, SAMN 16521381, SAMN 16521382, SAMN 16521383, SAMN 16521384 and SAMN 16521385.

4.1.1: Soil Bacterial Profiles Under Different Cropping Systems

A total of twenty (20) bacterial phyla were detected as shown in figure 4.1 out of which only 12 bacterial phyla were found at a relative abundance more than 1% and 8 phyla at a relative abundance lesser than 1% as shown in figure 4.1. The 4 most abundant identifiable bacterial phyla were *Acidobacteria* (30.2%), *Proteobacteria* (19.6%), *Verrucomicrobia* (12.3%) and *Actinobacteria* (7.8%) and they were considered as the dominant groups in all the samples (Figure 4.1).

Other bacterial phyla with relative abundance > 1% were Bacteroidetes (6.6%), Planctomycetes (4.9%), Chloroflexi (4.5%), Firmicutes (2.4%), Nitrospirae (1.7%), Armatimonades (1.4%) and Gemmatimonades (1.3%) as illustrated in figure 4.1.

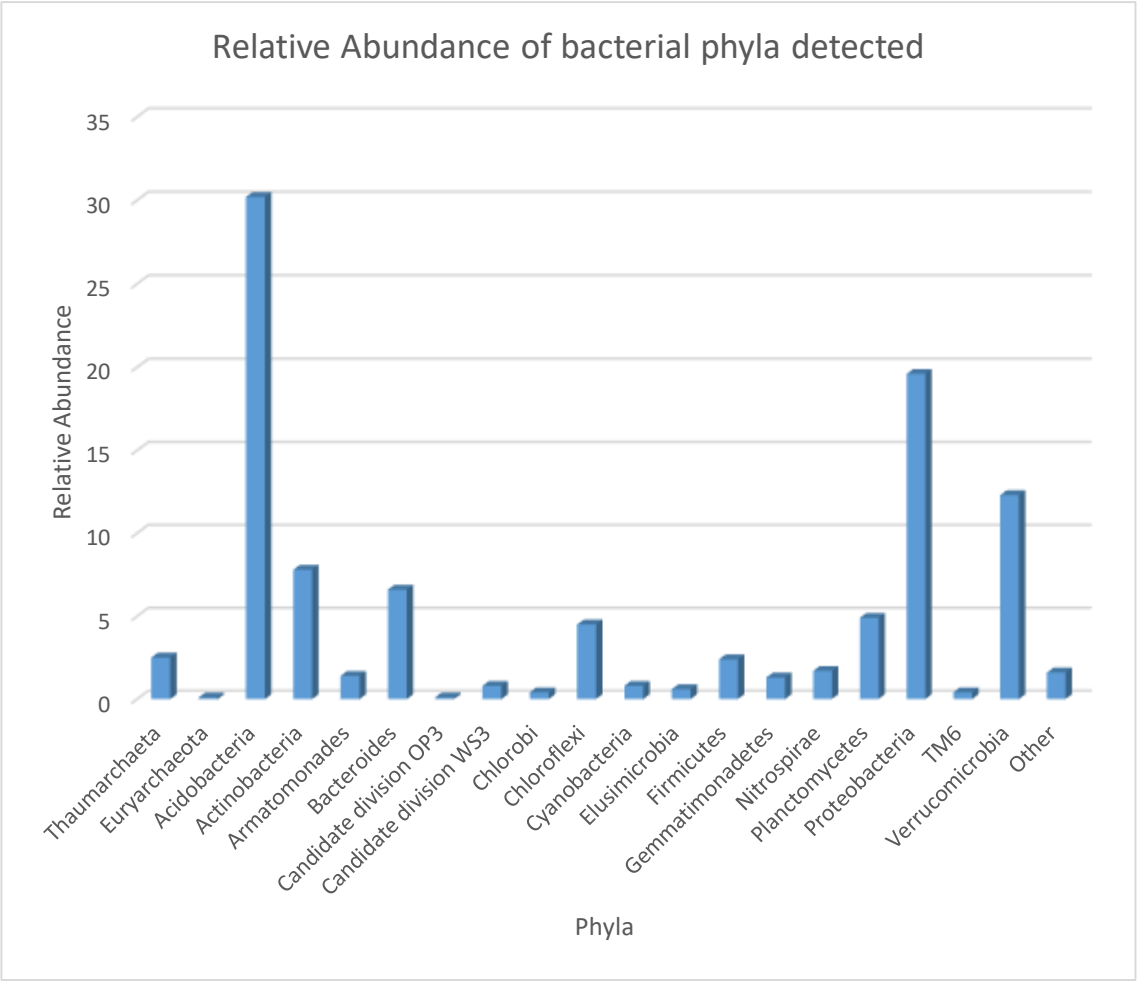


Figure 4. 1: Relative Abundance of Bacterial Phyla Observed

The relative abundance for *Proteobacteria* was recorded highest in forest (24.8%) and lowest in sugarcane (16.4%) whereas the relative abundance for *Acidobacteria* was highest in maize and beans cropping system (31%) and the lowest in forest (29%) as shown in figure 4.2. *Verrucomicrobia* and *Actinobacteria,* recorded the highest relative abundance in sugarcane cropping system (15.1%) and (11.8%) respectively whereas the lowest was observed in forest (10.3%) and (5.3%) respectively. At subsequent level, 68 classes, 119 orders, 169 families and 205 genera were identified.

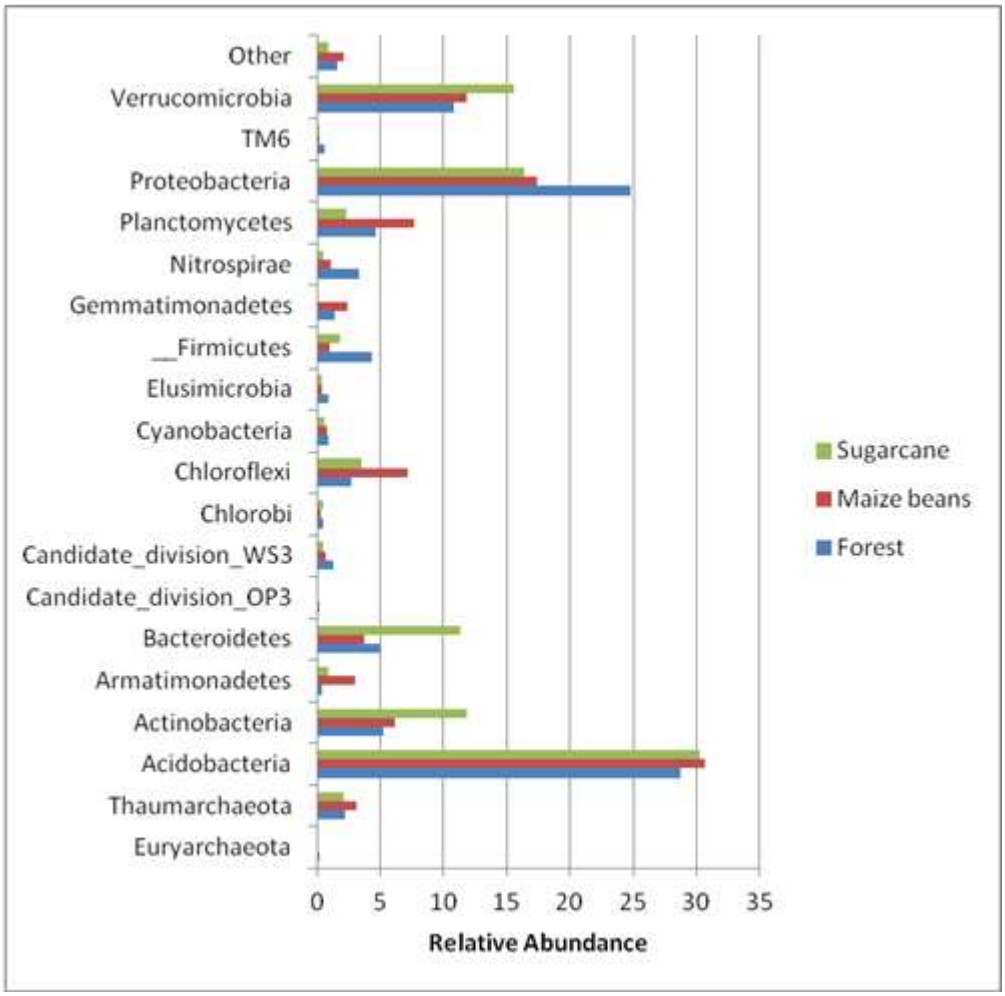


Figure 4. 2: Bacterial Phyla Observed under Different Cropping Systems

4.1.2: Soil Fungal Profiles Under Different Cropping Systems

For fungal sequences, *Ascomycota* (65.4% of all fungal sequence reads) represented the most abundant fungal phylum, followed by *Basidiomycota* (19.2%) and 11.3% of the sequences were unidentified as shown in Figure 4.3. The *Ascomycota* and *Basidiomycota* therefore formed majority of the fungal sequences observed in this study.

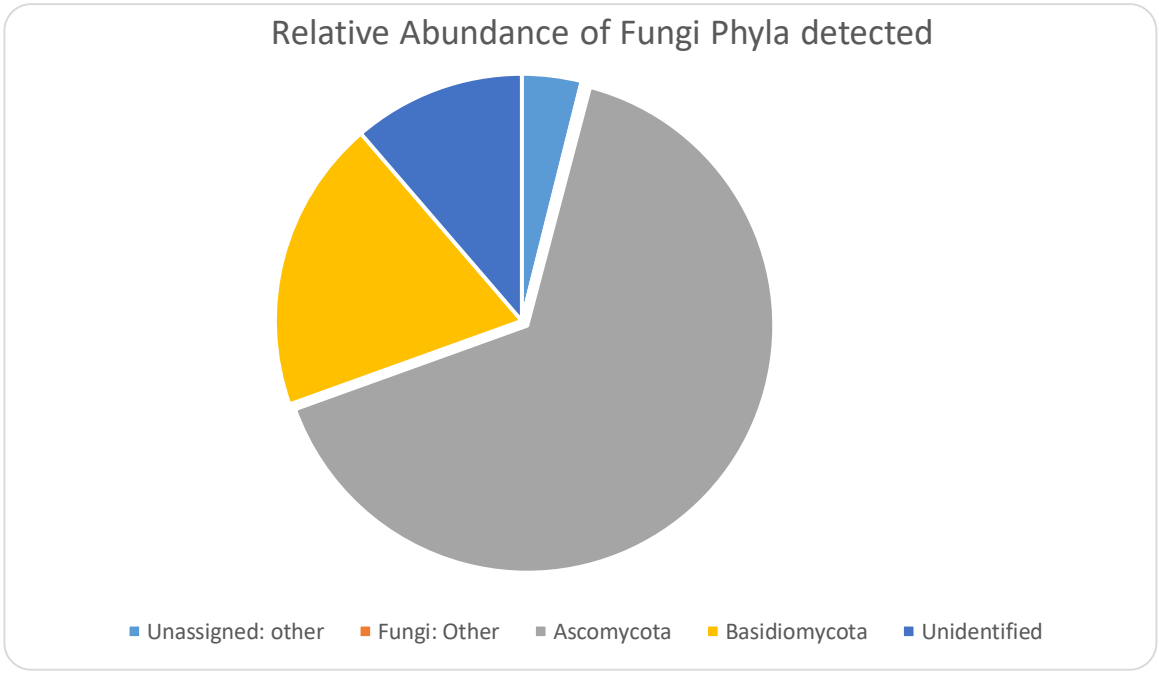


Figure 4. 3: Relative Abundance of Fungal Phyla Observed

The relative abundance for *Ascomycota* was greater in maize and beans intercrop and lowest in napier whereas that of *Basidiomycota* was highest in napier and least in maize beans intercrop as shown in figure 4.4.

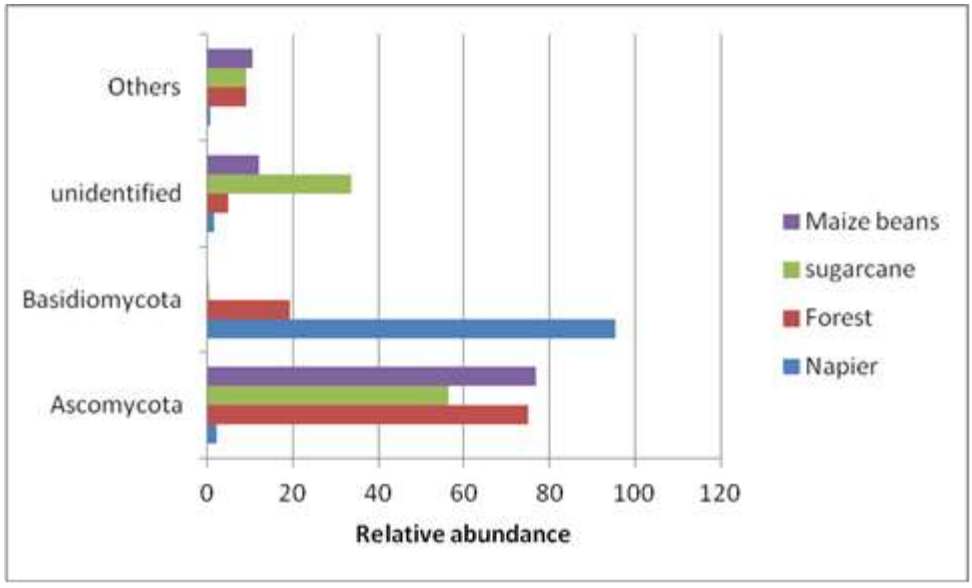


Figure 4. 4: Fungal Phyla under Different Cropping Systems

At the succeeding fungal taxonomical levels, 14 classes, 31 orders, 47 families and 64 genera were detected.

4.2: Effects of Cropping Systems on Soil Organic Carbon, Total Nitrogen and pH

Organic carbon of the soils under different cropping systems varied from 37.03 Mg/ha to 27.15 Mg/ha as shown in Table 4.1. The soils under maize and beans intercrop showed the highest amount of organic carbon, followed by the sugarcane soils. The lowest organic carbon contents were recorded in soils under napier after the soils under forest cover. Significant differences ($p = 0.001$) were obtained among the SOC levels observed under the various cropping systems studied and those obtained from the forest. Generally, soil organic carbon increased in the order of maize and beans > sugarcane > forest > napier. However, using the Tukeys Post Hoc test, forest and napier soils showed no significance difference.

The total nitrogen values followed a pattern similar to soil organic carbon (Table 4.1) with maize beans intercrop having the highest total nitrogen values and the least being shown under napier soils, and showed significant differences ($p = 0.014$) among the cropping systems and forest The levels of soil total nitrogen increased in the order of maize beans > forest > sugarcane > napier. Nevertheless, when subjected to the Tukey's test, forest and sugarcane soils showed no significant differences. The highest pH value was noted in soil samples under maize and beans and the least being the forest. The pH levels showed significant difference ($p = 0.0001$) among the different cropping systems and forest soils. However, the Tukey's test showed that the pH values for forest soils were significantly different from the other soils sampled in the study.

Table 4. 1: Soil Physicochemical Properties Based on Cropping Systems.

Cropping Systems	SOC Mg/ha	TN Mg/ha	pH
Forest	27.23±2.3b	2.90±0.3ab	5.22±0.2b
Maize&beans	37.03±1.0a	3.27±0.2a	6.24±0.1a
Napier	27.15±1.6b	2.38±0.1b	6.11±0.1a
Sugarcane	31.89±2.2ab	2.89±0.3ab	6.20±0.1a
Test values	**df-3;f-7.1; p= 0.001**	**df-3;f-4.3; p= 0.014**	**df-3;f-13.7; p=0.0001**

Textural profiles for the soils observed in this study varied in their percentage silt, clay and sand as shown in in Table 4.2. Significant differences were observed (df-3, f-3.37, p = 0.034) in the percentage sand content among the different cropping systems with the highest percentage of sand recorded among the forest (77.67%) and the lowest recorded among maize and beans intercrops (55.89%). There was no statistical difference observed among soils under maize and beans intercrops, napier and sugarcane with respect to percentage sand.

Table 4. 2: Soil Texture of the Cropping Systems Sampled

Cropping systems	Texture		
	% sand	% silt	% clay
Forest	77.67±1.7a	4.67±0.7b	17.33±1.5b
Maize & beans	55.89±2.7b	13.56±1.8a	29.22±1.8a
Napier	58.33±4.6b	10.00±1.5ab	29.33±3.4a
Sugarcane	63.44±3.9b	10.00±1.6ab	26.89±3.4ab
Test values	df-3; f-3.37 p = 0.034	df-3; f-2.90 p = 0.054	df-3; f-1.67 p = 0.19

Key: Values are means ± standard error. Means followed by the same letter are not significantly different (P< 0.05); Tukey's HSD test.

4.3 Relationship Between Physicochemical Properties and Microbial Profiles.

The study observed that the community structure of the soil microbes remained quite stable across all the different cropping systems but only few phyla correlated with the investigated soil properties irrespective of the treatments.

Table 4. 3: Spearman's Correlation Coefficients Between Soil Physicochemical Properties and Abundant Phyla (relative abundance > 2%)

	TN	SOC	pH
Bacteria			
Acidobacteria	0.80	1.00**	0.90**
Proteobacteria	0.80	0.40	-0.20
Verrucomicrobia	0.40	0.80	0.60
Actinobacteria	0.40	0.80	0.60
Bacteroidetes	0.20	0.40	0.00
Planctomycetes	1.00**	0.80	0.40
Chloroflexi	0.8	1.00**	0.80
Firmicutes	0.40	0.20	-0.40
Fungi			
Ascomycetes	1.00**	0.80	0.40
Basidiomycetes	-0.80	-1.00**	-0.8

Value represents correlation coefficients ** $p < 0.01$

Strong correlations were detected between various microbial phyla and some soil parameters like pH, SOC and TN as shown in Table 4.3. Of the bacterial phyla with relative abundance > 2% only the *Acidobacteria* and *Chloroflexi* were positively correlated to SOC (Table 4.3). *Acidobacteria* was also positively correlated to soil pH ($p < 0.05$). *Proteobacteria, Verrucomicrobia* and *Actinobacteria, Bacteroidetes* and *Firmicutes* were not apparently correlated to any of the soil properties ($p > 0.05$). Planctomycetes also showed a positive correlation with TN. Under fungi, *Ascomycetes* were positively correlated with TN while *Basidiomycetes* showed a negative correlation with SOC ($p < 0.5$).

Based on the Shannon diversity index maize-beans cropping system and the forest had the highest diversity and indices were almost similar (Table 4.4). Sugarcane had almost four (4) lower diversity than the two systems. Similarly, Chao1 index showed that sugarcane cropping system had the least diversity forest and maize -beans cropping system had the highest (Table 4.4).

For fungi, the highest Shannon diversity indices for was found in the sugarcane cropping system (7.7) followed by maize-beans (6.19) and forest (4.79). Napier grass had the least diversity (Table 4.6). Based on the Chao1 diversity index, sugarcane had the highest followed by the forest and napier. On the contrary the least diversity of fungi was in the maize-beans intercrop (Table 4.4).

Table 4. 4: Microbial Alpha Diversity under the Different Treatments

	Bacteria	
Cropping System	Chao 1	Shannon
Sugarcane	55	3
Maize and Beans	76624.57	11.17
Forest	87037.94	11.09
	Fungi	
Sugarcane	9593.02	7.7
Maize and beans	6385.88	6.19
Forest	7889.22	4.79
Napier	7447.23	1.65

CHAPTER FIVE

DISCUSSION

5.1 Microbial Profiles Under Different Cropping Systems

Microbes play an essential role in the cycling of material and transformation of energy in the soil ecosystem (Zhang *et al*., 2019) and the soil microbial profiles therefore represents an important aspect to be put into consideration when investigating the effects of different cropping systems on properties of soil. This study determined and compared the composition of soil microbial profiles present under different cropping systems namely napier grass, sugarcane and maize and beans intercrop, together with a conserved portion of the tropical forest along River Isiukhu watershed in Kakamega County. In the present study, a high throughput method of metagenomics was employed to understand the microbial structure and diversity of soils in an equatorial rain forest ecosystem, influenced by different agricultural practices.

5.1.1 Soil Bacterial Profiles Under Different Cropping Systems

The first hypothesis of this study that cropping systems practiced in small holder farms along river Isiukhu watershed do not influence soil microbial profiles was tested by investigating microbial community profiles under the different cropping systems in the region. The results obtained revealed that *Acidobacteria, Proteobacteria, Verrucomicrobia* and *Actinobacteria* represented the most predominant bacterial phyla in the study site. This finding is in agreement with previous researches done by Nie *et al.,* (2018), Cui *et al.,* (2017) and Sun *et al.,* (2015) who also found that *Acidobacteria, Proteobacteria, Actinobacteria* and *Verrucomicrobia* as among the dominant bacterial

phyla in soils representing between 60% - 80% of all the phyla detected. Meisinger *et al.*, (2007) has shown that some members of the *Acidobacteria* phylum are always in association with those of *Gammaproteobacteria,* a class under the *Proteobacteria.* This association has been observed to forms the root of ecological relationship that exists between *Proteobacteria* and *Acidobacteria* which influences the position of each other in the community (Kielak *et al.,* 2016). The composition of the dominant bacteria across all the treatments were similar at the phylum level although their taxa relative abundance varied among the different cropping systems thus demonstrating that different cropping systems influenced taxonomical microbial community soil structure below the phyla levels. Generally, microbial profiles of soil have been observed to be affected by land use and farming practices including cropping systems. In this study, forest soil presented higher abundance of *Proteobacteria* than sugarcane and maize and beans intercrop whereas *Acidobateria* was lowest in forest when compared to sugarcane and maize and beans intercrop. These findings contradict those observed by Jangid *et al.*, (2008) in the USA who observed that *Proteobacteria* showed high relative abundance in forest, whereas, *Acidobacteria* were found in less abundance in cropland soils as compared to the observations made under forest soils. This two studies may have differed in terms of the different cropping systems and practices studied. In the current study, forest ecosystems, farms under sugar cane, maize and beans and napier were studied whereas in the study by Jangid *et al* (2008), different systems of agricultural management namely grazed and hayed pasture and conventionally tilled crop land were investigated. The *Alphaproteobacteria* was among the most abundant classes comprising of order *Rhizobiales,* which plays a critical role in nitrogen fixation (Liu and Liu, 2013).

The relative abundance of *Acidobacteria* was observed to be highest under maize and beans cropping system. This may be due to the high SOC and TN contents present in these soils attributable to high levels of residue return under maize and beans cropping systems. This was in line with study done by Navarrette *et al.*, (2013), who also found high relative abundance of *Acidobacteria* under continuous maize cropping system with high levels of SOC and TN and attributed this to attributed this to high volumes of residues returned to the soil. *Acidobacteria* prefer soil environment that has high carbon levels (Voriskova and Baldrian, 2013) and are therefore connected to availability of soil carbon and nitrogen (Jones *et al.*, 2009).

Additionally, cropping systems may impact on soil biological characteristics thereby influencing microbial characteristics. Sugarcane crop residue for instance influence the biological characteristics of soil by influencing verrucomicrobial abundance in soils (Graham *et al.*, 2002). In this current study, *Verrucomicrobia* relative abundance was highest in sugarcane cropping system and lowest in forest. Our results contradicted with those documented by Brewer *et al.*, (2016) and Fierer *et al.*, (2013) who observed that *Verrucomicrobia* are reactive to soil disturbances and therefore often show lesser abundance under intensively managed conditions like those under agricultural use. It is however not clear why *Verrucomicrobia* were more abundant in disturbed systems than relatively pristine ecosystems like those under forest cover in this study and therefore calls for more investigations.

Variations in soil physicochemical properties, also may influence microbial diversity in soils, decline in the relative abundance of *Actinobacteria* in soils has been associated with reduction in the soil pH (Lauber *et al.*, 2009, Rousket *et al.*, 2010). In this study the

phylum *Actinobacteria* was highest in sugarcane and lowest under the forest soils, this finding could be therefore be attributed to soil pH conditions as the forest soils in this study were characterized by a lower pH values.

5.1.2 Soil Fungal Profiles Under Different Cropping Systems

The fungal taxa relative abundance observed in this study was in line with those observed in soils of other tropical regions (Kerfahi *et al.,* 2014, McGuire *et al.,* 2014). Kerfahi *et al.,* (2014), McGuire *et al.,* (2014) have also reported that *Ascomycota* and *Basidiomycota* are the most main phyla in the tropics. Lynd *et al.,* (2002) has attributed dominance of *Basidiomycota* and *Ascomycota* in soils to their capability to degrade dissolved organic matter including polyphenolic compounds and cellulose aerobically.

5.2 Effects of Cropping Systems on Soil Physicochemical Properties

The second hypothesis that cropping systems practiced in small holder farms along River Isiukhu watershed do not influence the physicochemical parameters of the soil was verified by analyzing soil physicochemical parameters namely total nitrogen, organic carbon, pH and texture. The results obtained from this study demonstrates a major soil nutrient attribute that show direct link between soil nitrogen and organic carbon content. The levels of soil total nitrogen and organic carbon were found to be highest under maize and beans intercrop cropping system. This conformed with the trends observed in other studies which documented that soil nitrogen is high where carbon concentrations are also high (Sakin 2012). Factors that increase availability of organic matter input into soils play an important role of increasing levels of both soil organic carbon and total nitrogen. In this study, the high soil nitrogen and carbon levels under the maize and beans intercrop

and sugarcane can be attributed to great residue returned to the soil and probably effective root depth of these cropping systems (Cunha *et al.,* 2011). The dense root system built by sugarcane offers protection of the soil from losses of nitrogen and carbon stored in the topsoil layer (Cunha *et al.,* 2011). Additionally, the nitrogen input from the legume crop, beans, serves important role in cycling of nutrients and soil organic carbon accumulation thus raising the levels of organic carbon and total nitrogen in the maize/beans intercrop soils.

Generally, soil organic matter levels are dependent on the balance between the degradative effect of tillage, harvest that entails the removal of above ground vegetation and organic input (Zotarelli *et al,* 2007). Forested soils had lower levels of organic carbon as compared to maize and beans intercrop and the sugarcane soils. Zotarelli *et al,* (2007) demonstrated that non tillage slows down organic matter decomposition in soils. Non tillage of soils offers a physical protection and storage of organic carbon, which normally occurs through aggregation of soil. Physical protection of soil organic carbon therefore leads to inhibition of organic matter decomposition, thus lowering down the decomposition rate of organic matter (Van Groenigen *et al.,* 2010; Zotarelli *et al.,* 2007) and this could have contributed to the low levels of carbon recorded under forested soils. Much carbon in the tropical forests is often stored in live biomass rather than the soils as opposed to other biomes in which soils forms the dominant carbon storage as revealed by Rachel (2014) which might account for the low levels of carbon witnessed under the forest soil, even though the current study did not investigate on carbon storage in live biomass. In the forest ecosystem, there is quick recycling of nutrients as a result of ants, termites and soil microbes action over the scale of weeks (Rachel, 2014) and these might have also

contributed to the reduced levels of nitrogen and carbon experienced under the natural forest.

Soil types also influence soil organic matter content as it has been shown that the very sandy nature of the soils stimulates organic matter loss and decomposition (Guimaraes *et al.*, 2014), whereas clay soils possess a protective effect on the organic matter (Zhao *et al.*, 2006). Clay particles have been reported to hold as much as 50% of the soil humus in organo-mineral complexes and thus protected from rapid decomposition (Muller and Hope, 2004) and it is on this basis that possibly in this study low organic carbon and nitrogen content was reported for soils under forest that are e characterized by very sandy soils.

Removal of above ground vegetation contributes significantly in reduction of carbon and nitrogen pools in soils (Chen *et al.*, 2014). The low total nitrogen and organic carbon levels reported for soils under napier in this current study may be attributable to continuous removal of vegetation as pasture for domesticated livestock in the study area. Above ground vegetation removal limits the recycling of carbon and nutrients through decomposition of litter therefore resulting to the lowest soil total nitrogen and organic carbon (Wan and Luo, 2003), and therefore explain why soils under napier farming in this study recorded low levels of SOC and TN.

In the current study, the forest showed the lowest pH of 5.2 as compared to maize and beans intercrop, sugarcane and napier cropping systems. The low pH of forest soils recorded in the current study was in line with other researches done in tropical rain forests (Pereira *et al.*, 2013 and Posada and Schuur, 2011). The acidic conditions under forest are

believed to be generated by the accumulation of both basic and acidic cations due to environmental factors such as high average annual precipitation (Posada and Schuur, 2011).

5.3 Relationship Between Physicochemical Properties and Microbial Profiles.

In this study, pH was observed to be less important as an environmental force in the shaping of fungal community structure as no relationship was established. Generally, fungi have the capacity to tolerate wider pH range for optimal growth and extracellular enzyme activity (Beales, 2004), and thus may not be influenced by pH as demonstrated in this study. Zhong *et al.,* (2015) in a recent study has shown that SOC and TN are more closely correlated to the bacterial community profile as compared to pH. This study nonetheless observed that *Ascomycetes* are positively correlated with TN, while *Basidiomycetes* showed a negative correlation with SOC. Wang *et al.,* (2017) also observed positive correlation between *Ascomycota* and TN. Overall, different farming practices on the communities of soil bacteria have shown that soil bacteria are greatly affected by soil physical and chemical characteristics, whereas the fungal communities have proven to be affected negatively or remain unchanged by these practices (Luo *et al.,* 2015).

This study demonstrated that the maize and beans cropping system showed the greater bacterial diversity. This conquered with study done by Hamamoto *et al*., (2018) who revealed greater Shannon bacterial diversity under soils of intensively cultivated maize sites as compared to forest and watermelon sites. The high diversity of bacteria observed under maize and beans cropping system could be as a result of direct contact between

substrates under agricultural practices and bacteria which facilitates the growth of bacteria and may reflect the direct contact of crop roots which causes stimulation of release of more nutrients by the roots (Song *et al.,* 2007). Beans are leguminous plants and therefore nitrogen fixing bacteria in association with their root nodules would be released into the soil in rotting root material and this could therefore account for the greatest richness under this cropping system. In this study, maize and beans intercrop soils had a higher pH which might also have contributed to the greater bacterial diversity under this cropping system as it has been shown by Lauber *et al.,* (2009) that a higher pH is frequently linked to greater soil bacterial diversity. The high bacterial diversity observed under the maize and beans system however could indicate that certain cropping systems may not necessarily reduce the diversity of soil microbes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study intended to determine the microbial profiles, soil physicochemical characteristics and establishing the relationship between the microbial profiles and the physicochemical properties of small holder farms under different cropping systems along river Isiukhu watershed in Western Kenya. It is therefore the first study to be carried in the region highlighting the impacts of different cropping systems on microbial profiles. The relative abundance and microbial profiles in different cropping systems was determined by Next Generation Sequencing. In conclusion:

Results from this study showed that *Acidobacteria, Proteobacteria, Verrucomicrobia* and *Actinobacteria* were the most abundant bacterial phyla while *Ascomycota* and *Basidiomycota* were the most abundant under fungi. Contrary to our first hypothesis, the soil microbial profiles were influenced by the different cropping systems although the dominant bacteria were similar at the phylum level but their abundances varied under the different treatments thus demonstrating that the different cropping systems influenced taxonomical microbial community soil structure below the phyla levels.

Soil physicochemical properties were markedly influenced by the different cropping systems whereby both soil total nitrogen and organic carbon were highest under maize and beans intercrop and lowest under napier soils while pH was lowest under forest soils and highest under maize and beans intercrop. These results also contradicted with our second hypothesis in that significant differences were recorded under the soil organic carbon, soil total nitrogen and pH among the different cropping systems.

Acidobacteria, Chloroflexi, Basidiomycota were affected by SOC whereas *Planctomycetes* and *Ascomycota* were affected by TN. Only *Acidobacteria* was affected by pH. This implied that there was a unique relationship between the specific subset of bacterial phyla rather than all and the selected soil physicochemical properties. Shannon diversity index confirmed greater bacterial diversity under maize and beans intercrop and lowest under sugarcane while considering the fungal diversity, a greater diversity was observed under sugarcane and lowest in napier soils. This shows that different cropping systems affects underground soil microbial diversity.

6.2 Recommendations

Degradation of soil due to cultivation is becoming severe in sub-Saharan Africa and there currently exist few reports on soil microbial profiles in this region. More studies are therefore needed to better understand soil microbial community profiles and how they differ with various cropping systems. Cropping systems such napier grass have a great negative impact on the microbial diversity in the watershed, and appropriate farming approaches should be explored that can reduce this effect.

Since this study only analyzed samples from the short rain season, future studies should consider longer periods of time in order to gain a better understanding of microbial profiles and diversity in response to different cropping systems.

Cropping systems that reduces the levels of SOC and TN in soil should be noted and therefore enhanced with organic manure in order to increase the relative abundance and diversity of soil microbes.

REFERENCES

Acinas, S. G., Marcelino, L. A., Klepac-Ceraj, V., & Polz, M. F. (2004). Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *Journal of bacteriology, 186*(9), 2629-2635.

Amadalo, B., & Jama, B. (2003). *Improved fallows for western Kenya: an extension guideline*. World Agroforestry Centre.

Anderson, J. M. and J. S. I. Ingram. 1993. 'Tropical Soil Biology and Fertility: A Handbook of Methods'. P. 221 in. Wallingford, Oxfordshire: Cambridge University Press (CUP).

Andre P Masella, Andrea K Bartram, Jakub M Truszkowski, Daniel G Brown and Josh D Neufe Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G., & Neufeld, J. D. (2012). PANDAseq: paired-end assembler for illumina sequences. *BM Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G., & Neufeld, J. D. (2012). PANDAseq: paired-end assembler for illumina sequences. BMC bioinformatics, 13(1), 31.C bioinformatics, 13*(1), 31.ld.

Asemaninejad, A., N. Weerasuriya, G. B. Gloor, Z. Lindo, and G. Thorn. 2016. 'New Primers for Discovering Fungal Diversity Using Nuclear Large Ribosomal DNA'. PLoS ONE 11(7).

Babujia.L.C., Hungria.M., Franchini. J.C., Brookes. P.C. (2010). Microbial biomass and activity at various soil Babujia, L. C., Hungria, M., Franchini, J. C., & Brookes, P. C. (2010). Microbial biomass and activity at various soil depths in a Brazilian

oxisol after two decades of no-tillage and conventional tillage. *Soil Biology and Biochemistry*, *42*(12), 2174-2181. depths in a Brazilian Oxisol after two decades of no-tillage and conventional tillage. *Soil Biol. Biochem*. 42,2174–2181.

Bakker, M. G., Manter, D. K., Sheflin, A. M., Weir, T. L., & Vivanco, J. M. (2012). Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil*, *360*(1-2), 1-13.

Beales, N. (2004). Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comprehensive reviews in food science and food safety*, *3*(1), 1-20.

Boddey, R. M., Jantalia, C. P., Conceicao, P. C., Zanatta, J. A., Bayer, C., Mielniczuk, J., ... & Giacomini, S. J. (2010). Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. *Global Change Biology*, *16*(2), 784-795.

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., ... & Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature methods*, *10*(1), 57.

Bouyoucos, G. J. (1936). Directions for making mechanical analyses of soils by the hydrometer method. Soil Science, 42(3), 225-230.

Brackin, R., Robinson, N., Lakshmanan, P., & Schmidt, S. (2013). Microbial function in adjacent subtropical forest and agricultural soil. *Soil Biology and Biochemistry*, *57*, 68-77.

Brewer, T. E., Handley, K. M., Carini, P., Gilbert, J. A., & Fierer, N. (2017). Genome reduction in an abundant and ubiquitous soil bacterium 'Candidatus Udaeobacter copiosus'. *Nature microbiology*, *2*(2), 16198.

Brimecombe, M. J., De Leij, F. A., & Lynch, J. M. (2000). The effect of root exudates on rhizosphere microbial populations. In *The rhizosphere* (pp. 111-156). CRC Press.

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and environmental microbiology*, *74*(3), 738-744.

Brown G.G., Hungria M., Oliveira l.J., Bunning S., Montanez A. (2002). International technical workshop on biological management of soil ecosystems for sustainableagriculture: Program, abstracts and related documents. Embrapa/FAO, Londrina.

Bucher, A. E., & Lanyon, L. E. (2005). Evaluating soil management with microbial community-level physiological profiles. *Applied Soil Ecology*, *29*(1), 59-71.

Butler, J. L., Bottomley, P. J., Griffith, S. M., & Myrold, D. D. (2004). Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. *Soil Biology and Biochemistry*, *36*(2), 371-382.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Huttley, G. A. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335.

Carrera, L. M., Buyer, J. S., Vinyard, B. R. Y. A. N., Abdul-Baki, A. A., Sikora, L. J., & Teasdale, J. R. (2007). Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. *Applied Soil Ecology*, *37*(3), 247-255.

Chen, Yong-Liang, Hang-Wei Hu, Hong-Yan Han, Yue Du, Shi-Qiang Wan, Zhu-Wen Xu, and Bao-Dong Chen. 2014. 'Abundance and Community Structure of Ammonia-Oxidizing Archaea and Bacteriain Response to Fertilization and Mowing in a Temperate Steppe in Inner Mongolia'. FEMS Microbiology Ecology 89(1):67–79.

Cui, J., Wang, J., Xu, J., Xu, C., & Xu, X. (2017). Changes in soil bacterial communities in an evergreen broad-leaved forest in east China following 4 years of nitrogen addition. *Journal of Soils and Sediments*, *17*(8), 2156-2164.

Cunha, E. D. Q., Stone, L. F., Moreira, J. A., Ferreira, E. D. B., Didonet, A. D., & Leandro, W. M. (2011). Soil tillage systems and cover crops in organic production of common bean and corn. I-Soil physical properties. *Revista Brasileira De Ciencia Do Solo*, *35*(2), 589-602.

Depret, G., Houot, S., Allard, M. R., Breuil, M. C., Nouaïm, R., & Laguerre, G. (2004). Long-term effects of crop management on Rhizobium leguminosarum biovar viciae populations. *FEMS Microbiology Ecology*, *51*(1), 87-97.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., ... & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database

and workbench compatible with ARB. *Appl. Environ. Microbiol.*, *72*(7), 5069-5072.

Drijber, R. A., Doran, J. W., Parkhurst, A. M., & Lyon, D. J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology and Biochemistry*, *32*(10), 1419-1430.

Edmeades, D. C. (2003). The long-term effects of manures and fertilisers on soil productivity and quality: a review. *Nutrient cycling in Agroecosystems*, *66*(2), 165-180.

FAO (Food and Agriculture Organization of the United Nations. (2012). Agri-culture and consumer protection department. Conservation agriculture, http://www.fao.org/nr/cgrfa/cthemes/cgrfa-micro-organisms/en/ (accessed02/2010).

Ferreira, M. C., Andrade, D. D. S., Chueire, L. M. D. O., Takemura, S. M., & Hungria, M. (2000). Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biology and Biochemistry*, *32*(5), 627-637.

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, *88*(6), 1354-1364.

Fierer, N., Ladau, J., Clemente, J. C., Leff, J. W., Owens, S. M., Pollard, K. S., ... & McCulley, R. L. (2013). Reconstructing the microbial diversity and function of

pre-agricultural tallgrass prairie soils in the United States. *Science*, *342*(6158), 621-624.

Follett, R. F. (2001). Soil management concepts and carbon sequestration in cropland soils. *Soil and Tillage Research*, *61*(1-2), 77-92.

Graham, M. H., Haynes, R. J., & Meyer, J. H. (2002). Soil organic matter content and quality: effects of fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. *Soil biology and biochemistry*, *34*(1), 93-102.

Guimaraes, D. V., Gonzaga, M. I., & Melo Neto, J. D. O. (2014). Management of soil organic matter and carbon storage in tropical fruit crops. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *18*(3), 301-306.

Hamamoto, T., Chirwa, M., Nyambe, I., & Uchida, Y. (2018). Small-Scale Variability in the Soil Microbial Community Structure in a Semideveloped Farm in Zambia. *Applied and Environmental Soil Science*, *2018*.

Hopkins, D. W., & Gregorich, E. G. (2005). Carbon as a substrate for soil organisms. *Biological diversity and function in soils*, 57-82.

Hugenholtz, P. (2002). Exploring prokaryotic diversity in the genomic era. *Genome biology*, *3*(2), reviews0003-1.

Jangid, K., Williams, M. A., Franzluebbers, A. J., Sanderlin, J. S., Reeves, J. H., Jenkins, M. B., ... & Whitman, W. B. (2008). Relative impacts of land-use, management

intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology and Biochemistry*, *40*(11), 2843-2853.

Jones, D. L., Nguyen, C., & Finlay, R. D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant and soil, 321(1-2), 5-33.

Kerfahi, D., Tripathi, B. M., Lee, J., Edwards, D. P., & Adams, J. M. (2014). The impact of selective-logging and forest clearance for oil palm on fungal communities in Borneo. PloS one, 9(11).

Kielak, A. M., Barreto, C. C., Kowalchuk, G. A., van Veen, J. A., & Kuramae, E. E. (2016). The ecology of Acidobacteria: moving beyond genes and genomes. *Frontiers in microbiology*, *7*, 744.

Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H., & Trevors, J. T. (2004). Methods of studying soil microbial diversity. *Journal of microbiological methods*, *58*(2), 169-188.

Kowalchuk, G. A., Drigo, B., Yergeau, E., & van Veen, J. A. (2006). Assessing bacterial and fungal community structure in soil using ribosomal RNA and other structural gene markers. In *Nucleic acids and proteins in soil* (pp. 159-188). Springer, Berlin, Heidelberg.

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.*, *75*(15), 5111-5120.

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.*, *75*(15), 5111-5120.

Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., ... & Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New phytologist*, *199*(1), 288-299.

Liu, X., Zhang, J., Gu, T., Zhang, W., Shen, Q., Yin, S., & Qiu, H. (2014). Microbial community diversities and taxa abundances in soils along a seven-year gradient of potato monoculture using high throughput pyrosequencing approach. *PloS one*, *9*(1), e86610.

Liu, Z., & Liu, J. (2013). Evaluating bacterial community structures in oil collected from the sea surface and sediment in the northern Gulf of Mexico after the Deepwater Horizon oil spill. *MicrobiologyOpen*, *2*(3), 492-504.

Lombard, N., Prestat, E., van Elsas, J. D., & Simonet, P. (2011). Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. *FEMS microbiology ecology*, *78*(1), 31-49.

Luo, P., Han, X., Wang, Y., Han, M., Shi, H., Liu, N., & Bai, H. (2015). Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Annals of microbiology, 65(1), 533-542.

Lynd, L. R., P. J. Weimer, W. H. van Zyl, and I. S. Pretorius. 2002. 'Microbial Cellulose Utilization: Fundamentals and Biotechnology'. Microbiology and Molecular Biology Reviews 66(3):506–77.

Mandal, A., Patra, A. K., Singh, D., Swarup, A., & Masto, R. E. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource technology*, *98*(18), 3585-3592.

Martyniuk, S., Koziel, M., Stalenga, J., & Jonczyk, K. (2016). Labile factions of soil organic matter and microbial characteristics of soil under organic and conventional crop management systems. *Biological agriculture & horticulture*, *32*(1), 1-6.

McGuire, K. L., & Treseder, K. K. (2010). Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biology and Biochemistry*, *42*(4), 529-535.

McGuire, K. L., H. D'Angelo, F. Q. Brearley, S. M. Gedallovich, N. Babar, N. Yang, C. M. Gillikin, R. Gradoville, C. Bateman, B. L. Turner, P. Mansor, J. W. Leff, and N. Fierer. 2014. 'Responses of Soil Fungi to Logging and Oil Palm Agriculture in Southeast Asian Tropical Forests'. Microbial Ecology 69(4):733–47.

Meisinger, D. B., Zimmermann, J., Ludwig, W., Schleifer, K. H., Wanner, G., Schmid, M., ... & Lee, N. M. (2007). In situ detection of novel Acidobacteria in microbial mats from a chemolithoautotrophically based cave ecosystem (Lower Kane Cave, WY, USA). *Environmental microbiology*, *9*(6), 1523-1534.

Meliani, A., Bensoltane, A., & Mederbel, K. (2012). Microbial diversity and abundance in soil: related to plant and soil type. *American Journal of Plant Nutrition and Fertilization Technology*, *2*(1), 10-18.

Meriles, J. M., Gil, S. V., Conforto, C., Figoni, G., Lovera, E., March, G. J., & Guzman, C. A. (2009). Soil microbial communities under different soybean cropping systems: characterization of microbial population dynamics, soil microbial activity, microbial biomass, and fatty acid profiles. *Soil and Tillage Research*, *103*(2), 271-281.

Metzker, M. L. (2010). Sequencing technologies—the next generation. *Nature reviews genetics*, *11*(1), 31-46.

Miki, T., Ushio, M., Fukui, S., & Kondoh, M. (2010). Functional diversity of microbial decomposers facilitates plant coexistence in a plant–microbe–soil feedback model. *Proceedings of the National Academy of Sciences*, *107*(32), 14251-14256.

Moon, J. B., Wardrop, D. H., Bruns, M. A. V., Miller, R. M., & Naithani, K. J. (2016). Land-use and land-cover effects on soil microbial community abundance and composition in headwater riparian wetlands. *Soil Biology and Biochemistry*, *97*, 215-233.

Müller, T., & Höper, H. (2004). Soil organic matter turnover as a function of the soil clay content: consequences for model applications. *Soil Biology and Biochemistry*, *36*(6), 877-888.

Myers, R. T., Zak, D. R., White, D. C., & Peacock, A. (2001). Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Science Society of America Journal*, *65*(2), 359-367.

Navarrete, A. A., Kuramae, E. E., de Hollander, M., Pijl, A. S., van Veen, J. A., & Tsai, S. M. (2013). Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS microbiology ecology*, *83*(3), 607-621.

Negasa, T., Ketema, H., Legesse, A., Sisay, M., & Temesgen, H. (2017). Variation in soil properties under different land use types managed by smallholder farmers along the toposequence in southern Ethiopia. *Geoderma*, *290*, 40-50.

Nie, Y., Wang, M., Zhang, W., Ni, Z., Hashidoko, Y., & Shen, W. (2018). Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment. *Science of the total environment*, *624*, 407-415.

Ojiem, John O. 2006. 'Exploring Socio-Ecological Niches for Legumes in Western Kenya Smallholder Farming Systems'. Wageningen Universiteit.

Omoro, L. M., Starr, M., & Pellikka, P. K. (2013). Tree biomass and soil carbon stocks in indigenous forests in comparison to plantations of exotic species in the Taita Hills of Kenya. Silva Fennica, 47(2), 1-18.

Osborne, C. A., Zwart, A. B., Broadhurst, L. M., Young, A. G., & Richardson, A. E. (2011). The influence of sampling strategies and spatial variation on the detected

soil bacterial communities under three different land-use types. *FEMS Microbiology Ecology*, *78*(1), 70-79.

Oyamo, S., Serrem, C., & Otinga, A. N. (2017). Restoring soil fertility in previously sugarcane cropped farms for maize production in Butere-Kakamega County. *African Journal of Agricultural Research*, *12*(33), 2598-2607.

Patel, R. K., & Jain, M. (2012). NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. *PloS one*, *7*(2), e30619.

Paustian, K., Six, J., Elliott, E. T., & Hunt, H. W. (2000). Management options for reducing CO2 emissions from agricultural soils. *Biogeochemistry*, *48*(1), 147-163.

Pereira, J. D. M., Baretta, D., Bini, D., Vasconcellos, R. L. D. F., & Cardoso, E. J. B. N. (2013). Relationships between microbial activity and soil physical and chemical properties in native and reforested Araucaria angustifolia forests in the state of São Paulo, Brazil. *Revista Brasileira de Ciência do Solo*, *37*(3), 572-586.

Pontes, D. S., Lima-Bittencourt, C. I., Chartone-Souza, E., & Nascimento, A. M. A. (2007). Molecular approaches: advantages and artifacts in assessing bacterial diversity. *Journal of industrial microbiology & biotechnology*, *34*(7), 463-473.

Posada, J. M., & Schuur, E. A. (2011). Relationships among precipitation regime, nutrient availability, and carbon turnover in tropical rain forests. *Oecologia*, *165*(3), 783-795.

Rachel E. Gallery (2014). Ecology of Tropical Rain Forests. *Ecology and the Environment.* 10.1007/978-1-4614-7612-2_4-1.

Rajendhran, J., & Gunasekaran, P. (2008). Strategies for accessing soil metagenome for desired applications. *Biotechnology advances*, *26*(6), 576-590.

Rhodes, J.D. (1982). In methods of soil analysis, part 2. Second edition (A.L. Page, R.H.M and D.R.Keeney, (Eds.). American Society of Agronomy, Madison, USA, 102-105.

Rideout, J. R., He, Y., Navas-Molina, J. A., Walters, W. A., Ursell, L. K., Gibbons, S. M., ... & Clemente, J. C. (2014). Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ*, *2*, e545.

Roesti, D., Gaur, R., Johri, B. N., Imfeld, G., Sharma, S., Kawaljeet, K., & Aragno, M. (2006). Plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. *Soil Biology and Biochemistry*, *38*(5), 1111-1120.

Romaniuk, R., Giuffré, L., Costantini, A., Bartoloni, N., & Nannipieri, P. (2012). A comparison of indexing methods to evaluate quality of soils: the role of soil microbiological properties. *Soil Research*, *49*(8), 733-741.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., ... & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME journal*, *4*(10), 1340.

Sakin, E. 2012. 'Relationships between of Carbon, Nitrogen Stocks and Texture of the Harran Plain Soils in Southeastern Turkey'. Bulgarian Journal of Agricultural Science 18(4):626–34.

Shi, Y., Yang, H., Zhang, T., Sun, J., & Lou, K. (2014). Illumina-based analysis of endophytic bacterial diversity and space-time dynamics in sugar beet on the north slope of Tianshan mountain. *Applied microbiology and biotechnology*, *98*(14), 6375-6385.

Silva, A. P., BABUJIA, L., Matsumoto, M. F., Guimarães, M. F., & Hungria, M. (2013). Bacterial diversity under different tillage and crop rotation systems in an oxisol of Southern Brazil. *Embrapa Soja-Artigo em periódico indexado (ALICE)*.

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., ... & Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences*, *103*(32), 12115-12120.

Sombroek, W. G., Braun, H. M. H., & Van der Pouw, B. J. A. (1982). Exploratory soil map and agro-climatic zone map of Kenya, 1980. Scale 1: 1,000,000. Kenya Soil Survey.

Song, Y., Deng, S. P., Acosta-Martínez, V., & Katsalirou, E. (2008). Characterization of redox-related soil microbial communities along a river floodplain continuum by fatty acid methyl ester (FAME) and 16S rRNA genes. *applied soil ecology*, *40*(3), 499-509.

Spiegelman, D., Whissell, G., & Greer, C. W. (2005). A survey of the methods for the characterization of microbial consortia and communities. *Canadian Journal of Microbiology*, *51*(5), 355-386.

Steenwerth, K. L., Jackson, L. E., Calderón, F. J., Stromberg, M. R., & Scow, K. M. (2002). Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry*, *34*(11), 1599-1611.

Sun, R., Zhang, X. X., Guo, X., Wang, D., & Chu, H. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry, 88*, 9-18.

Tolli, J., & King, G. M. (2005). Diversity and structure of bacterial chemolithotrophic communities in pine forest and agroecosystem soils. *Appl. Environ. Microbiol.*, *71*(12), 8411-8418.

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current opinion in microbiology*, *5*(3), 240-245.

Treseder, K. K. (2008). Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology letters*, *11*(10), 1111-1120.

Ushio, M., Kitayama, K., & Balser, T. C. (2010). Tree species effects on soil enzyme activities through effects on soil physicochemical and microbial properties in a tropical montane forest on Mt. Kinabalu, Borneo. *Pedobiologia*, *53*(4), 227-233.

Van Groenigen, K. J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., ... & Jones, M. B. (2010). Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biology and Biochemistry*, *42*(1), 48-55.

Vineela, C., Wani, S. P., Srinivasarao, C. H., Padmaja, B., & Vittal, K. P. R. (2008). Microbial properties of soils as affected by cropping and nutrient management practices in several long-term manurial experiments in the semi-arid tropics of India. *Applied Soil Ecology*, *40*(1), 165-173.

Vitousek, P. M., Naylor, R., Crews, T., David, M. B., Drinkwater, L. E., Holland, E., & Nziguheba, G. (2009). Nutrient imbalances in agricultural development. *Science*, *324*(5934), 1519-1520.

Voříšková, J., & Baldrian, P. (2013). Fungal community on decomposing leaf litter undergoes rapid successional changes. *The ISME journal*, *7*(3), 477.

Walkley, A. and I. Armstrong Black. 1934. 'An Examination of the Degtjareff Method for Determining Soil Organic, and a Proposed Modification of the Chromic Acid Titration Method'. Soil Science 37(1):29–38.

Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindström, K., Hartikainen, H., & Niemi, R. M. (2011). Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. *Soil Biology and Biochemistry*, *43*(7), 1464-1473.

Wan, S., & Luo, Y. (2003). Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment. *Global biogeochemical cycles*, *17*(2).

Wang, F., Ding, Y., Sayer, E. J., Li, Q., Zou, B., Mo, Q., & Li, Z. (2017). Tropical forest restoration: Fast resilience of plant biomass contrasts with slow recovery of stable soil C stocks. *Functional Ecology*, *31*(12), 2344-2355.

Wang, J., Song, Y., Ma, T., Raza, W., Li, J., Howland, J. G., & Shen, Q. (2017). Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. *Applied Soil Ecology*, *112*, 42-50.

Wang, J., Song, Y., Ma, T., Raza, W., Li, J., Howland, J. G., & Shen, Q. (2017). Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. Applied Soil Ecology, 112, 42-50.

Wang, Y., Zeng, X., Iyer, N. J., Bryant, D. W., Mockler, T. C., & Mahalingam, R. (2012). Exploring the switchgrass transcriptome using second-generation sequencing technology. *PloS one*, *7*(3), e34225.

Williams, A., Birkhofer, K., & Hedlund, K. (2014). Above-and below-ground interactions with agricultural management: Effects of soil microbial communities on barley and aphids. *Pedobiologia*, *57*(2), 67-74.

Wood, S. A., Almaraz, M., Bradford, M. A., McGuire, K. L., Naeem, S., Neill, C., & Zhou, J. (2015). Farm management, not soil microbial diversity, controls nutrient loss from smallholder tropical agriculture. *Frontiers in microbiology*, *6*, 90.

Yesilonis, I., Szlavecz, K., Pouyat, R., Whigham, D., & Xia, L. (2016). Historical land use and stand age effects on forest soil properties in the Mid-Atlantic US. *Forest Ecology and Management*, *370*, 83-92.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., & Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: are there any links?. *Ecology*, *84*(8), 2042-2050.

Zak, D. R., Pregitzer, K. S., Burton, A. J., Edwards, I. P., & Kellner, H. (2011). Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. *Fungal Ecology*, *4*(6), 386-395.

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., & van der Lelie, D. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio*, *6*(2).

Zhang, B., Li, Y., Ren, T., Tian, Z., Wang, G., He, X., & Tian, C. (2014). Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. *Biology and fertility of soils*, *50*(7), 1077-1085.

Zhang, J., Wang, P., Tian, H., Xiao, Q., & Jiang, H. (2019). Pyrosequencing-based assessment of soil microbial community structure and analysis of soil properties

with vegetable planted at different years under greenhouse conditions. *Soil and Tillage Research*, *187*, 1-10.

Zhao, D., Huang, R., Zeng, J., Yu, Z., Liu, P., Cheng, S., & Wu, Q. L. (2014). Pyrosequencing analysis of bacterial community and assembly in activated sludge samples from different geographic regions in China. *Applied microbiology and biotechnology*, *98*(21), 9119-9128.

Zhao, L., Sun, Y., Zhang, X., Yang, X., & Drury, C. F. (2006). Soil organic carbon in clay and silt sized particles in Chinese mollisols: relationship to the predicted capacity. *Geoderma*, *132*(3-4), 315-323.

Zhong, Y., Yan, W., & Shangguan, Z. (2015). Impact of long-term N additions upon coupling between soil microbial community structure and activity, and nutrient-use efficiencies. *Soil Biology and Biochemistry*, *91*, 151-159.

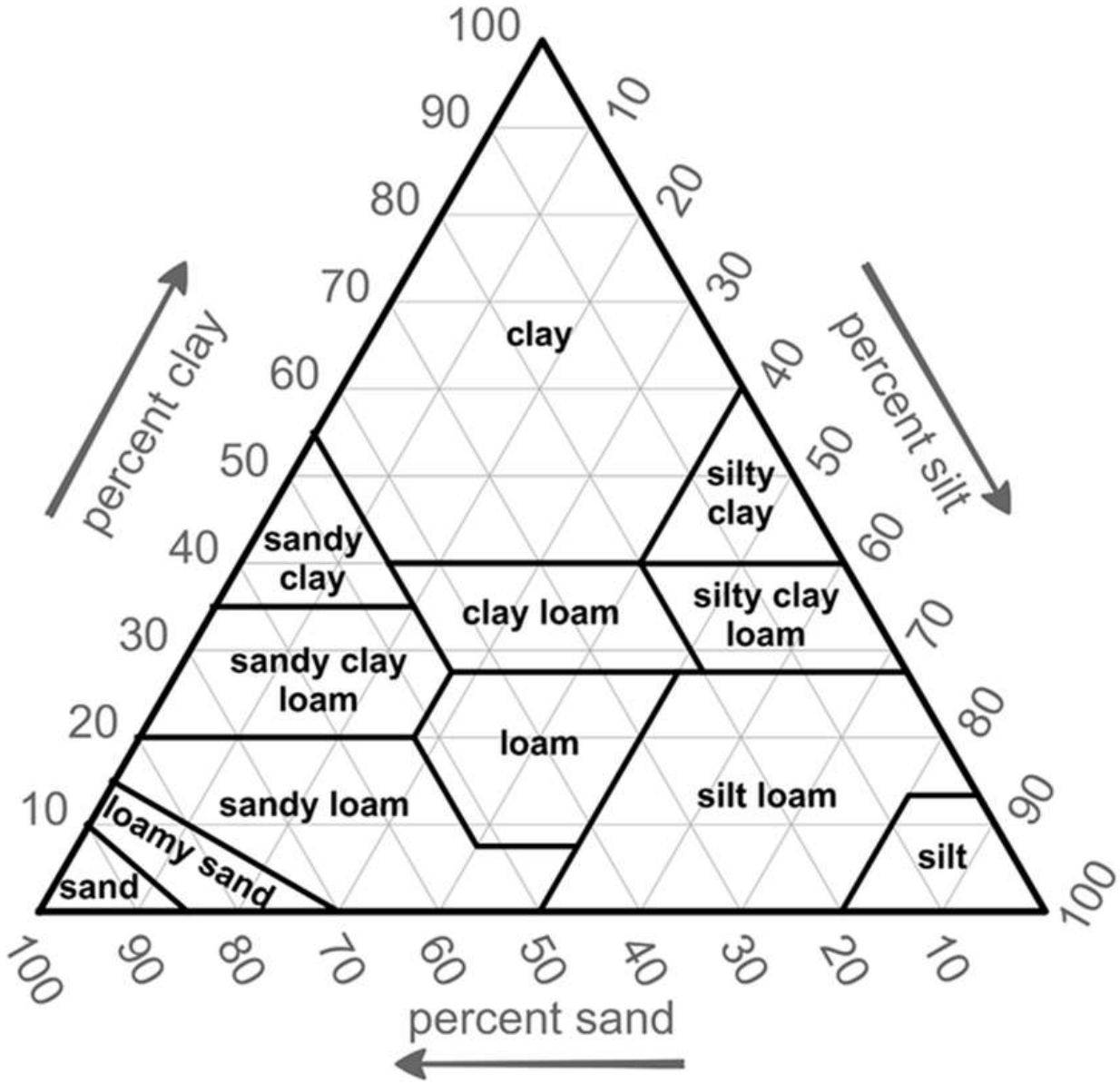
Zhou, X., Liu, J., & Wu, F. (2017). Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. *Plant and Soil*, *415*(1-2), 507-520.

Zhou, X., Yu, G., & Wu, F. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. *European Journal of Soil Biology*, *47*(5), 279-287.

Zotarelli, L., B. J. R. Alves, S. Urquiaga, R. M. Boddey, and J. Six. 2007. 'Impact of Tillage and Crop Rotation on Light Fraction and Intra-Aggregate Soil Organic Matter in Two Oxisols'. Soil and Tillage Research 95(1–2):196–206.

Zwolinski, M. D. (2007). DNA sequencing: strategies for soil microbiology. *Soil Science Society of America Journal*, *71*(2), 592-600.

APPENDICES



Appendix 1: Soil texture triangle (format-USDA) used to assign textural classes of soil under study based on particle size distribution.

Appendix 2: NCBI Links to the Submitted Sequences

https://www.ncbi.nlm.nih.gov/biosample/16521379
https://www.ncbi.nlm.nih.gov/biosample/16521380
https://www.ncbi.nlm.nih.gov/biosample/16521381
https://www.ncbi.nlm.nih.gov/biosample/16521382
https://www.ncbi.nlm.nih.gov/biosample/16521383
https://www.ncbi.nlm.nih.gov/biosample/16521384
https://www.ncbi.nlm.nih.gov/biosample/16521385